

Survey and Assessment of Cotton Leaf Curl Disease Severity in North-Cotton Growing Zone of India

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ABSTRACT

Cotton is one of the major agricultural cash crops worldwide as well as in the Indian subcontinent. Cotton production in India is severely affected by cotton leaf curl virus disease (CLCuD), caused by a complex of CLCuD-associated begomoviruses (CABs) transmitted by whiteflies (*Bemisia tabaci*). Lately, CLCuD incidence was found to occur in some unpredictable patterns. To assess the disease incidence, the survey was conducted in different locations in the north cotton growing zone of India during the years 2022 and 2023. The per cent disease incidence and per cent disease index (PDI) were recorded based on the morphological symptoms of CLCuD and disease severity grades. The CLCuD incidence was 100% in all the three states during 2022, however, during 2023 it was low and ranged from 13.8 to 23.3%. The PDI of CLCuD varied from location to location in all the three states. During 2022, it ranged from 53.38 to 61.09 % in Haryana, from 49.77 to 54.38% in Punjab and from 53.08 to 56.42% in Rajasthan. However, the PDI was drastically low during 2023 and ranged from 10.27 to 12.7% in Haryana, 5.97 to 10.27% in Punjab and 9.47 to 12.11% in Rajasthan. The huge variability in incidence and PDI indicates the possible role of climate change and recombination in CABs and emphasizes the need to continuously monitor, and to map the disease incidence with climate as well as with the emergence of recombinants to develop suitable management strategies.

Key words: Cotton leaf curl disease, per cent disease incidence, DRS, begomovirus

INTRODUCTION

Cotton is a major commodity worldwide and one of the dominant cash crops of the Indian sub-continent. India is the largest producer and exporter of cotton worldwide, with an estimated cultivated area of 130.49 lakh hectares with a productivity of 439 kg lint/ha (Anonymous, 2022-23). Based on geographical location, different cotton production zones have been identified like the North zone (Haryana, Punjab and Rajasthan), Central zone (Madhya Pradesh, Maharashtra, and Gujarat) and South zone (Andhra Pradesh, Telangana, Karnataka, Tamil Nadu and Odisha). The North zone contributes to more than 50% of the total yield, with a cultivation area of 1.67 million hectares (Anonymous, 2022-23). India is the only country in the world having the cultivation of all four domesticated species two diploids viz., *Gossypium arboreum*, *G. herbaceum* and two tetraploids viz., *G. hirsutum* and *G. barbadense*. In the North zone, the two species viz., *G.*

hirsutum and *G. arboreum* are the commonly cultivated ones, and of these two, the former is more popular due to its higher yield (Hasan *et al.*, 2019). The cotton production in North zone is severely affected by cotton leaf curl disease (CLCuD), which is caused by a complex of CLCuD-associated begomoviruses (CABs) transmitted by whiteflies (*Bemisia tabaci*; Soumia *et al.*, 2021; Sain *et al.*, 2023). CLCuD symptoms are characterized by the thickening, and darkening of leaf veins, leaf enations, and tiny delicate leaf-like structures on the undersurface of the leaves and upward or downward curling. Severe symptoms include stunted growth of the plants and result in reduced flowering, boll formation and boll size (Sain *et al.*, 2020). Cotton productivity is adversely affected by CLCuD infection, which leads to the deterioration of fiber quality and seed cotton yield. Since its first appearance in the 1990s in the Indian sub-continent, CLCuD has caused several epidemics. The Multan epidemic from 1990 to 1999, and the

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Burewala epidemic from 2004 to 2015 were devastating to cotton production in the subcontinent and caused an estimated loss of 630-670 million US dollars to the Indian economy in 2016 (Sattar *et al.*, 2017). CLCuD continues to damage the Indian economy (Anonymous, 2019-20).

