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# Study on Correlation between Population of Viruliferous Whitefly and the Percent Intensity of Cotton Leaf Curl Disease in Cotton

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**A B S T R A C T**

*Cotton Leaf Curl Disease* (CLCuD) is a devastating disease in cotton and cause seed cotton yield loss upto 80% in Northern India. Transmission of CLCuD by *Bemisia tabaci*, the role of alternative weed hosts, infested cotton plants is well understood. However, the relationship of viruliferous whitefly population and CLCuD incidence is still not clear. We aimed to pursue a detailed study on the effect of general whitefly population and viruliferous whitefly population on CLCuD percent disease index (PDI) and their correlation. Three years study showed a decreasing trend in general whitefly population and increasing trend in viruliferous whitefly population in correspondence with the increase in CLCuD intensity from August to October. A highly significant and positive correlation between viruliferous whitefly population on cotton plant and percent diseases index of CLCuD ($r^2$ = 0.945) was observed both at on-station and on-farm multilocation trials. A non-significant positive correlation between whitefly population and CLCuD PDI ($r^2$ = 0.796) and between whitefly population and viruliferous whitefly population ($r^2$ = 0.633) was recorded at on-station trials. Thus, it's one of its first kinds of research study which shows a positive correlation between viruliferous whitefly population and the level of CLCuD intensity in cotton field for the first time. These results advance our understanding on timely detection of viruliferous whitefly level in the cotton field during the off-season as well as during the crop season. This would help in managing the transmission of CLCuV through the judicious and timely application of management strategies for viruliferous whitefly.

## Introduction

Cotton (*Gossypium* spp.) known as "White Gold" is worlds' one of the most important commercial and natural textile fibre crops and a significant contributor of oilseeds. India is a leading producer of cotton in the world and is the only country in the world to cultivate all four cultivable *Gossypium* species *i.e.*, *Gossypium arboreum* and *G. herbaceum*, *G. barbadense* and *G. hirsutum* besides hybrid cottons. Cotton is cultivated in three distinct agro-ecological regions (north, central and south) of the country. Cotton Leaf Curl Disease (CLCuD) is caused by *Cotton leaf curl virus* (CLCuV) which belongs to begomovirus group, family Geminivirideae and has emerged as a serious threat to cotton

cultivation in North India and Pakistan (Mansoor *et al*., 2003; Sattar *et al*., 2013; Varma and Malathi, 2003). In India, CLCuD is presently restricted to 1.2 - 2.0 million ha in northern cotton growing states i.e. Haryana, Punjab and Rajasthan (Varma and Malathi, 2003) and is reported to cause potential yield losses from 25.2 - 81.4% (Monga *et al*., 2013; Monga, 2014; Narula *et al*., 1999). A number of geminiviruses have been reported to infect cotton in the Indian subcontinent, including *Cotton leaf curl Multan virus* – Rajasthan (CLCuMuV-Ra), *Cotton leaf curl Kokhran virus* - Burewala (CLCuKoV-Bu), *Papayaleaf curl virus* (PaLCuV), *Okra enation leaf curl virus* (OEnLCV), *Tomato leaf curl Bangalore virus* (ToLCuBaV), *Tomato leaf curl New Delhi virus* (ToLCNDV). However, CLCuMuV and CLCuKoV or their strains such as with CLCuMuV-Ra, CLCuKoV-Bu are predominantly associated with CLCuD epidemics in the Indian subcontinent (Brown *et al*., 2017; Sattar *et al*., 2017). During 2004-2005, CLCuMuV-Ra was the major in northwestern India, but in 2009-10, resistant breaking CLCuKoV-Bu caused a severe outbreak of CLCuD in Punjab and Rajasthan states of India (Rajagopalan *et al*., 2012). However, during 2015-16, replacement of the 'virulent resistance breaking' CLCuKoV-Bu by the re-emerging CLCuMuV recombinants were recorded to cause an epidemic in North India (Datta *et al*., 2017).

*Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) assumed major importance on cotton in India after severe outbreaks during 1984 - 85 and 1985 - 86 seasons in Andhra Pradesh, Karnataka and Tamil Nadu and later as a vector in transmission of CLCuD in Rajasthan, Haryana and Punjab during 1990s onward. Thus, the whitefly itself as well as a vector of CLCuD is assuming serious pest status of almost all cotton varieties. It was observed that the percent disease incidence (PDI) increased slowly during the month of June and reached up to 80% during July and August in Sudan (Idris, 1990). Similarly, the CLCuV incidence increased rapidly from 4.3 to12.3% during the end of July and first week of August in Pakistan (Ali *et al*., 1995). However, the progress of the disease was reported to be maximum during the month of August as compared to July and September in Northern India (Monga *et al*., 1998). In a recent study three *B. tabaci* biotypes were recorded from India include Asia-II-7 in Pusa -Delhi, Asia-I in south and central India and Asia-II-1 in north India (Naveen *et al*., 2017).

An effective management of this important disease and its vector is possible by the development of resistant varieties and suppression of whitefly along with the eradication of weed hosts carrying this disease. At present there is no source of absolute resistance against CLCuV in *G hirsutum* cotton varieties and there is no chemical control for the CLCuD except the management of its vector whitefly.

