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Leaf curl disease of *Capsicum annuum* and its impact on secondary metabolites

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Abstract
Leaf curl disease of *Capsicum annuum* has been recognized serious problem, wherever crop is grown. Experiment was designed to observe the leaf curl incidence and biochemical changes in infected and healthy plants of nine cultivars. The lowest leaf curl incidence was recorded 2.27% and highest 85.83%. The chlorophyll content were found highest in healthy leaves (0.684 mg g^{-1}) then infected leaves(0.511 mg g^{-1}).Whereas, total phenol content was significantly lowest in leaf curl infected leaves (5.582 mg g^{-1}) as compared to healthy leaves (7.544 mg g^{-1}). The average total tannin content was observed significantly higher in healthy leaves (4.028 mg g^{-1}) as compared with infected leaves (3.128 mg g^{-1}). Out of nine cultivars total phenol (9.905 mg g^{-1}) and tannin (5.27 mg g^{-1}) contents were found maximum in Gucchedar.

Keywords: *Capsicum annuum*, Leaf curl virus**,** incidence, chlorophyll, phenol, tannin

Introduction
Chilli (*Capsicum annuum* L.) is an important spices crop belongs to the family solanaceae and is one of the important widely cultivated crops grown for the value of its green and ripe fruits in India. India is rich in maximum diversity of chilli varieties with differing habit, size, shape, colour and pungency of fruit. Besides, traditional use of chilli as vegetables, spices, condiments, souces and pickles, it is also used in pharmaceuticals, casmatics and beverages [24]. India produces approximately 12.60 thousands metric tonns from an area of 792.1 thousands hectare [1]. Uttar Pradesh occupies an area of 13.47 thousand hectares with production of 10.30 metric tonns [1]. The yield of chilli is much lower than potential yield of improved varieties, it is due to several other factors, lack of improved varieties against incidence of different pest and diseases are the main constraints for getting low productivity [8]. Viral diseases of plants cause economic losses to the tune of 15 billion dollars per annum on global basis [25], particularly in tropical and semitropical regions. These provide ideal conditions for the perpetuation and transmissions of viruses through vectors. Natural occurrence of several viruses have been reported on chilli (*Capsicum annuum* L.) by various workers [14], among them leaf curl virus has been reported most destructive for chilli, tomato, potato, okra, cotton and cucumber cultivation in terms of incidence and yield losses [11]. Leaf curl virus affecting chilli in India was first reported from Jodhpur during December, 2004 and emerged as a serious problem in major chilli growing area of Rajasthan with very high disease incidence upto 100 per cent plants infection in farmers' fields [18]. Leaf curl virus is a member of genus *Begomovirus,* family geminiviridae. The genome of *Begomoviruses* have a single or double stranded circular DNA of c. 2.7kb, which is encapsulated in a quasi-isometric geminate particles of C.20-30nm. During the past two decades, agricultural intensification has resulted *Begomoviruses* inciting disease out breaks in tropical and subtropical regions, causing 80 per cent yield losses of many crops [20]. The leaf curl disease was first reported from India on tomato by Vasudeva and Samraj [26]. The virus is transmitted in nature by the white fly (*Bemisia tabaci*) in a semipersistant (Circulative) manner. The minimum acquisition and inoculation feeding periods are 15-30 minutes, the latent period in the vector is more than 20 hours and virus is retained by the vector upto 20 days. Single white fly can carry a finite number of virions in the range of 600 million [27]. The investigation of plant responses to elicitors is one of the most rapidly developing lines of injury in the plant physiology. The elicitors stimulate the contact between plants and phytopathogens and thereby trigger defensive mechanisms that constrain the invasion of pathogenic viruses. The phenol and tannins are more studied biochemical, which regulate the expression of resistance genes and induced its synthesis in plant [23]. These techniques for the detection of chlorophyll, phenol and tannin have provided an opportunity to develop methods for the diagnosis of plant resistance.

Analysis of bio-chemical provides very useful information on the defense mechanism of plant against pathogen infection.