Genetic diversity within the CLCuD-begomovirus species in cotton-growing regions of India has been extensively studied (Sattar *et al.*, 2017; Zubair *et al.*, 2017). More than nine species of CLCuD-begomoviruses have been identified in the Indian subcontinent. The core viruses are *Cotton leaf curl Alabad virus* (CLCuAlV), *Cotton leaf curl Kokhran virus* (CLCuKoV) and *Cotton leaf curl Multan virus* (CLCuMuV) being the predominant and widely distributed types (Saleem *et al.*, 2016; Godara *et al.*, 2017; Sattar *et al.*, 2017; Zubair *et al.*, 2017). Other less prevalent non-core viruses i.e. CLCuD-begomoviruses, include *Cotton leaf curl Bangalore virus* (CLCuBaV), *Cotton leaf curl Gezira virus* (CLCuGeV), *Okraenation leaf curl virus* (OEnLCV), *Papaya leaf curl virus* (PaLCuV), *Tomato leaf curl Bangalore virus* (ToLCuBaV) and *Tomato leaf curl New Delhi virus* (ToLCNDV) have been found to be occasionally associated with the CLCuD complex (Brown *et al.*, 2017).

The sole beta satellite species detected in association with the CLCuD complex in the Indian subcontinent is the *Cotton leaf curl Multan beta satellite* (CLCuMB; Godara *et al.*, 2016; Datta *et al.*, 2017; Sattar *et al.*, 2017; Qadir *et al.*, 2019). Various alpha satellites, such as *Cotton leaf curl Multan alpha satellite* (CLCuMuA), *Cotton leaf curl Lucknow alpha satellite* (CLCuLuA), *Gossypium darwinii symptomless alpha satellite* (GDarSLA), *Gossypium mustelinum symptomless alpha satellite* (GMusSLA) and *Guar leaf curl alpha satellite* (GLCuA) have also been identified in association with the CLCuD complex in this region (Datta *et al.*, 2017; Briddon *et al.*, 2018; Qadir *et al.*, 2019). CLCuD-associated begomoviruses (CABs) have single-stranded (ss) DNA genome (identified as DNA-A, ~ 2.8kb in size), along with two ss circular satellite DNA molecules: alpha satellite (of ~1.2 kb size) and beta satellite (of ~1.3 kb size) frequently associated with non-viral, single-stranded circular ss DNA, together representing as an infectious (Jain *et al.*, 2023). Alpha satellites are still enigmatic, whereas beta satellites are instrumental in determining disease severity,

symptoms and adaptability to the host (Sain *et al.*, 2020).

During the last decade, considerable variations in the severity of CLCuD prevailing in North India have been observed in farmer fields, CLCuD recently re-emerged as the most devastating disease in the entire 1.1 Mha cotton growing areas of North India (Biswas *et al.*, 2020). The incidence of CLCuD is increasing year by year, and causing a massive loss to cotton yield characterized with a significant reduction in the number of bolls per plant, branches per plant, boll weight, seed cotton yield and deterioration in lint quality (Monga and Sain, 2021). Because the yield loss corresponding to CLCuD symptom severity grades/scale, which is used to arrive at Percent Disease Incidence (PDI), may vary depending upon season and location, the present study was conducted for assessment of disease severity across various locations at three CLCuD hot-spot fields in Haryana, Punjab and Rajasthan for the years 2022 and 2023.

MATERIALS AND METHODS

To estimate the CLCuD incidence and severity at farmers' fields from August to September for the two consecutive years, 2022 and 2023, the survey was conducted in the farmers fields of Haryana, Punjab and Rajasthan. From each state, five different locations were selected. During 2022, the selected locations for the survey were Madhosighana, Ellenabad, Dabwali, Bhodiya Khera and HAU of Haryana; Chapriwala, Gobindgarh, Sarawan, Fatuhiwala and Faridkot of Punjab; and Bhadra, Gandhi Bari, Nohar, Nethrana and Sri Ganganagar of Rajasthan. During 2023, the selected locations were Umedpura, Chopta, Dabwali, Bhodiyakhera and Balsamand of Haryana; Chapriwala, Abohar, Sarawan, Fatuhiwala and Faridkot of Punjab; and Bhadra, Gandhi Bari, Malwani, Nethrana and Sri Ganganagar of Rajasthan. These 30 locations (Fig. 1) were surveyed and observations were recorded for two consecutive years.