Earlier studies conducted on correlation of whitefly, weather factors and CLCuD, reveal that the percent CLCuD incidence and whitefly population shows a negative correlation with maximum and minimum temperature and rainfall while positive correlation with morning and evening relative humidity and sunshine hours (Maharshi *et al*., 2017). Non-significant correlation between CLCuD intensity and whitefly population were reported on different cotton varieties (Varma and Malathi, 2003). The real positive correlation of whitefly populations *vis-à-vis* CLCuD development and its severity has not been established (Akhtar *et al*., 2004). However, disease severity of *rice stripe virus* (RSV) transmitted by small brown planthopper in paddy was reported to have a positive correlation with viruliferous rate of the vector but not with the population density of the insect. This suggests that the proportion

of vectors infected by the virus rather than the total number of vectors plays an important role in RSV epidemics and could be used for disease forecasting (He *et al.*, 2016). The literature available indicates that there is no such study available which could establish the correlation of the proportion of non-viruliferous and viruliferous whiteflies with CLCuD incidence and its percent severity or PDI in cotton. Hence, we intended to study the effect of general whitefly population and viruliferous whitefly population on CLCuD percent disease index (PDI) as well as to study their correlation. It was also felt that understanding the inoculum source becomes an important step in epidemiological studies, disease development and decision making for management of CLCuD transmission vector. The present studies were planned with a view to elucidate and understand the correlation of levels of viruliferous whiteflies population out of the existing whitefly populations in cotton leaf curl disease intensity.

## Materials and Methods

### Selection of cotton fields for whitefly and CLCuD data recording

The two types of field experiments, one at on-station and another at the hot spot locations were decided for conducting the study to understand the relationship between total whitefly population - viruliferous whitefly population and CLCuD PDI. These experiments were conducted twice during 2010 and 2011 at on-station and during 2011 and 2012 at hot spot locations. On-station trial was conducted at ICAR Central Institute for Cotton Research- Regional Station (ICAR-CICR-RS), Sirsa experimental farm (29°32'39.5"N 75°02'24.6"E). CLCuD and whitefly susceptible variety HS 6 was sown with row to row and plant to plant spacing of 67.5 x 30 cm in an area of 393 $m^2$ with 1940 plants (twenty rows of 97 plants each). Standard package and practices were followed to raise the crop. Another, on-farm hot spot location trial was conducted during 2011 and 2012 at three fixed locations situated in whitefly and CLCuD hot spot areas in Haryana, Rajasthan and Punjab. In Haryana, three locations, i.e. Umedpura, Jagmalwali and Fatehpuria were selected as hot spots based on previous research experience (Monga personal communication). In Rajasthan, three locations, namely Kaluwali, Sadhuwali and Agriculture Research Station-Sriganganagar and in Punjab- five locations, i.e. Mansa, Bathinda, Fazilka, Wander Jatna and Nihalkhera locations were selected for recording of whitefly population and CLCuD per cent disease index (PDI).

### Recording of whitefly population and progress in PDI of CLCuD at ICAR-CICR-RS, Sirsa

Observations on total whitefly population-viruliferous whitefly population and CLCuD PDI were recorded at weekly interval during 2010 from the 25$^{th}$ Standard Meteorological Week (SMW) to 41$^{st}$ SMW and during 2011 from 25$^{th}$ SMW to 44$^{th}$ SMW (June to October). Data on whitefly population were taken on 50 randomly selected tagged cotton plants of HS-6 variety in five plots by selecting 10 plants in each. For determination of PDI of CLCuD, a total of 100 cotton plants were selected randomly in five plots of one acre field. 20 cotton plants were selected in each plot for the observation of CLCuD PDI. Observation of CLCuD on cotton plants from each point were recorded by observing CLCuD symptoms using 0-6 scale (Monga, 2014). The observations were taken at 0-6 disease rating scale where 0 = complete absence of symptoms; 1 = symptoms of vein thickening (VT) on few upper leaves; 2 = symptoms of VT, cupping and curling on few upper leaves; 3 = one fourth of a plant affected with VT, cupping and curling, leafy enations;

4 = half plant of a plant affected with VT, cupping and curling, leaf enations; 5 = three fourth of a plant affected with VT, cupping and curling, leafy enation; and 6 = Plants stunted severely and complete plant affected with VT, cupping and curling and leafy enation. The average grade was calculated by using the formula: Average disease rating grade = (Sum of all disease rating grades/ Total number of plants), and percent disease index (PDI) was calculated for each entry/plot by using the following formula: PDI= [Average disease rating grade /Maximum disease rating grade] x 100

## Recording of whitefly population, viruliferous whitefly population and progress in PDI of CLCuD at different farmer field locations

This study was undertaken to observe the relationship between disease progress and presence of the percent viruliferous population of whitefly at different locations and at different time intervals. Observations on whitefly population, viruliferous whitefly and CLCuD progress were recorded only from one cotton field from each location (hot spots) during 2011 and 2012. Three observations on whitefly population per three leaves (top, middle and bottom strata) on tagged plants were taken during the months of July, August and October in 2011 and 2012. Data were recorded from 40 randomly selected plants at each location of one acre by selecting 10 plants from four points in each field. CLCuD disease incidence and severity was recorded from the same plants using 0-6 scale (Monga, 2014). The PDI was calculated using the formula mentioned under section 2.2.