Materials and Methods

The experiment was carried out during the month of February to May in 2014 and 2015 at student's instructional farms of N.D. University of Agriculture and technology, Kumarganj, Faizabad, India. Thirty days old seedlings were transplanted in first week of February at the spacing of 60x45 cm in augmented design under field condition for evaluation of their resistance against leaf curl virus. Total 36 chilli germplasm/ cultivars were collected from Vegetable Farm, N. D. University of Agriculture and Technology, Kumarganj, Faizabad and Indian institute of vegetable research Varanasi, U.P. Seeds of all the cultivars were sown in single lines 2.0x2.5 cm deep furrow for nursery. Two rows of chilli leaf curl susceptible (check) cultivar (IC 119797) were transplanted all around the field to create epiphytotic conditions. Recommended package of cultural practices were followed to raise the crop and to promote natural infection. Periodical fungicidal sprays were given to avoid fungal diseases. Ten plants in each plots were randomly selected and tagged for visual observations on symptoms appearance at 30, 45, 60, 75 and 90 days after transplanting. The leaf curl disease intensity noted on each cultivars and was rated following a intensity scale 0 - 5. Percent Disease Intensity (PDI) was calculated by formula given by Banerjee and Kallo [4]:

$$\text{Per cent disease intensity (PDI)} = \frac{\text{Sum of total numerical ratings}}{\text{Total no. of leaves examined x maximum disease grade}} \text{x100}$$

Biochemical analysis of diseased and healthy leaves

The healthy and infected leaves of chilli cultivars *viz.,* Bydagi Kaddi, Musa Badi, Phuley Joyti, CA-960, Gucchedar, POL-75, NPKT-2, PDC-54 and Pusa Sadabhar were collected from field at the time of flowering and fruiting stage for the estimation of chlorophyll. The leaves were washed with distilled water and excess water of the leaves was soaked by filter paper. Then 100mg leaves were grind into 80 per cent acetone with the help of mortar and pestle The grind solutions were taken into test tubes and final volume made to 10 ml by adding 80 per cent acetone. The solutions were centrifuged at 5000rpm for 10 minutes. The supernatant was taken in clean test tubes separately and repeated the step for two times until the pellet is clear. The absorbance was recorded at 663 and 645 nm in a spectrophotometer (SL 159 UV VIS spectrophotometer) against 80 per cent acetone blank. The amount of chlorophyll a, b, and total chlorophyll (mg/g fresh weight) were calculated according to Arnon's formula [2]. Total phenols were estimated using Folin-Ciolcalteu reagent, according to the modified method of Bray and Thorpe [5]. The crude powders of the leaves were prepared for photometric determination of tannins. The standard procedure was followed by Folin-Denis method described as Tambe and Bhambar [22].

Results and Discussion

Results on per cent disease incidence of leaf curl virus on chilli consisting 36 cultivars under natural field condition are given in table (1). The lowest leaf curl incidence was recorded in Surajmukhi (2.27%, 3.81%), Japani long (2.72%, 3.77%), Pusa jawala (2.71%, 3.79%), DOH-2 (7.64%, 9.23%), PBC-473 (9.96%, 10.38%) and Arka lohit (10.55%, 10.02%) in compression to susceptible check IC-119797 (81.35%, 85.83%) during 2014 and 2015 respectively. Kumar *et al.*, (2006) reported the symptoms less reaction against leaf curl disease for certain genotypes under artificial screening. Whereas, highest per cent disease incidence was recorded in Musa badi (80.03, 82.41) followed by CA-960 (79.09, 81.35), Musala badi (78.56, 79.76), IC-119485 (77.03, 76.55), NBKTA 1-2 (79.25, 77.55), and Gucchedar (77.27, 76.66). The finding revealed that no significant difference in disease intensity between the years was observed. The capsicum cultivars screening are reported similar based on occurrence of leaf curl disease under field conditions [3, 21].

Chlorophyll content

Results presented in Table (2) showed that average chlorophyll a was significantly higher in healthy leaves (0.684 mg g^{-1}) in compression to infected leaves (0.511 mg g^{-1}). In case of variety, it was found maximum in NPKT-2 and Musa Badi (0.73 mg g^{-1}) followed by Phuley Joyti (0.70 mg g^{-1}), Pusa Sadabhar (0.69 mg g^{-1}), CA-960 (0.69 mg g-$^{-1}$), Pol-75 (0.68 mg g^{-1}) and Gucchedar (0.66 mg g^{-1}) in healthy leaves. NPKT-2 and Musa Badi have similar amount of chlorophyll a. Whereas infected leaves, the highest chlorophyll a contents was found in Phuley Joyti and Gucchedar (0.74 mg g^{-1}) and lowest in Pusa Sadabahar (0.60 mg g^{-1}) and NPKT-2 (0.60 mg g^{-1}). The mean chlorophyll b content was significantly highest in healthy leaves (0.984 mg g^{-1}) and lowest in infected leaves (0.832 mg g^{-1}). However, the average chlorophyll b content was found highest in Pusa Sadabhar (1.135 mg g^{-1}) followed by CA-960 (1.058 mg g^{-1}), Gucchedar (1.048 mg g^{-1}) and Phuley Joyti (0.979 mg g^{-1}) in healthy leaves. In case of infected leaves, the maximum chlorophyll b content was found CA-960 (1.08 mg g^{-1}) as compared with Pusa Sadabhar (1.06 mg g^{-1}), Gucchedar (1.02 mg g^{-1}) and Pol-75 (0.80 mg g^{-1}). The total chlorophyll content was also found highest in healthy leaves (1.678 mg g^{-1}) as compared with infected leaves (1.508 mg g^{-1}). The average total chlorophyll content was recorded maximum in Pusa Sadabhar (1.78 mg g^{-1}) followed by Gucchedar (1.77 mg g^{-1}), CA-960 (1.72 mg g^{-1}) and Phuley Joyti (1.70 mg g^{-1}) in healthy leaves. Whereas infected leaves, it was highest in Gucchedar (1.77 mg g^{-1}) and lowest in NPKT-2 (1.27 mg g^{-1}). Chlorophyll a, and b contents were found either similar or at par between most of the cultivars. During host pathogen infection considerable decrease in the chlorophyll content was observed in infected leaves which could be the consequence of disorganization of the chloroplast membrane system and breakdown of the chloroplast envelope during infection as demonstrated by earlier researchers [7, 10, 12]. The disease development in Mesta also altered by the ratio between chlorophyll a and b, that is probably affecting the photosynthetic efficiency of plants [6, 9].