The random field survey was conducted using the zigzag pattern to select the plants for phenotypic observations of the CLCuD symptoms. Plants were randomly selected in each field and observations were recorded thrice during the season. The CLCuD incidence

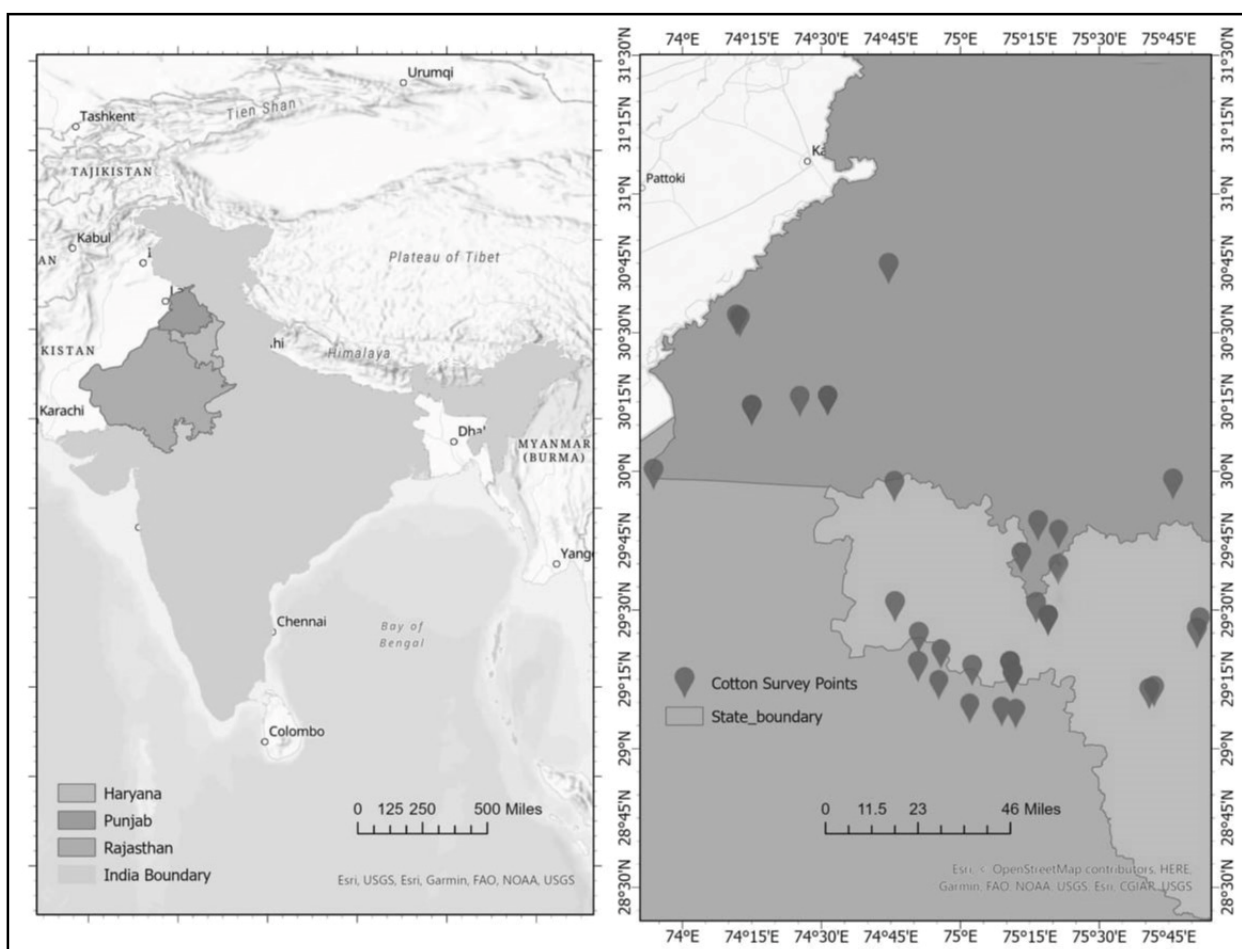


Fig. 1. The map location of the selected sites of cotton cultivation area of north India (GPS coordinates are shown with a location indication in tag, created by ArcGIS Pro). Observation on disease incidence and per cent disease incidence.

