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Temporal and spatial dynamics of Tomato leaf curl disease at Chitrakoot region in India

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Abstract
Tomato is the most important vegetable crop next to potato cultivated across the world. A severe leaf curl disease with puckering, mottling, chlorosis and distorted growth was observed in tomato (*Solanum lycopersicum*) with significantly high disease incidence (15.2 to 35.9% in summer crop and 12.7 to 31.3% in *Rabi* crop) at Chitrakoot region during survey 2015-16 and samples were collected. Presence of huge population of whitefly indicated the possibility of begomovirus infection. The causal agent was amplified by PCR (~536 bp and ~1.1 kb) from 102 symptomatic leaves and 63 whiteflies DNA using two set of begomovirus specific degenerate primers. Randomly sequenced four amplicon showed highest similarity (96-99%) with *Tomato leaf curl New Delhi virus* (KF537780: India: Varanasi). In temporal dynamics study, transplanting of 5th November had minimum disease incidence ranged from 3.3 to 12.2 per cent and low whitefly population than other two early planted crops. A positive linear correlation (y = 2.841x R^2 = 0.374) was recorded between whitefly and leaf curl incidence. The study covered survey, molecular diagnosis of Tomato leaf curl disease along with temporal dynamics and development of eco-friendly ToLCD management strategy in Chitrakoot area for the first time.

Keywords: Polymerase Chain Reaction (PCR), Tomato leaf curl disease, *Tomato leaf curl New Delhi virus*, *Solanum lycopersicum*, whitefly

Introduction
Tomato (*Solanum lycopersicum*) belongs to the family *solanaceae* is commercially cultivated throughout the years by the local farmers in rural area of Chitrakoot, situated on the bank of river Mandakini. Agriculture is the main occupation of this area. Lycopene, a flavonoid antioxidant, present in tomato. It has antioxidant and anticancerous properties. Tomato leaf curl disease is most common problem to the farmers of this area for tomato production and facing 40 to 60 per cent economic loss. Which was first reported from North India (Vasudeva and Sam Raj 1948) and subsequently from central India and Southern India (Sastry and Singh 1973). Tomato leaf curl disease (ToLCD) caused by Tomato leaf curl viruses (Persistent plant virus) transmitted by whitefly (*Bamisia tabaci*) belongs to genus begomovirus, family *Geminiviridae*. Several leaf curl virus has been reported for infecting tomato as *Tomato leaf curl Sudan virus* (Jarullah *et al*., 2017), *Tomato leaf curl Palampur virus* (Heydarnejad *et al*., 2012), *Tomato leaf curl New Delhi virus* (Padidiam *et al*., 1995; Snehi *et al*., 2016), *Tomato yellow leaf curl virus* (Basak, 2016), *Tomato leaf curl Bangalore virus* (Muniyappa *et al*., 2000), *Tomato leaf curl Karnataka virus* (Chatchawankaphanich and Maxwell, 2002), *Tomato leaf curl Gujarat virus* (Chakraborty *et al*., 2003), *Tomato leaf curl Bangladesh virus* (Green *et al*., 2001), *Tomato leaf curl Joydebpur virus* (Maruthi *et al*., 2006), *Tomato leaf curl Palampur virus* (Kumar *et al*., 2008), *Tomato leaf curl Kerala virus* (Pandey *et al*., 2010), *Tomato leaf curl Patna virus* (Kumari *et al*., 2010).
As per current review of literature, Tomato leaf curl virus (ToLCV) infection most commonly found in every part of Indian subcontinent responsible for considerable yield loss in several solanaceous crops with huge temporal variability. Tomato leaf curl disease dynamics depends on various biotic and abiotic factors. Among them, conducive environment as temperature, relative humidity and date of planting play most significant role for growth, development and multiplication of vector insects. As per many reports, maximum whitefly infestation was observed at high temperature and low relative humidity, while low temperature and high relative humidity inhibits the activities of whitefly (Ali *et al*., 2005, Aktar *et al*., 2008; Kaushik, 2012). The most favorable temperatures range was 25 °C to 30 °C for the development of egg and nymph stages of whitefly. (Darwish *et al*., 2000). So, here to understand the temporal dynamics and molecular identity of the pathogen, tomato leaf curl disease was studied with different time interval on *Punjab Chhuhara* tomato cultivars and developed environmental-friendly disease management package at Chitrakoot in India.