## Detection of viruliferous whitefly

Detection of viruliferous whitefly population from the total whitefly population collected from infected plants was achieved by PCR technique using CLCuV specific coat protein (CP) primer pair CP -F and CP –R and the relationship between viruliferous whiteflies and CLCuD PDI was worked out. To determine percent viruliferous population of whitefly the DNA was isolated from collected whitefly samples. Fifty whitefly samples were collected from each site at the time of each observation from where the whitefly population and CLCuD PDI recording was done. For DNA isolation, a single whitefly was crushed in 25 µl extraction buffer [50 mM Tris-Cl (pH 8.4) -1 ml, 50mM KCl -1 ml, 0.45% Tween-20 -1 ml, 0.45% NP-40 1-ml, Proteinase K (10 mg/ml) -30 µl and 970 µl distilled water] in 1.5 ml eppendorf tube by the help of micro pestle (Tarsons). 25 µl of extraction buffer was added to wash micro pestle and incubated at 65$^{o}$C and 95$^{o}$C for 45 min and 10 min, respectively. After incubation the crude extracts were centrifuged at 12,300 rpm for 3 min. DNA isolated from single whiteflies was stored at -20 $^{o}$C. The purified DNA isolated from single whitefly was subjected to PCR to detect the presence of CLCuV using CLCuV specific coat protein (CP) primer pair CP - F and CP – R. The nucleotide sequence of these primers are: primer F- 5'-CGG GAT CCA TGT CGA AGC GAG CTG CC - 3'and primer -R- 5'-CCG GAA TTC ATA TCA ATT CGT TAC AGA GTC A -3' (Imperial Life Sciences). PCR amplification was achieved using the 50 µl reaction mixture using: Genomic DNA (50ng) - 2 µl; CP primer (Forward) CP-F and CP primer (Reverse) CP-R 1.5 µl each, PCR master mix (1 X) - 45 µl. PCR amplification was performed in a thermocycler (model PTC-100, M. J. Research Inc., USA) under the following parameters: one cycle for initial denaturation at 95 $^{0}$C for 4 min., 29 cycles of denaturation at 94$^{0}$C for 30 sec., annealing at 55$^{0}$C for 30 sec, and extension at 72$^{0}$C for 45 sec. An additional cycle at 72 ºC for 10 min. was run at the end of these cycles (Chakrabarty *et al*., 2005). After PCR, the

PCR products (10 µl) were resolved by submerged horizontal electrophoresis (Tarsons India Ltd) in 1% (w/v) Agarose gel (containing ethidium bromide) in Tris-Acetate-EDTA buffer (pH 8.0) and electrophoresis was carried out at constant voltage of 80 Volt for 1h. The gel was visualized in ultraviolet light (260 nm) and photography was done by using gel documentation system. The 1000 bp DNA ladder was used to determine the size of the CLCuV DNA bands. Out of the total whitefly samples collected from each location at every observation, positive and negative samples were used for calculation of percent viruliferous whitefly population at each location and analysis of correlation. Along with viruliferous whitefly the CLCuD infection of cotton was also confirmed for CLCuV detection from each location.

## Statistical analyses

Simple T test was applied to determine the statistical significance of differences among the mean data of each experiment. Similarly, the correlation among whitefly population on cotton plants, CLCuD PDI and among whitefly population, percent viruliferous whitefly population and CLCuD PDI was determined using Pearson Spearman Rank and Kendall's Tau with the help of computer program OP Stats (Sheoran *et al*., 1998).

## Results and Discussion

### Percent viruliferous whitefly population and CLCuD PDI on cotton variety HS-6 during 2010 and 2011 at ICAR-CICR Sirsa

The presence of 771 bp DNA band of CLCuV was recorded in 1% (w/v) agarose gel in viruliferous positive whitefly samples as well as cotton plant showing CLCuD infection. The data on percent viruliferous whitefly were calculated based on presence and absence of CLCuV in each of the collected whitefly samples out of the total whiteflies samples and was used for analysis of correlation (Fig. 1). When the percentage of viruliferous whiteflies was compared during 2010 and 2011, the population was more during 2010 than 2011. In June 2010, among the total whitefly population at ICAR-CICR Sirsa, the viruliferous whiteflies population was 7.14%, while in 2011, they were 4.14 % and the corresponding CLCuD PDI was 0.17 and 0.08%, respectively. In July, percent viruliferous whiteflies were 17.64% and 14.13% and the corresponding CLCuD PDI was 7.86 and 4.98 during 2010 and 2011, respectively. In August, viruliferous whiteflies were 26.5% and 18.06 % and CLCuD PDI were 59.32 % and 27.41%, recorded during 2010 and 2011, respectively. In September 2010, viruliferous whitefly was observed to be 33.53%, while in 2011 viruliferous whitefly was 22.22%. The CLCuD PDI was 79.24 % and 42.64% during September 2010 and 2011, respectively. During October 2010, 34.48% viruliferous whiteflies were detected; while in 2011 at this time 27.72% viruliferous whitefly were detected. CLCuD PDI was 86.10% and 50.47% during October 2010 and 2011, respectively (Table 1).

The whitefly population during 2010 and 2011 from May to October varied from 0.0 to 4.33 and 0.41 to 5.14, respectively, without showing any particular trend in increase or decrease in population. However, the percent viruliferous whiteflies and CLCuD PDI showed an increasing trend from May to October. The pooled mean of two year data of whitefly population per three leaves per cotton plant and PDI of CLCuD had a positive correlation among each other. There was a significant positive correlation at p=0.01 among viruliferous whitefly population and PDI CLCuD ($r^2$ = 0.945). However, non significant positive correlation was recorded among whitefly population per three leaves

per cotton plant and PDI of CLCuD ($r^2$ = 0.633) and among whitefly population per three leaves per cotton plant and viruliferous whitefly population ($r^2$ = 0.796) (Table 2).