was recorded based on the morphological symptoms of CLCuD in the plants, such as the upward and downward curling of leaves, the thickening of veins, the presence of leaf enations and the cupping of leaves. The disease severity was observed based on the standard disease rating scale (Table 1). The symptoms noted for each field were

converted to the disease incidence as per the following formula: Disease Incidence (DI) – (Sum of all infected plants/ Total number of plants observed) × 100
Percent Disease Index (PDI) was calculated using the following standard formula: PDI – [Sum of all grades/ (Total plants observed x Maximum grade)] x 100

Table 1. Symptom and disease rating scale (Sain *et al.*, 2020)

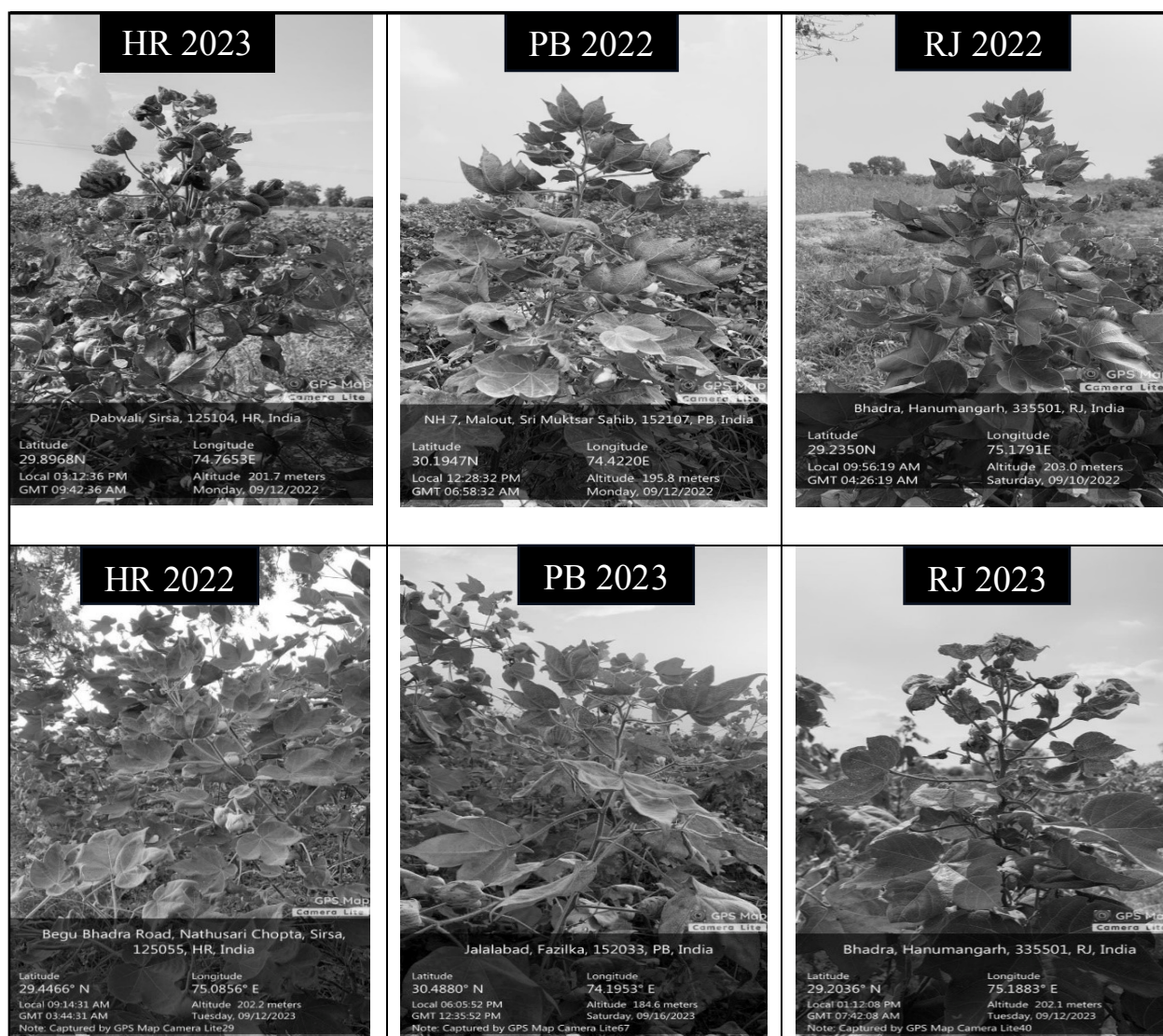
S. No.	Symptoms	Disease rating scale (DRS)/ symptom severity scale
1.	Complete absence of symptoms	0
2.	Symptoms of vein thickening on a few upper leaves	1
3.	Symptoms of vein thickening, cupping and curling on a few upper leaves	2
4.	25% of the plant affected by vein thickening, cupping, curling and leaf enations	3
5.	50% of plants affected by vein thickening, cupping, curling and leaf enations	4
6.	75% of plants affected 5 with vein thickening, cupping, curling and leafy enation	5
7.	Plants stunted severely and completely plant affected by vein thickening, cupping and curling, and leafy enation	6