Materials and Methods

Survey, collection of symptomatic leaf samples and whiteflies

To cover maximum area, Extensive survey was conducted at nine locations of Chitrakoot (Pathra, Paldev, Bhaganpur, Shivrampur, Balapur, Rajoula, Rani Bhatt, Semaria and Sitapur) during 2015-16 to observe any viral symptoms on the tomato plants. Tomato leaves exhibited naturally severe leaf curl disease with puckering, mottling, chlorosis and distorted growth like symptoms were collected at different time intervals (For March-April and October-November transplanted crop) along with one healthy sample and ten whiteflies of each location in every attempt using an aspirator and brought into lab for molecular analysis. Presence of whitefly population in much quantity suspected the infection of begomovirus pathogen.

Total g-DNA isolation and PCR assay

Total genomic DNA extracted using 100 mg symptomatic leaves sample with mortar and pestle in liquid nitrogen using CTAB method. For the confirmation of suspected pathogen, PCR was performed using two set of degenerate primer specific to begomovirus (Deng-541F/540R and PARIv722/PALIc1960) following the respective annealing temperature (Table 1). PCR reaction were carried out in a total of 25 µl volume containing, 12.5 µl Dream Taq Green Master Mix (2X) Fermentas, 1 µl each primer (20 pmole/µl), 2 µl template DNA (20ng/ µl) and 8.5 µl dH_2O in Master Cycler Nexus, Eppendorf, (Germany). Amplicon were visualized in 1% agarose gel electrophoresis in TAE buffer containing 0.1% ethidium bromide. The gel examined under gel documentation system (UV Tech Cambridge). Similarly molecular diagnosis was done for collected whiteflies from field and randomly four amplified product was subjected for sequencing.

Table 1: Primer pairs used for diagnosis of Tomato leaf curl disease (ToLCD) in Polymerase chain reaction assay

Primer I D	Primer sequence (5'-3')	Target virus	Annealing temperature (ºC)	Expected size of amplicon	Reference/ Remark
Deng 541-F	TAATATTACCKGWKGVCCSC	Begomoviruses Specific	49	~530 bp	Deng *et al*.,1994
Deng540-R	TGGACYTTRCAWGGBCCTTCACA				
PARIv722	GGNAARATHTGGATGGA	Begomoviruses (DNA-A genome specific)	52	~1.1 kb	Reddy *et al.*,2005
PALIc1960	ACNGGNAARACNATGTGGGC				

Observations of Tomato leaf curl disease in *Summer* and *Rabi*

In 2015-16, Field survey was conducted in Chitrakoot area consisting nine locations. Tomato leaf curl disease (ToLCD) data was recorded and documented in different time interval (for March-April and October-November transplanted crop) at Chitrakoot in local farmer's tomatoes field. For which, randomly three fields A, B and C were selected in (3 X 3) mt^2 size and number of total plants, infected plants were counted from each field. Young leaves showed clear cut symptoms of severe leaf curling with puckering; chlorosis; distorted growth and slight crinkling were sampled (Table 2). The average disease incidence (DI) per field was calculated in percentage. Disease incidence data were calculated using following formula

$$\text{Disease Incidence (\%)} = \frac{\text{No. of infected plants}}{\text{Total No. of plants}} \times 100$$

Temporal dynamics of ToLCD and monitoring of whitefly population

To know the temporal dynamics of tomato leaf curl disease and whitefly population, 60 plants (highly susceptible tomato variety *Punjab Chhuhara*) were planted in three different date at 15 days gap (05th Oct, 20th Oct and 05th Nov, 2016) with three replications at Nana Ji Deshmukh Research Farm of Mahatma Gandhi Chitrakoot Gramoday Vishwavidyalaya (MGCGV), Chitrakoot. Each experiment was followed the all cultural practices except spray of insecticides and monitored regularly for disease appearance and vector population after transplanting (DAT). Moreover, Disease incidence and whitefly population data was documented at 15 days interval up to crop maturity and analyzed. Prevalence of whiteflies was also recorded in tomato plants by considering 5 leaves per plant (2 middle, 2 lower and 1 top leaves).