Total Phenol

Data presented in Table (3) revealed that average total phenol content was significantly lowest in leaf curl infected leaves (5.582 mg g^{-1}) as compared to healthy leaves (7.544 mg g^{-1}). Whereas in variety, the average total phenolic content was found maximum in Gucchedar (9.905 mg g^{-1}) followed by POL-75 (9.480 mg g^{-1}), Byddagi Kaddi (8.32 mg g^{-1}) and Pusa Sadabhar (6.935 mg g^{-1}). While in healthy leaves, it was found highest in Gucchedar (10.35 mg g^{-1}) followed by POL-75 (10.06 mg g^{-1}), Bydagi Kaddi (9.72 mg g^{-1}), Pusa Sadabhar (7.96 mg g^{-1}), NPKT-2 (7.91 mg g^{-1}), PDC-54 (6.88 mg g^{-1}), CA-960 (6.17 mg g^{-1}), Phuley Jyoti (4.mg g^{-1}) and Musa Badi (3.80 mg g^{-1}). But in infected leaves, total phenolic content was significantly lowest in Musa Badi (1.74 mg g^{-1}) in

comparison to Phuley Joyti (2.84 mg g^{-1}), NPKT-2 (4.34 mg g^{-1}) and CA-960 (4.67 mg g^{-1}).The higher accumulation of phenolic compounds in resistant varieties and lower amount in susceptible varieties of virus infected plants were observed because of it may have a fungistatic effect through their involvement in metabolic reactions associated with disease resistance [17, 6].

Total Tannin

Results are described after the critical examination of data presented in Table (4). The average total tannin content was observed significantly higher in healthy leaves (4.028 mg g^{-1}) as compared with infected leaves (3.128 mg g^{-1}). Whereas in variety, the average total tannin content was highest in Gucchedar (5.27 mg g^{-1}) followed by CA-960 (4.18 mg g^{-1}), Phuley Joyti (4.11 mg g^{-1}), Pusa Sadabhar (4.08 mg g^{-1}) and Bydagi Kaddi (3.72 mg g^{-1}). In Infected leaves it was highest in Gucchedar (4.74 mg g^{-1}) and lowest in Musa Badi (1.18 mg g^{-1}). While in healthy leaves it showed maximum 5.80 mg g^{-1} tannin content in Gucchedar and minimum 1.57 mg g^{-1} in Musa Badi. Tannin content was observed an enhanced quantity in healthy leaf as compared to leaf curl infected leaf, it is consequence of extremely complex interaction that exists between plant pathogenic virus and their host plants [13, 15]. Enhanced amount of tannic acid has observed in healthy roots than root knot infected roots by Singh *et al.* [19].

Table 1: Incidences of chilli leaf curl complex viruses on chilli cultivars during summer season (March-May).