**Whitefly population, percent viruliferous whiteflies and CLCuD PDI in Punjab, Haryana and Rajasthan during 2011 and 2012**

Among the three hot spot areas, comparatively the whitefly population was maximum in Rajasthan and minimum in Punjab during 2011, while it was maximum in Punjab and minimum in Haryana during 2012. Whitefly population was observed to be in increasing trend from July to August, and then it decreased in October at all locations considered for the study and during both the year.

During 2011 in the month of July and August viruliferous whiteflies were recorded, but there was no CLCuD PDI among all the locations except in Punjab in August. During July 2011, maximum percent viruliferous population was recorded from Rajasthan (4.0%), followed by Punjab (3.2%) and Haryana (2.0%). In August the population of viruliferous whitefly increased to the tune of 6.7%, 4.8%, 3.3 % in Rajasthan, Punjab and Haryana, respectively. The viruliferous whitefly population was further reached to 10.7% and 6.4% in Rajasthan and Punjab, respectively, in the month of October, while in Haryana percent viruliferous population remained 3.3% only. During July 2012 higher viruliferous whitefly population in Punjab (16.0%) and Rajasthan (18.7%) and which was further increased in the month of August to the tune of 28.7% and 27.0% in Rajasthan and Punjab, respectively. During October, percent viruliferous increased upto 35.3% in Rajasthan and 15.3% in Haryana. PDI of CLCuD was recorded to be slightly higher in Rajasthan in comparison to Haryana and Punjab during 2011 while the PDI of CLCuD was much higher in Rajasthan followed by Punjab during 2012. During 2011 in the month of July and August, CLCuD was not observed in Haryana and Rajasthan, but in Punjab PDI of CLCuD was only 0.6% in August. During October 2011, PDI of CLCuD was also observed in Rajasthan (4.0%), Punjab (2.2%) and Haryana (1.7%). During 2012 in the month of July, maximum PDI of CLCuD was recorded in Rajasthan (24.3%), followed by Punjab (18.5%) and Haryana (3.3%). During August 2012, the PDI was increased upto 42.3% in Rajasthan, 33.0% in Punjab and 10.7% in Haryana. Which was further increased in the month of October upto 55.7% in Rajasthan and 17.7% in Haryana (Table 3).

Pooled mean of two year data indicates that overall viruliferous whitefly population and PDI of CLCuD were recorded to be higher in Rajasthan and Punjab compared to Haryana. Similarly, an increasing trend of per cent viruliferous population and PDI of CLCuD was observed from July to October, during both the years. Percent viruliferous whitefly per three leaves per cotton plant and PDI of CLCuD showed a significant positive correlation (p=0.05) among each other. However, highly significant correlation (p=0.01) was recorded in between CLCuD PDI (%) and percent viruliferous whiteflies ($r^2$=0.995) (Table 4).

Results of two years data collected during 2010 and 2011 showed an increasing trend in whitefly population from the month of May to September and later decreased in on-station experiments carried out at ICAR-CICR, Sirsa while increasing trend in viruliferous whitefly population as well as CLCuD PDI from May to October was recorded. By observing the two years data, it is clear that during 2010, CLCuD PDI was high in comparison to 2011. Disease progression and viruliferous whitefly population were higher in 2010 from June to

October compared to 2011 at ICAR-CICR. The CLCuD PDI increased with the increase in viruliferous whitefly population. The total whitefly population in general does not correlate with PDI of disease, whereas it is the viruliferous nature of the whitefly which is important and has correlation for CLCuD PDI. Earlier studies have also reported that single *B. tabaci* is able to transmit the leaf curl virus agent (Sharma and Rishi, 2003), but greater transmission efficiency is observed when a higher number *B. tabaci* (more than 10 whiteflies per plant) is present (Cauquil and Follin, 2003). The general whitefly population during 2010 was peaked in July however, during 2011 it peaked only in September. The reasons behind this are higher relative humidity (>82%), minimum temperature (~25 $^{o}$C), rainfall (3-10 mm) and sunshine hours (Table 5). In the current study, whitefly population showed significant negative correlation with maximum temperature, significant positive correlation with relative humidity (morning and evening) and sunshine, while non-significant negative correlation was observed with minimum temperature and positive non-significant correlation with rainfall. Similarly, the PDI of CLCuD showed significant negative correlation with maximum and minimum temperature, significant positive correlation with relative humidity in the morning and sunshine, while non-significant positive-non-significant correlation with evening temperature, evening relative humidity, rainfall and whitefly population (Table 6). Janu and Dhiya (2017) have reported whitefly population in cotton to be significantly and positively correlated with the minimum temperature, morning and evening relative humidity while, significantly and negatively correlated with maximum temperature. This study also indirectly confirms that the more whiteflies probably will have more viruliferous ones, thereby leading to more transmission (Singh *et al*., 1994; Mann and Singh, 2004). However, at on-station trial in May 2011, 2.78% viruliferous whiteflies were recorded but CLCuD PDI was negligible. During the beginning correlation observed between percent viruliferous whitefly and PDI at one moment could not necessarily explain what is happening in field at the same moment. However, this indicates that there may be delay between inoculation of the virus through whitefly and symptoms appearance, and the CLCuD PDI values depend on this delay. Moreover, the CLCuD symptoms appear only on young leaves one month after inoculation which results in very low PDI (Khan and Ahmad 2005). Subsequently, two months after inoculation of the virus the CLCuD PDI will increase. The pooled data of the two years study at on-station trial suggest that increase in viruliferous whitefly population increases CLCuD PDI in subsequent time i.e. about 3-4 week time later which might be due to incubation period required for symptom appearance. Previous serial transmission studies showed that *B. tabaci* adults could retain the virus for 9 d to entire life span. However, the serially transferred viruliferous whiteflies were not consistent in transmitting the virus in new plants *i.e.*, the whiteflies transmitted the virus to new plants on day 1, 2, 5 or 8, but not transmitted on day 3, 4, 6 or 7 (Mann and Singh 2004). The exact reasons why all whiteflies in a population do not become viruliferous are not understood. All these studies indicate the various factors influencing cotton leaf curl virus disease incidence and its intensity. Percent viruliferous whiteflies population correlation with PDI of CLCuD in our studies shows a new and very important observation which can help us in prediction of disease and its management more accurately. The virus transmission is also shown to be a direct fraction of the number of viruliferous whiteflies per plant in several virus transmission studies including CLCuV. It has been demonstrated that when single whitefly