Relationship between whitefly and disease incidence

In temporal dynamic study, we recorded level of disease pressure and whitefly population in different date of transplanted crop at Research Farm of MGCGV, Chitrakoot. To know the significant relationship, data of DI and whitefly population were recorded at 15 days interval and a linear regression study was performed to understand the correlation between DI and whitefly population using statistical software SPSS.

Results & Discussion

Survey, samples collection and PCR assay

In survey 2015-16, total 135 symptomatic tomato leaves and 90 whiteflies were collected from nine different locations of Chitrakoot area. All samples were processed for PCR mediated amplification in order to confirm the presence of virus. Positive amplification was found in 102 symptomatic leaves and 63 whiteflies DNA for begomovirus genome (~530 bp for Deng-541F/540R (Fig. 1) and ~1.1 kb for PARIv722/PALIc1960) as mentioned (Table 1). All healthy leaves sample of each location was found negative for begomovirus amplification. Obtained sequence data of randomly selected four amplicon showed maximum (96-99%) similarity with *Tomato leaf curl New Delhi virus* (KF537780: India: Varanasi). This result clearly indicated that the suspected pathogen was begomovirus infection.

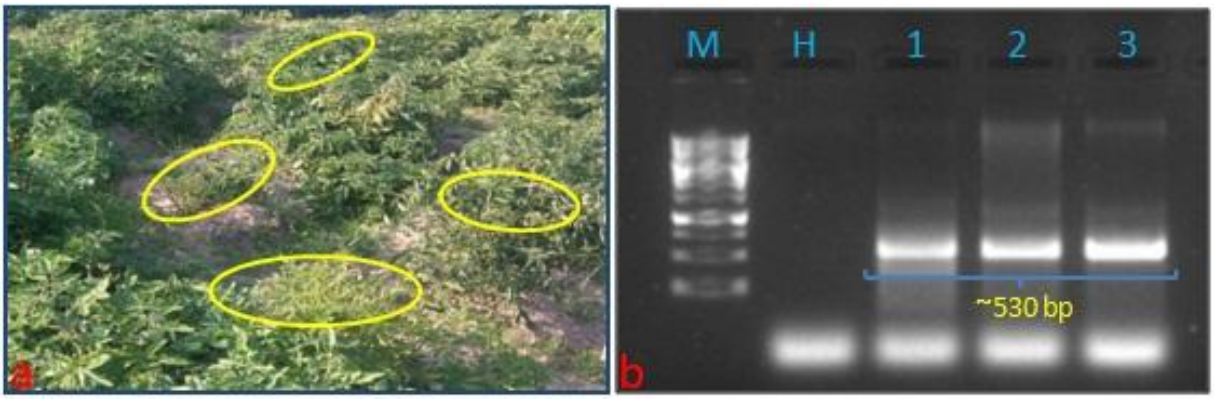

Fig 1: Incidence of leaf curl disease with distorted growth in tomato at farmer's field of Rajoula location in Chitrakoot area (a) and gel image of randomly three symptomatic samples (lanes 1-3) with Deng541-F/540-R primer along with healthy H, Lane M: 1 kb DNA ladder (b).

Data collection of tomato leaf curl disease in two season

During extensive survey 2015-16, the collected data of tomato leaf curl revealed disease incidence mean ranged 15.2 to 35.0 per cent for March-April transplanted crop (*Summer*) and 12.7 to 31.3 per cent for October-November crop (*Rabi*). In summer season, the location Rajoula having maximum 35.9 per cent DI followed by Bhaganpur (32.4%), Sitapur (31.7%), Pathra (29.4%), Semaria (27.7%), Paldev (21.3%), Rani Bhatt (19.5%), Shivrampur (16.3%) and Balapur (15.2%).