Analysis of variance was performed to statistically analyze the experimental data. Treatment means were compared by one-factor analysis at 0.05%. Statistical analysis was performed using software OPSTAT, HAU, India. The graphical representation was made through Microsoft Excel 2019.

RESULTS AND DISCUSSION

Surveys carried out in August and September for two consecutive years (2022 and 2023), showed that the symptoms varied drastically from field to field across different years (Fig. 2).

The current investigation observed a 100% disease incidence (DI) in 2022 in Haryana Punjab and Rajasthan (Table 2). However, in the same year, a great variation was observed in PDI; the highest and lowest PDI for Haryana state was observed in Ellenabad, Sirsa (76.6%) and Madhosighana, Sirsa (64.4%), respectively. In Punjab, the highest and lowest PDI was observed in Sarawan, Mukatsar (66.1%) and Chapriwala, Fazilka (58.3%), respectively, while for Rajasthan, the highest and lowest PDI was observed in Sri Ganganagar, Sri Ganganagar (70.6%) and Nethrana, Hanumangarh (63.3%), respectively (Table 2 and Fig. 3).



Where: HR - Haryana, PB - Punjab and RJ - Rajasthan.

Fig. 2. Field survey during 2022 and 2023, CLCuD affected cotton plants exhibiting different symptoms in the farmers' field of Haryana, Punjab and Rajasthan.

Table 2. Field survey for CLCuD in the major cotton growing areas of Haryana, Punjab and Rajasthan for the year 2022