per plant was allowed an acquisition access period of 24 h., 20% of the experimental whiteflies acquired the virus to induce CLCuV symptoms on healthy cotton plants. The percentage of transmission increased to 80, 87, 85, 90 and 88 when the number of whiteflies was increased to 5, 10, 15, 20 and 25 per plant, respectively (Singh *et al*., 1994; Mann and Singh, 2004). In addition to host suitability and plant age, CLCuV acquisition is influenced by the severity of disease symptoms or the virus titer present in plants. *B. tabaci* acquired CLCuV more efficiently from heavily diseased plants than from less severely infected plants (Singh *et al*., 2000; Singh *et al*., 2001). Disease severity of the rice strip virus (RSV) was reported to be positively correlated with viruliferous rate of the vector but not with the population density of the insect, suggesting that the percentage of vectors infected by the virus rather than the total number of vectors play an important role in RSV epidemics and could be used for disease forecasting (He *et al*., 2016). The epidemic and outbreak of rice stripe disease are closely related to the occurrence of viruliferous small brown planthopper populations- *Laodelphax striatellus* Falle´n (Hibino 1996). These findings support the current study where we have also found that more the viruliferous whitefly population and severe is the CLCuD PDI at later crop stage.

A significant positive correlation (P=0.01) among pooled mean of two years data on viruliferous whitefly population and PDI CLCuD ($r^2$ = 0.945) was recorded. Non significant positive correlation among whitefly population per three leaves per cotton plant and PDI of CLCuD ($r^2$ = 0.633) and among whitefly population per three leaves per cotton plant and viruliferous whitefly population ($r^2$ = 0.796), indicates that the increase in whitefly population also has positive relationship with viruliferous whiteflies as well as with PDI. However, these correlations were not significant. A non-significant correlation between CLCuD intensity and whitefly population on different varieties studied were also reported by Varma and Malathi (2003) however, Aktar *et al*., (2004) have not been able to eastablish a significant positive correlation of whitefly populations *vis-à-vis* disease development and its severity. Many other researchers also found non-significant relationship of whitefly population with disease incidence (Briddon and Markham 1994; Hameed *et al*., 1994; Iqbal, 2003). Some workers have found non-significant correlation of weekly maximum air temperature ($^0$C), % relative humidity (5 p.m.), wind velocity, rainfall, sunshine and whitefly population on thirteen mutant/varieties and negative significant correlation between minimum air temperature and wind velocity (8 a.m.) for CLCuV disease development (Khan and Khan, 2000). Maharshi *et al*., (2017) have reported that percent CLCuD incidence and whitefly population have a significant negative correlation with temperature maximum and minimum, while positively correlated with relative humidity morning and evening. Monga *et al.*, (2010) have not observed any correlation between general whitefly population and CLCuD incidence from 1999 to 2009, however, they observed that minimum temperature and sunshine hours have significant negative correlation whereas morning/evening relative humidity and rainfall have positive correlations with incidence and progress of CLCuD and developed regression equation which could be helpful in understanding factors affecting disease development and its prediction. The present study, we found a non-significant though positive correlation among whitefly population and CLCuD PDI, however, a significant positive correlation between population level of viruliferous whitefly and CLCuD. Hence, the results clearly indicate the role of the level of viruliferous whitefly and

CLCuD PDI. The findings of the current study will support in CLCuD management and to minimize the cotton crop loss due to CLCuD and suggests the monitoring and estimation of viruliferous whitefly are essential rather than non-viruliferous whitefly.

To further to confirm the correlation between the whitefly population, viruliferous whitefly population and CLCuD PDI the study was conducted in farmer fields in Punjab (five location), Rajasthan (three location) and Haryana (three locations) during 2011 and 2012.

The study indicated that whitefly population was maximum in Rajasthan and minimum in Punjab during 2011 while it was maximum in Punjab and minimum in Haryana during 2012. A similar trend was observed in term of whitefly population increase from July to August, and then decrease in October at all locations and in both the year.