In *Rabi*, similar trends of disease incidence were recorded at each location of Chitrakoot area. However, it was found comparatively low than summer crop. For which, Rajoula showed maximum incidence (31.3%) followed by Bhaganpur (27.6%), Sitapur (26.2%), Semaria (24.3%), Pathra (21.4%), Paldev (19.3%), Rani Bhatt (14.9%), Shivrampur (13.4%) and Balapur (12.7%) (Fig. 2 and Table 2).

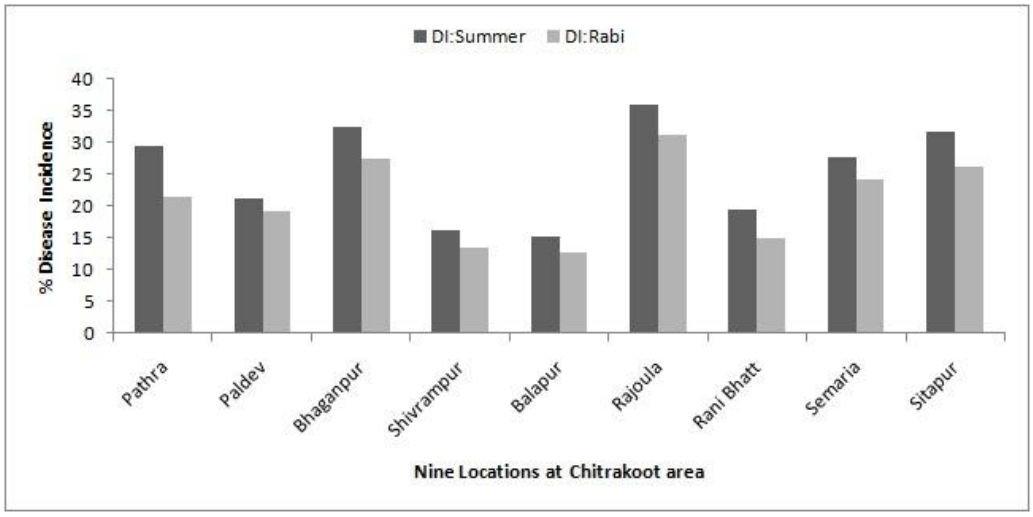

Fig 2: Prevalence of tomato leaf curl disease incidence (mean) during *summer* and *rabi* at different locations of Chitrakoot.

Table 2: Details of sample collected at Chitrakoot region; PCR analysis of ToLCD through two set of whitefly transmitted *Geminivirus* specific degenerate primers along with disease incidence and symptoms.

Location	No. of selected field (3X3) mt^2	No. of collected sample/ No. of positive sample with both primers (Deng541-F/540-R and PARIv722 / PALIc1960)	DI (%) (*Summer*)	DI (%) (*Rabi*)	Symptoms
Pathra	A	5/4	25.4	17.1	Cu, Ch
	B	5/3	33.2	25.7	Cu, Dg
	C	5/5	29.6	21.4	Pk, Mo
Paldev	A	5/3	25.3	16.3	Cu, Ch
	B	5/3	17.2	19.3	Pk, Mo
	C	5/3	21.4	22.3	Cu, Ch
Bhaganpur	A	5/5	26.4	32.4	Cu, Ch
	B	5/4	34.5	25.8	Cu, Mt
	C	5/5	36.3	24.6	Cu, Pk
Shivrampur	A	5/4	11.3	11.7	Cu, Ch
	B	5/3	21.1	13.1	Cu, Dg
	C	5/3	16.5	15.4	Pk,Ch
Balapur	A	5/3	15.2	15.9	Cu,Ch
	B	5/4	17.0	9.5	Pk, Mt
	C	5/1	13.4	12.7	Cu, Ch
Rajoula	A	5/5	26.8	25.1	Cu, Dg
	B	5/5	45.0	33.3	Cu, Mt