Haryana					
S. No.	Location	District	GPS coordinates	DI mean	Mean PDI* \pm SE
1.	Madhosighana (Sirsa)	Sirsa	29.4321N,75.9304E	100	64.4 (53.3) \pm 0.8
2.	Ellenabad (Sirsa)	Sirsa	29.4548N,74.7652E	100	76.7 (61.0) \pm 0.6 ^b
3.	Dabwali (Sirsa)	Sirsa	29.8966N,75.7653E	100	69.4 (56.4) \pm 1.2 ^a
4.	Bhodiya Khera (Fatehabad)	Fatehabad	29.4913N,75.4163E	100	71.1 (57.4) \pm 0.9 ^a
5.	HAU (Hisar)	Hisar	29.1412N,75.6797E	100	72.2 (58.1) \pm 0.9 ^{ab}
C. D.					2.889
S.E.(m)					0.872
S.E.(D)					1.234
C. V.					2.63
Punjab					
1.	Chapriwala (Jalalabad)	Fazilka	30.4818N,74.2069E	100	58.3 (49.7) \pm 0.5 ^c
2.	Gobindgarh (Abohar)	Fazilka	30.1625N,74.2496E	100	60.6 (51.0) \pm 1.1 ^{ac}
3.	Sarawan (Malout)	Mukatsar	30.1947N,74.4225E	100	66.1 (54.3) \pm 0.8 ^b
4.	Fatuhwala (Malout)	Mukatsar	29.9882N,74.6800E	100	58.9 (50.1) \pm 0.8 ^{ac}
5.	Faridkot (Faridkot)	Faridkot	30.6738N,74.7421E	100	62.2 (52.0) \pm 0.8 ^b
C. D.					1.737
S.E.(m)					0.525
S.E.(D)					0.742
C. V.					1.765
Rajasthan					
1.	Bhadra (Hanumangarh)	Hanumangarh	29.2390N,75.1790E	100	63.9 (53.0) \pm 0.8
2.	Gandhi Bari (Bhadra)	Hanumangarh	29.1974N,75.1886E	100	65.0 (53.8) \pm 0.4
3.	Nohar (Nohar)	Hanumangarh	29.3594N,75.8513E	100	70.0 (54.7) \pm 1.5
4.	Nethrana (Bhadra)	Hanumangarh	29.2253N,75.0427E	100	63.3 (56.4) \pm 0.3
5.	Sri Ganganagar (Sri Ganganagar)	Sri Ganganagar	29.9322N,73.8963E	100	70.6 (52.7) \pm 1.1
C. D.					N/A
S.E.(m)					1.084
S.E.(D)					1.533
C. V.					3.467

DI – Disease incidence, PDI – Per cent Disease Index (PDI* values in parentheses are angular transformed; error bar represents SD, n=3), Data with similar superscript are not significantly different.

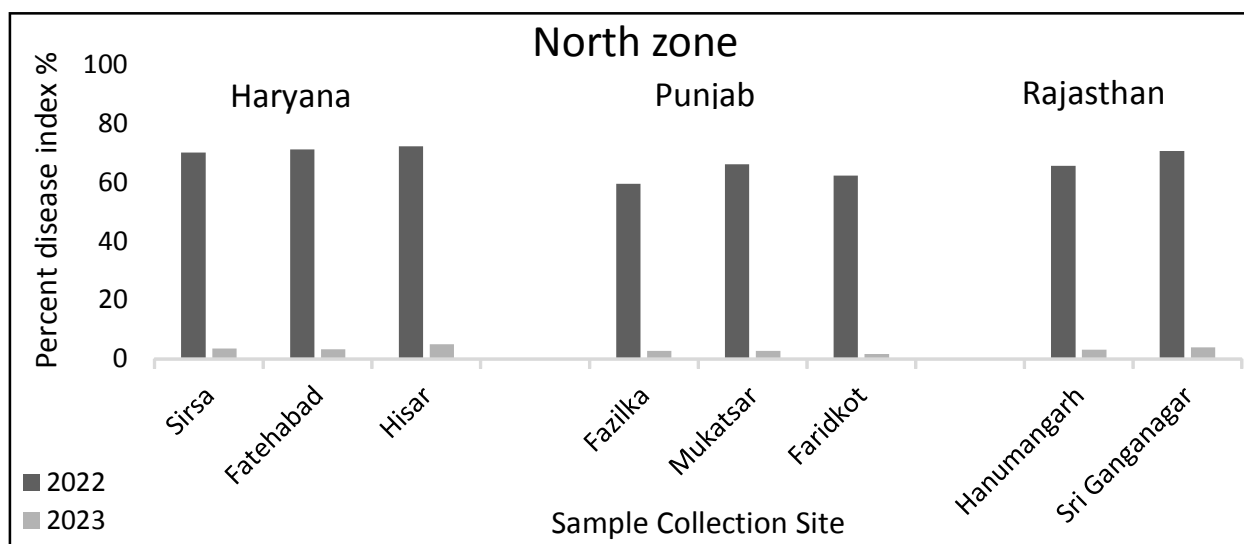


Fig. 3. District-wise per cent disease index observed in Haryana, Punjab and Rajasthan during the years 2022 and 2023.