**Table.1** Effect of percent viruliferous population on PDI of CLCuD during 2010 and 2011 at ICAR-CICR Sirsa

| **Observation Months** | Whitefly[a] | | Pooled mean | Viruliferous whiteflies (%) | | Pooled mean | CLCuD PDI (%) | | Pooled mean |
|---|---|---|---|---|---|---|---|---|---|
| | 2010 | 2011 | | 2010 | 2011 | | 2010 | 2011 | |
| **May** | 0.00 | 0.41 | 0.21 | 0.00 | 2.78 | 1.39 | 0.00 | 0.00 | 0.00 |
| **June** | 1.84 | 0.54 | 1.19 | 7.14 | 4.14 | 5.64 | 0.17 | 0.08 | 0.13 |
| **July** | 4.33 | 1.86 | 3.10 | 17.64 | 14.13 | 15.89 | 7.86 | 4.98 | 6.42 |
| **August** | 3.42 | 3.68 | 3.55 | 26.5 | 18.06 | 22.28 | 59.32 | 27.41 | 43.37 |
| **September** | 2.40 | 5.14 | 3.77 | 33.53 | 22.22 | 27.88 | 79.24 | 42.64 | 60.94 |
| **October** | 2.08 | 2.58 | 2.33 | 34.48 | 27.72 | 31.1 | 86.1 | 50.47 | 68.29 |
| **Mean** | 2.35 | 2.37 | 2.36 | 19.88 | 14.84 | 17.36 | 38.78 | 20.93 | 29.86 |
| **T value= 0.05** | 3.80 | 3.09 | 4.01 | 3.42 | 3.66 | 3.54 | 2.34 | 2.28 | 2.32 |
| **Probability** | 0.0126 | 0.0271 | 0.0102 | 0.0187 | 0.0147 | 0.0166 | 0.0667 | 0.0714 | 0.068 |

**[a] average of whitefly population recorded from 3 leaves (upper, middle and lower strata) per plant**

**Table.2** Correlation of whitefly population, percent viruliferous population on PDI of CLCuD during 2010 and 2011 at ICAR-CICR Sirsa

| **Observations** | Whitefly[a] | Viruliferous whiteflies (%) | CLCuD PDI (%) | S. Error |
|---|---|---|---|---|
| **Whitefly[a]** | 1.000 | | | 0.576 |
| **Viruliferous whiteflies (%)** | 0.796[NS] | 1.000 | | 4.893 |
| **CLCuD PDI (%)** | 0.633[NS] | 0.945[**] | 1.000 | 12.846 |

**[a] average of whitefly population recorded from 3 leaves (upper middle and lower) per plant**
**[**] Statistically significant at p=0.01**

**Table.3** Whitefly population, per cent viruliferous whiteflies and PDI of CLCuD in cotton fields in Punjab, Haryana and Rajasthan during 2011 and 2012

| Observations | Observation Months | Punjab* | | | Haryana** | | | Rajasthan** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 2011 | 2012[a] | Pooled mean | 2011 | 2012 | Pooled mean | 2011 | 2012 | Pooled mean | T values = 0.05 | Probability |
| **Whitefly/3 leaves/plant** | July | 3.2 | 5.2 | 4.2 | 4.4 | 4.3 | 4.35 | 5.9 | 5.1 | 5.5 | 16.98 | 0.0000 |
| | August | 4.3 | 6.4 | 5.35 | 5.3 | 5.8 | 5.55 | 6.7 | 5.7 | 6.2 | 23.93 | 0.0000 |
| | October | 4.0 | - | 2 | 4.7 | 4.9 | 4.8 | 6.1 | 5.5 | 5.8 | 6.28 | 0.0002 |
| **Viruliferous whiteflies (%)** | July | 3.2 | 16 | 9.6 | 2.0 | 4.0 | 3.0 | 4.0 | 18.7 | 11.35 | 3.83 | 0.005 |
| | August | 4.8 | 27 | 15.9 | 3.3 | 10 | 6.65 | 6.7 | 28.7 | 17.7 | 4.22 | 0.0029 |
| | October | 6.4 | - | 3.2 | 3.3 | 15.3 | 9.3 | 10.7 | 35.3 | 23 | 3.14 | 0.0137 |
| **CLCuD PDI (%)** | July | 0.0 | 18.5 | 9.25 | 0.0 | 3.3 | 1.65 | 0.0 | 24.3 | 12.15 | 2.54 | 0.0345 |
| | August | 0.6 | 33 | 16.8 | 0.0 | 10.7 | 5.35 | 0.0 | 42.3 | 21.15 | 2.82 | 0.0226 |
| | October | 2.2 | - | 1.1 | 1.7 | 17.7 | 9.7 | 4.0 | 55.7 | 29.85 | 2.18 | 0.0613 |

***Mean of five locations**
**** Mean of three locations**
**[a] During the October 2012 the data could not be recorded in Punjab.**

**Table.4** Correlation matrices among whitefly/3 leaves/plant, percent viruliferous whiteflies and CLCuD PDI (%) in cotton fields in Punjab, Haryana and Rajasthan during 2011 and 2012

| Observations | Whitefly[a] | Viruliferous whiteflies (%) | CLCuD PDI (%) | Std. Error |
|---|---|---|---|---|
| **Whitefly[a]** | 1.000 | | | 0.571 |
| **Viruliferous whiteflies (%)** | 0.698[*] | 1.000 | | 2.020 |
| **CLCuD PDI (%)** | 0.689[*] | 0.995[**] | 1.000 | 2.729 |