	C	5/5	35.9	35.5	Cu,Mo
Rani Bhatt	A	5/3	16.5	12.7	Cu, Pk
	B	5/4	19.8	17.9	Cu, Mo
	C	5/2	22.2	14.1	Cu, Ch
Semaria	A	5/4	23.9	20.3	Cu, Mo
	B	5/5	31.4	29.1	Cu, Ch
	C	5/3	27.8	23.5	Cu, Mo
Sitapur	A	5/5	31.7	21.2	Cu, Ch
	B	5/4	29.5	27.0	Pk, Mo
	C	5/4	33.9	30.4	Cu, Ch

Cu=curling, Pk=puckering, Ch=chlorosis, Mo=mosaic, Dg= distorted growth, Mt=mottling

Temporal dynamics of tomato leaf curl disease and whitefly population

During temporal dynamics study in different date transplanting we noticed, at initial stages (upto 25 DAT) of tomato crop, no disease incidence was recorded. Disease appeared after 30 DAT. Maximum incidence of leaf curl was observed in 5th October transplanted crop. The disease was initially noticed at 5th November with 4.7 per cent DI and it was progressively increased up to first week of April with 35.0 per cent DI. The crop transplanted after 15 days interval (20th October), a moderate disease progress was found with maximum 23.5 per cent leaf curl incidence at crop maturity stage. A slow progress of disease was recorded in delayed planting (5th November) with maximum 12.2 per cent incidence upto maturity of tomato crop (Fig. 3a).

The staggered planted tomato crop was monitor for whitefly population at periodic interval (15 DAT). The collected data of whitefly prevalence revealed that maximum whitefly population was recorded in third week of November. Which was 7.5, 6.1 and 4.5 (whitefly/plant) for 5th October, 20th October and 05th November transplanting respectively (Fig. 3b). After that, Population was rapidly deceased up to 20th December and remains in minimum number (range 3.1- 4.9 whitefly/plant) till 20th February due to low temperature in atmosphere. Onward of 20th Feb, the population was again progressively increased. After crop maturity, whitefly population was noticed in decreasing order in every experiment. Overall, Lower whitefly population and lower disease incidence was recorded in 5th November transplanting tomato seedling. It clearly indicated that as the temperature decreases, whitefly population also decreases but incidence gradually increases and a unique pattern of ToLCD and whitefly dynamics was observed in all planting under the study.

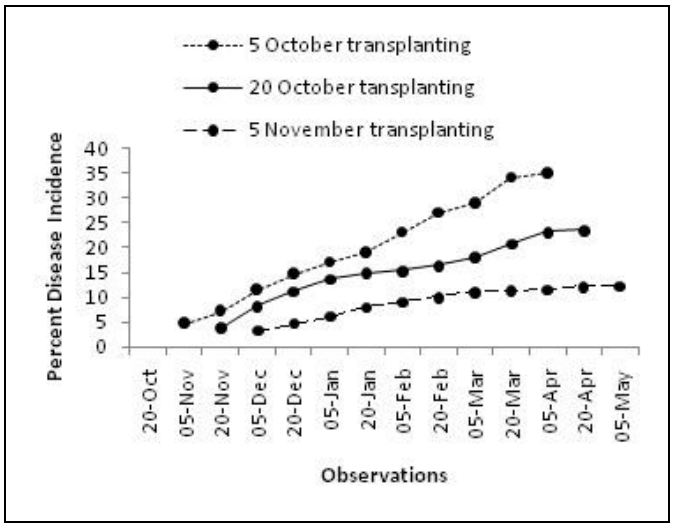

Fig 3a: Effect of different dates of planting on the disease incidence (DI) in cv. *Punjab Chhuhara* (tomato)