For the survey conducted during 2023, very different observations were recorded as compared to the previous year in different

locations of Haryana, Punjab and Rajasthan. In Haryana, the highest and lowest DI was observed in Balsamand, Hisar (23.3%), and

Dabwali, Sirsa (13.3%), respectively. In Punjab, the highest and lowest DI was observed in Chapriwala, Fazilka (20.0%), and Faridkot, Faridkot (10.0%), while for Rajasthan, the highest and lowest DI were observed in Malwani, Hanumangarh (20.0%) and Bhadra, Hanumangarh (13.3%), respectively (Table 3). Likewise, a great variation was observed in PDI. The highest and lowest PDI was observed in Balsamand, Hisar (4.4%) and Umedpura, Sirsa (2.8%) of Haryana in Chapriwala, Fazilka (3.3%) and Faridkot, Faridkot (1.7%) of Punjab, and Malwani, Hanumangarh (4.4%) and Sri Ganganagar, Sri Ganganagar (3.9%) of Rajasthan, respectively (Table 3 and Fig. 3). In Haryana, Punjab and Rajasthan, during 2009-12, yield loss ranged between 25.2 - 46.6% from 15.7 to 56.7% during 2012-2014 (Monga 2016). In 2013, CLCuD appeared as an epidemic form in India, with 100% disease incidence with boll number reduction of 32.3 -

82.3% with about 40% yield loss (Bhattacharyya *et al.*, 2017). It was also reported as an overall disease incidence of 77.5 and 49.6% in Haryana; 54.1 and 57.8% in Punjab and 55.9 and 10.8% in Rajasthan between 2013 and 2014, respectively. With the rising frequency of CLCuD, there has been a significant loss in cotton cultivation and the occurrence of CLCuD-begomovirus variations in North India. Similarly, Biswas *et al.* (2020) found a CLCuD incidence of 37.5, 63.6, and 38.8% PDI in Haryana, Punjab and Rajasthan, respectively, from 2012 to 2014. Similarly, Monga and Sain (2021) observed PDI 51.3-57.8% in Punjab, and in Haryana it ranged from 32.7 to 77.5% and in Rajasthan it was 8.9 to 59.2%, respectively.

A correlation of CLCuD was identified with whitefly. Qadir *et al.* (2019) and Soumia *et al.* (2021) found that the prevalence of whiteflies in North India's cotton-growing areas was

Table 3. Field survey for CLCuD in the major cotton growing areas of Haryana, Punjab and Rajasthan for the year 2023

Haryana					
S. No.	Location	District	GPS coordinates	DI mean	Mean PDI* \pm SE
1.	Dabwali (Sirsa)	Sirsa	29.8880N,74.7628E	13.3	4.4(11.9) \pm 1.4
2.	Nathusari Chopta (Nathusari Chopta)	Sirsa	29.4517N,75.2727E	16.6	3.3(10.2) \pm 1.5
3.	Umedpura(Sirsa)	Sirsa	29.4056N,75.3160E	16.6	2.8(9.4) \pm 1.0
4.	Bhodiyaakhera (Fatehabad)	Fatehabad	29.4913N,75.4169E	16.6	3.3(10.2) \pm 1.5
5.	Balsamand (Hisar)	Hisar	29.1488N,75.6973E	23.3	5.0(12.7) \pm 1.2
C.D.					N/A
S.E.(m)					0.861
S.E.(D)					1.217
C.V.					13.60
Punjab					
1.	Chapriwala, (Jalalabad)	Fazilka	30.4881N,74.1958E	20.0	3.3(10.2) \pm 1.5
2.	Abohar, (Abohar)	Fazilka	30.1625N,74.2497E	13.3	2.2(8.4) \pm 1.0
3.	Sarawan, (Malout)	Mukatsar	30.1947N,74.4224E	16.6	2.8(9.4) \pm 1.0
4.	Fatuhwala, (Malout)	Mukatsar	29.9882N,74.6805E	13.3	2.8(9.4) \pm 1.0
5.	Faridkot, (Faridkot)	Faridkot	30.6738N,74.7401E	10.0	1.7(5.9) \pm 3.1
C.D.					N/A
S.E.(m)					1.89
S.E.(D)					2.68
C.V.					37.605
RAJASTHAN (2023)					
1.	Bhadra (Bhadra)	Hanumangarh	29.2376N,75.1786E	13.3	2.8(9.4) \pm 1.0
2.	Gandhi Bari (Bhadra)	Hanumangarh	29.2036N,75.1883E	16.6	3.3(10.2) \pm 1.5
3.	Nethrana (Bhadra)	Hanumangarh	29.2390N,74.8476E	13.3	2.2(8.4) \pm 1.0
4.	Malwani (Nohar)	Hanumangarh	29.3959N,75.8623E	20.0	4.4(12.1) \pm 1.0
5.	Sri Ganganagar (Sri Ganganagar)	Sri Ganganagar	29.9322N,73.8960E	16.6	3.9(11.3) \pm 1.0
C. D.					N/A
S.E.(m)					1.053
S.E.(D)					1.489
C.V.					17.4