**[a] average of whitefly population recorded from 3 leaves (upper middle and lower) per plant**
**[*] Statistically significant at p=0.005**
**[**] Statistically significant at p=0.01**

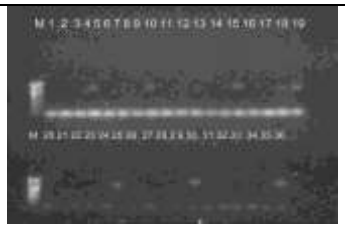
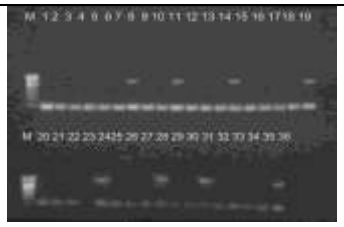
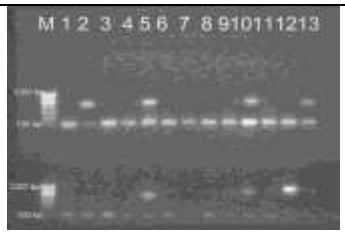
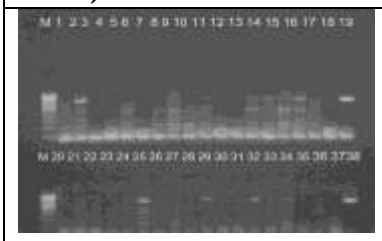
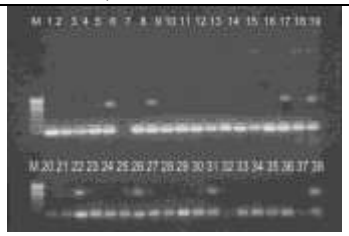
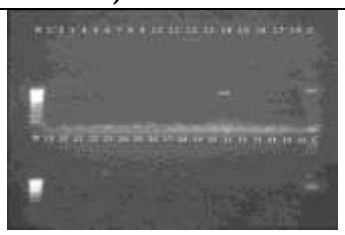
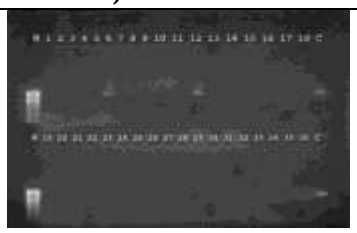
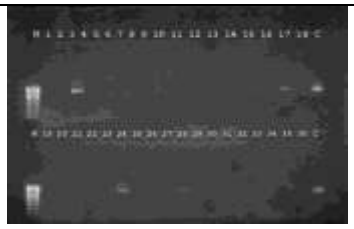
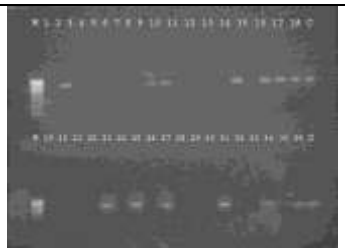
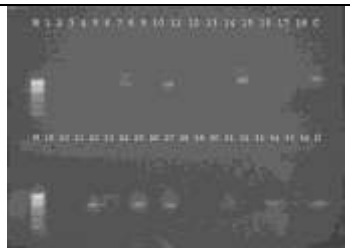
**Table.5** Whitefly population and disease progress in relation to weather factors at CICR Sirsa during 2010 and 2011

| | 2010 | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Months** | CLCuD Incidence % | WF/3 leaves | Max. Temp. | Min. Temp. | Morning RH | Evening RH | Rainfall | Sunshine hours |
| **June** | 0.2 | 1.8 | 40.0 | 26.5 | 56.0 | 29.0 | 0.0 | 2.2 |
| **July** | 3.3 | 4.3 | 36.3 | 27.3 | 81.3 | 52.8 | 10.1 | 3.7 |
| **Aug** | 48.2 | 3.4 | 34.1 | 27.1 | 89.0 | 65.5 | 17.3 | 3.8 |
| **September** | 77.5 | 2.4 | 33.8 | 25.4 | 87.0 | 59.0 | 7.6 | 6.4 |
| **October** | 86.1 | 2.1 | 34.5 | 22.9 | 83.0 | 39.0 | 0.0 | 5.4 |
| | **2011** | | | | | | | |
| **June** | 0.1 | 0.5 | 38.7 | 28.0 | 73.5 | 49.0 | 0.0 | 0.8 |
| **July** | 2.5 | 1.8 | 37.2 | 28.1 | 75.0 | 50.8 | 0.0 | 3.9 |
| **Aug** | 22.9 | 3.7 | 33.9 | 26.4 | 87.1 | 70.3 | 7.2 | 5.2 |
| **September** | 40.7 | 5.1 | 33.1 | 25.2 | 87.1 | 63.6 | 3.7 | 3.8 |
| **October** | 48.8 | 2.6 | 34.1 | 21.1 | 75.6 | 40.5 | 0.0 | 8.8 |