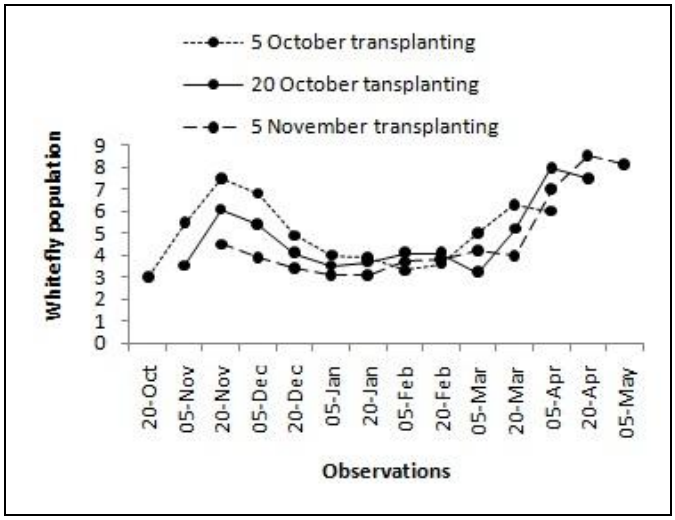

Fig 3b: Effect of different dates of planting on the whiteflies population in cv. *Punjab Chhuhara* (tomato)

Whitefly and disease incidence correlation

In response of whitefly infestation with the crop, leaf curl incidence was also increased. A positive linear correlation (y = 2.841x R^2 = 0.374) among whitefly and leaf curl incidence was recorded under periodic observation study (Fig. 4).

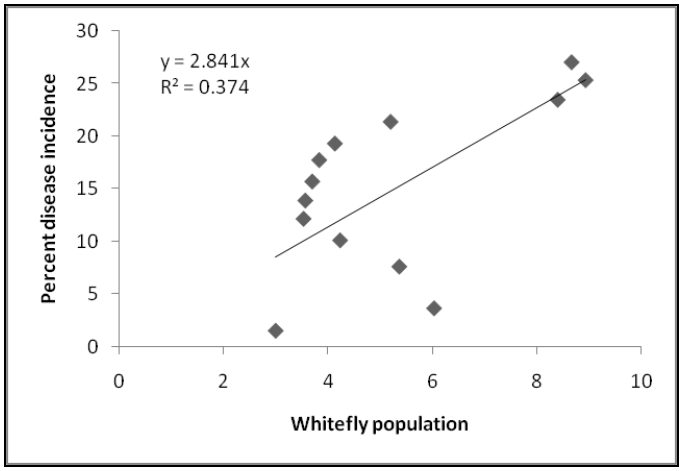

Fig 4: Positive linear correlation between whitefly and disease incidence of tomato

Tomato leaf curl disease is very serious constraint for tomato production across the world caused by whitefly transmitted geminivirus (begomovirus), resulted giant yield losses (Saha *et al*., 2014;
Inoue-Nagata *et al*., 2016; Das *et al*., 2017). Disease symptom of begomovirus infection in tomato commonly showed upward or downward leaf curling with puckering, chlorosis, distorted growth and stunting of the plant. The study covered the epidemiology of tomato leaf curl disease (ToLCD) and its identification at molecular level to know the pathogen at

Chitrakoot region. It was recorded that disease prevalence mean ranged from 15.2 to 35.9 per cent and 12.7 to 31.3 per cent for *Summer* (March-April) and *Rabi* (October-November) transplanted crop respectively across the nine location of Chitrakoot (Fig. 2). These results clearly suggested that tomato seedlings transplanted during March-April was open higher disease pressure due to accommodating environment for whitefly development and seedling transplanted during October-November infested with lower disease pressure due to adverse environment. So, In *Summer* crop, whiteflies attack on tomato crop with high infestation and severely attacked the sowing, transplanting, flowering and fruiting stage results low yield comparison to *Rabi* crop. So farmers are suggested to transplanting in *Rabi* season to obtain much tomato production for commercial purpose in Chitrakoot area.