DI – Disease incidence, PDI – Per cent Disease Index (PDI* values in parentheses are angular transformed; error bar represents SD, n=3).

Table 4. Field survey and estimation of disease incidence during the years 2022 and 2023

State/District	2022			2023		
	No. of fields surveyed	Average disease incidence (%)*	Average PDI surveyed	No. of fields surveyed	Average disease incidence (%)*	Average PDI
Haryana						
Sirsa	3	100	70.1	3	15.5	3.5
Fatehabad	1	100	71.1	1	16.6	3.3
Hisar	1	100	72.2	1	23.3	5.0
Mean	5	100	70.7	5	18.4	3.9
Punjab						
Faridkot	1	100	62.2	1	10.0	1.7
Fazilka	2	100	59.4	2	14.9	2.7
Mukatsar	2	100	62.5	2	16.6	2.8
Mean	5	100	61.3	5	13.8	2.4
Rajasthan						
Hanumangarh	4	100	65.5	4	15.8	3.1
Sri Ganganagar	1	100	70.6	1	16.6	3.9
Mean	5	100	68.0	5	16.2	3.5
Overall mean of north region	15	100	67.1	15	16.1	3.2

responsible for the region's disease severity. These findings were also as per the ICAR-All India Coordinated Research Project on Cotton (AICRP) report from 2019-2022. Kumar *et al.* (2019) discovered a highly substantial and positive link ($r^2 = 0.945$) between the virulent whitefly population on cotton plants and the per cent disease index of CLCuD, both in the CICR and farmer fields.

Saeed *et al.* (2018) reported that rainfall and higher air temperatures 34 to 35.5°C as maximum, and 25.8 to 26.2°C as minimum favoured vector populations and hence it led to increased disease severity. Highest PDI was recorded at 1-2 mm rainfall. Similarly, the ICAR-AICRP (2022-23) report supported the fact that temperature, relative humidity and rain favoured vector population, thereby increasing disease severity on CICR stations (Sirsa, Faridkot, Sri Ganganagar) as well as farmer fields in North India.

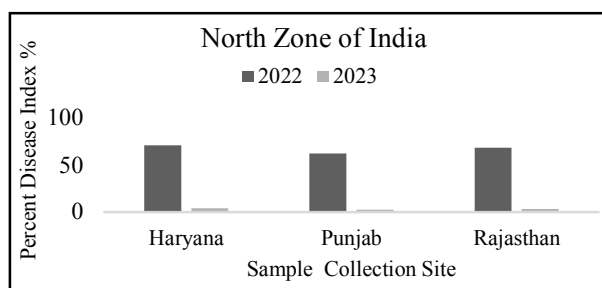


Fig. 4. Cotton growing zone of north India (Punjab, Haryana and Rajasthan) showing the per cent disease index during the years 2022 and 2023.

Fields had more healthy plants in 2023, and the severity of the symptoms on the infected plants was also low compared to the previous year in Haryana, Punjab and Rajasthan (Table 4 and Fig. 4). Whitefly population resulted in a lower incidence of the disease in 2023 as compared to the previous year, indicating a positive impact on disease control through the management of the vector responsible for transmitting the disease.

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