**Table.6** Correlation between different weather parameters with whitefly population build up and disease incidence (pooled over 2010 and 2011)

| **Weather parameters** | **Correlation coefficient** | | |
|---|---|---|---|
| | Whitefly | PDI (%) | Std Error |
| **Temperature** | | | |
| **Maximum** | -0.717* | -0.808* | 0.214 |
| **Minimum** | -0.044 $^{NS}$ | -0.751* | 1.526 |
| **Relative humidity** | | | |
| **Morning** | 0.574* | 0.530* | 1.237 |
| **Evening** | 0.683* | 0.281 $^{NS}$ | 2.310 |
| **Rainfall** | 0.189 $^{NS}$ | 0.086 $^{NS}$ | 2.162 |
| **Sunshine** | 0.484* | 0.498* | 3.214 |
| **Whitefly** | - | 0.481$^{NS}$ | 1.237 |

**Fig.1** PCR amplification of CLCuV-DNA (771bp) in whitefly collected from cotton field at ICAR-CICR Regional Station during 2010-2011 by PCR (Lane M-100 bp molecular weight marker, Lane C- positive check, Lane 1-36 whitefly DNA)

| | | | |
|---|---|---|---|
|  |  |  |  |
| **20 June 2010 (lane 14& 18 Check)** | **23 July 2010 (lane 19 & 36 Check)** | **31 July 2010 (lane 19 & 36Check)** | **5 August 2010 (lane 13 & 25 Check)** |
|  |  |  |  |
| **20 August 2010 (lane 19 & 38 Check)** | **6 September 2010 (lane 19 & 31 Check)** | **16 September 2010 (lane 19 & 38 Check)** | **10 October 2010/10** |
|  |  |  |  |
| **23 May 2011 (lane c-check)** | **06 June 2011(lane c-check)** | **19 June 2011(lane c-check)** | **05 July 2011(lane c-check)** |
|  |  |  |  |
| **19 July 2011 (lane c-check)** | **04 August 2011(lane c-check)** | **19 August 2011(lane c-check)** | **02 September 2011(lane c-check)** |
|  |  |  | |
| **19 October 2011 (lane c-check)** | **04 October 2011(lane c-check)** | **19 October 2011 (lane c-check)** | |

Pooled mean of two year data indicates that the overall viruliferous whitefly population and PDI of CLCuD were recorded to be higher in Rajasthan and Punjab compared to Haryana. Similarly, an increasing trend of per cent viruliferous population and PDI of CLCuD was observed from July to October, during both the years. Similarly, to the present study, the CLCuV incidence is reported to increase from 4.3 to 12.3% during the end of July and first week of August in Pakistan (Ali *et al*., 1995).

While, the progress of the disease was reported to be maximum during the month of August as compared to July and September in Northern India (Monga *et al*., 1998). As, in the present study, a less number of viruliferous whitefly (<7%) with negligible amount of PDI were recorded during July and August 2011 in all the three locations (Table 3). Moreover, in the on-station trail at ICAR-Sirsa, the presence of viruliferous whitefly was detected during the month of May 2011, however, the CLCuD symptoms were recorded during June. The absence of PDI during the corresponding months in the hot spot area may also be due to the lower whitefly population as well as delay between inoculation of the virus and the expression of the symptom level. These results are similar to the on-station trial and also previous transmission studies which showed that CLCuV could be acquired by whitefly within 4 h, transmit the virus within 1 hr of feeding and symptoms are recorded after 4 weeks (Khan and Ahmad, 2005). In this study conducted at multi-location trials, highly significant correlation (p=0.1) was recorded in between PDI of CLCuD PDI (%) and percent viruliferous whiteflies ($r^2$=0.995). Moreover, a significant positive correlation (p=0.5) was found among whitefly population per three leaves per plant, percent viruliferous whitefly per three leaves per cotton plant and PDI of CLCuD. However, from the this study it can be concluded that percent viruliferous whiteflies increases continuously from June to early October and decreases at the end of October. Corresponding to the percent viruliferous whiteflies population, the CLCuD PDI also increases in the same manner and becomes constant at the end of October.

The current study conducted both at on-station and hot spot areas of three different agroclimatic conditions, strengthen our understanding about the epidemiology of CLCuV for improved forecasting to manage the disease monitoring and detection of viruliferous whiteflies rather than total whitefly population. CLCuD PDI primarily depends on the presence of viruliferous whiteflies in the environment. Additionally, the study holds the importance in controlling the transmission of CLCuV through timely detection of viruliferous whitefly level in the field during the off-season as well as during the crop season from cotton and other alternate plant-hosts. Thus, timely detection of viruliferous whitefly level can serve as a useful tool which will help in timely application of the appropriate management strategies for management of viruliferous whitefly and ultimately reducing CLCuD PDI.

## Conflict of interest

This is to submit that that the work described in the paper has not been published before; that it is not under consideration for publication anywhere else; that its publication has been approved by all co-authors, as well as by the responsible authorities – tacitly or explicitly – at the institute where the work has been carried out. The publisher will not be held legally responsible should there be any claims for compensation. All authors are wishing to include figures, tables, or text passages that have not been published elsewhere.

## Key Message

This is one of its first kinds of research study which shows a positive correlation between viruliferous whitefly population and the level of CLCuD intensity in cotton field for the first time. The field and lab studies advances our understanding in making the decision for management of viruliferous whitefly and ultimately reducing CLCuD PDI. Regular monitoring and timely detection of viruliferous whitefly level in fields can serve as a useful tool for reducing transmission of CLCuD.

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