ToLCD was also diagnosed through PCR assay using two set of whitefly transmitted *geminivirus* (begomovirus) specific degenerate primers (Table 1) and found positive results with DNA of 75.55 per cent (102/135) leaves and 70 per cent (63/90) whiteflies, clearly indicated infection of begomovirus pathogen in tomatoes of Chitrakoot. Excepting this, the sequence data of randomly selected four amplicon revealed 96-99% similarity with *Tomato leaf curl New Delhi virus* (KF537780) isolated from Varanasi, India. These sequencing results makes strong hypothesis for involvement of Tomato leaf curl virus, which causing ToLCD in Chitrakoot.

In temporal dynamic study, It was found that much disease pressure present with early transplanting crop (05th October) while lower pressure observed with delayed transplanted crop (05th November). It means favorable environment (as temperature and relative humidity) was available in October month to whitefly development that causes high infestation with tomato crop as comparison to November.

It was also noticed that as whitefly population increased, disease incidence also increased. So, tomato leaf curl disease (ToLCD) multiplication was significantly correlated with the whitefly population with a positive linear correlation ($y = 2.841x$ $R^2 = 0.374$) at Chitrakoot (Fig. 4).

As per current review of literature, the temperature ranges between 25-30 °C is encouraging for the development of whitefly (Darwish *et al*., 2000). The temperature, exceed than minimum temperature and low relative humidity, enhanced the activity of whitefly to spreading the disease (Ali *et al*., 2005; Aktar *et al*., 2008).

Generally, the temperature range of Chitrakoot area having 15-35°C for the month of March to April and October of every year as previously recorded. So, greater chances for the development of whitefly population and increase vector activity to cause higher infestation with crop at this time.

The present investigation clearly indicated that disease incidence increased with the increase of minimum temperature. Simultaneously, as the temperature increases, whitefly population also increased but it decreased with the increase of relative humidity in winter. So, It could be clearly discussed that disease incidence was directly proportional to the temperature and inversely proportional to the relative humidity.

Conclusion

In present investigation, the distribution of tomato leaf curl disease by whitefly vector in different time interval at Chitrakoot region was studied for the first time. The study intimates that conducive environment in month of March, April and early October was the main factor to increase whitefly population and tomato leaf curl disease. So keeping this in mind, mid-October to mid-November was the most effective time for transplanting of tomato seedling at Chitrakoot. The study also suggested to validating some resistant tomato variety in this area. The information generated under the study may be useful in designing epidemiological model and eco-friendly leaf curl disease management strategy especially under temporal variation scenario to boost up the socio-economic status of tomato growers of this area.

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References

1. Aktar MM, Akhter MS, Akanda AM. Impact of insecticides and organic oil spray on the growth and yield of tomato under TYLCV infected condition. Bangladesh Res. Publ. J., 2008; 1:199-205.
2. Ali S, Khan MA, Habib A, Rasheed S, Iftikhar Y. Correlation of environmental conditions with okra yellow vein mosaic virus and Bemisia tabaci population density. Int. J. Agric. Biol., 2005; 7:142-144.
3. Basak J. Tomato Yellow Leaf Curl Virus: A Serious Threat to Tomato Plants World Wide. J Plant Pathol Microbiol., 2016; 7:346.
4. Chakraborty S, Pandey PK, Banerjee MK, Kallo G, Fauquet CM. Tomato leaf curl Gujarat virus, a new begomovirus species causing a severe leaf curl disease of tomato in Varanasi, India. Phytopathology. 2003; 93:1485-1495.
5. Chatchawankaphanich O, Maxwell DP. Tomato leaf curl Karnataka virus from Bangalore India appears to be a recombinant begomovirus. Phytopathology 2002; 92:637–645.
6. Darwish YA, Mannaa SH, Rehman MAA. Effect of constant temperature on the development of egg and nymphal stages of the cotton whitefly, B. tabaci (Genn.) (Homoptera; Aleyrodidae) and use of thermal requirements in determining its annual generation number. Assiut. J. agric. Sci., 2000; 31:207-216.
7. Das R, Chowdhury R, Singh A, Sarkar S. Diversity of Tomato Leaf Curl Virus (ToLCV), Bemisia tabaci and its Transmission. Int. J. Curr. Microbiol. App. Sci., 2017; 6(5):78-87.
8. Deng D, Mlgrath PF, Robinson DJ, Harrison BD. Detection and differentiation of whitefly-transmitted geminiviruses in plants and vector insects by the polymerase chain reaction with degenerate primers. Ann. Applied Biol., 1994; 125:327-336.
9. Green SK, Tsai WS, Shih SL, Black LL, Rezaian A, Rashid MH, Hong LTA. Molecular characterization of begomoviruses associated with leaf curl diseases of tomato in Bangladesh, Laos, Malaysia, Myanmar, and Vietnam. Plant Disease. 2001; 85(12):1286-1286.
10. Heydarnejad J, Hesari M, Massumi H, Varsani A. Incidence and natural hosts of Tomato leaf curl Palampur virus in Iran. Australasian Plant Pathol., 2012; 42:195-203.
11. Inoue-Nagata, Alice K, Lima, Mirtes F, Gilbertson, Robert L. A review of geminivirus diseases in vegetables

and other crops in Brazil: current status and approaches for management. Horticultura Brasileira. 2016; 34(1):8-18.
12. Jarullah B, Sohrab SS, Jarullah JS. First Report of Tomato leaf curl Sudan virus Infecting Tomato Plants in Gujarat State, India. Disease Notes. 2017; 101(9):1685.
13. Kaushik C. Incidence and abundance of whitefly, Bemisia tabaci, Genn. and the occurrence of tomato yellow leaf curl virus disease (TYLCV) in relation to the climatic conditions of Alipurduar, Jalpaiguri, West Bengal, India. J. entomol. Res., 2012; 36:35-40.
14. Kumar Y, Hallan V, Zaidi AA. Molecular characterization of a distinct bipartite begomovirus species infecting tomato in India. Virus Genes. 2008; 37:425-431.
15. Kumari P, Chattopadhyay B, Singh AK, Chakraborty S. A new begomovirus species causing tomato leaf curl disease in Patna, India. Plant Dis., 2010; 93:545.
16. Maruthi MN, Rekha AR, Alm SN, Kader KA, Cork A, Colvin J. A novel begomovirus with distinct genomic and phenotypic features infects tomato in Bangladesh. Plant Pathol., 2006; 55:290.
17. Muniyappa V, Venkatesh HM, Ramappa HK, Kulkarni RS, Zeidan M, Tarba CY, Ghanim M, Czosnek H. Tomato leaf curl virus from Bangalore (ToLCV-Ban4): sequence comparison with Indian ToLCV isolates, detection in plants and insects, and vector relationships. Arch. Virol., 2000; 145:1583-1598.
18. Padidam M, Beachy RN, Fauquet CM. Tomato leaf curl geminivirus from India has bipartite genome and coat protein is not essential for infectivity. J. Gen. Virol., 1995: 76:25-35.
19. Pandey P, Mukhopadhya S, Naqvi AR, Mukherjee SK, Shekhawat GS, Choudhury NR. Molecular characterization of two distinct monopartite begomoviruses infecting tomato in India. Virol., 2010; J.7:337.
20. Reddy RV, Colvin J, Muniyappa V, Seal S. Diversity and distribution of begomoviruses infecting tomato in India. Arch Virol., 2005; 150(5):845-67.
21. Saha B, Saha D, Biswas KK, Saha A. Distribution and molecular characterization of begomoviruses infecting tomato in sub-himalayan tarai region of West Bengal and Brahmaputra valley of Assam in northeast India. Indian Phytopath., 2014; 67(1):92-96.
22. Sastry KSM, Singh SJ. Assessment of loss in tomato by tomato leaf curlvirus. Ind. J. Mycol. Plant Pathol., 1973; 27:274-297.
23. Snehi SK, Parihar SS, Gupta G, Singh V, Purvia AS. Molecular Detection and Identification of Begomovirus Isolate on Tomato from Central Region of India. J Plant Pathol Microbiol., 2016; 7: 389.
24. Vasudeva RS, Sam Raj. Leaf curl disease of tomato. Phytopathology. 1948; 18:364-369.

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