S. No	Genotypes	2014			2015			Types of symptoms
		Total No. of plants	No. of infected plants	Percent disease intensity	Total No. of plants	No. of infected plants	Percent disease intensity	
1	Arka lohit	10	4	10.55	10	3	10.02	LC,LR
2	Faizabad kala	10	6	17.10	10	6	15.42	LC, LR
3	Super (Local)	10	6	20.36	10	6	22.85	LC, LR
4	BC 14-2	10	7	29.75	10	8	32.70	LC, LR
5	Kalyanpur Type-1	10	8	55.65	10	7	53.76	LC,LR, MM
6	JBT 17/99	10	8	57.26	10	9	66.23	LC,LR, M,N, DM, MM
7	Phuley joyti	10	10	77.56	10	10	79.37	LC,LR, M,N,DM, MM
8	Ajeet-1	10	2	10.30	10	3	11.37	LC, LR
9	Faizabad long	10	4	26.99	10	5	27.14	LC, LR
10	PDC-54	10	6	37.12	10	5	34.62	LC,LR, MM
11	PDC-64	10	8	52.32	10	7	53.18	LC,LR, MM
12	IC119362	10	8	56.85	10	9	58.10	LC,LR, MM
13	CA-960	10	10	79.09	10	10	81.35	LC,LR,M,N,DM, MM
14	IC119485	10	10	77.03	10	10	76.55	LC,LR,M,N, DM,MM
15	Musala badi	10	10	78.56	10	10	79.76	LC,LR,M,N, DM,MM
16	POL-75	10	10	71.51	10	10	70.63	LC,LR,M,N, DM, MM
17	Anand	10	2	10.54	10	2	11.01	LC, LR
18	Suhawal-17	10	4	21.25	10	3	18.53	LC,LR
19	NPKT-2	10	10	62.34	10	10	60.74	LC,LR,M,N, DM MM
20	NBKTA 1-2	10	10	79.25	10	7	77.55	LC,LR,M,N, DM, MM
21	IC 119444	10	6	51.57	10	5	54.06	LC,LR,M,N,MM
22	Agni	10	47	10.63	10	3	12.11	LC,LR
23	Surajmukhi	10	2	2.27	10	3	3.81	LC, LR
24	Jpaani long	10	2	2.72	10	2	3.77	LC, LR
25	Pant chilli-1	10	4	28.73	10	4	26.74	LC, LR
26	Bydagi Kaddi	10	10	73.56	10	10	69.47	LC,LR,M,N, DM, MM
27	Pusa jawala	10	2	2.71	10	2	3.79	LC,LR
28	LCA-357	10	8	53.44	10	7	51.07	LC,LR,M,N,MM
29	HC-44	10	4	22.65	10	4	20.89	LC,LR
30	DOH-2	10	4	7.64	10	5	9.23	LC, LR
31	PBC-473	10	4	9.96	10	4	10.38	LC, LR
32	IC 119349	10	6	33.56	10	6	29.95	LC, LR
33	Musa badi	10	10	80.03	10	10	82.41	LC,LR,M,N, DM, MM
34	Pusa sadabhar	10	8	49.08	10	7	\48.27	LC,LR, M,N
35	Gucchedar	10	10	77.27	10	10	76.66	LC,LR,M,N, DM, MM
36	IC119797 (CHECK)	10	10	81.35	10	10	85.83	LC,LR,M,N, DM, MM

LC=Leaf curling, LR= Leaf rolling, Mosaic, N=Necrosis of leaves, DM=Distortion mosaic and MM= Mosaic mottling

Table 2: Estimation of chlorophyll contents (mg/g leaf) in healthy and leaf curl virus infected leaves of chilli genotypes.

S. No.	Varieties	Chlorophyll a (mg/ g fresh weight)			Chlorophyll b (mg/ g fresh weight)			Total chlorophyll (mg/ g fresh weight)		
		Healthy leaf	Infected leaf	Average	Healthy leaf	Infected leaf	Average	Healthy leaf	Infected leaf	Average
1	Bydagikaddi	0.64	0.65	0.648	0.84	0.74	0.798	1.48	1.47	1.488
2	Musa badi	0.73	0.66	0.702	0.96	0.67	0.820	1.70	1.34	1.522
3	Phuleyjoyti	0.70	0.74	0.721	1.16	0.79	0.979	1.86	1.53	1.700
4	CA-960	0.69	0.63	0.666	1.03	1.08	1.058	1.73	1.71	1.723

5	Gucchedar	0.66	0.74	0.706	1.06	1.02	1.048	1.78	1.77	1.779
6	POL-75	0.68	0.70	0.686	0.92	0.80	0.860	1.58	1.50	1.548
7	NPKT-2	0.73	0.60	0.669	0.89	0.67	0.786	1.63	1.27	1.455
8	PDC-54	0.64	0.65	0.654	0.80	0.66	0.734	1.45	1.32	1.388
9	Pusasadabhar	0.69	0.60	0.653	1.20	1.06	1.135	1.90	1.67	1.787
	Average	0.684	0.511	0.678	0.984	0.832	0.913	1.678	1.508	1.598

Interaction							
		Chlorophyll a		Chlorophyll b		Total Chlorophyll	
		SEm±	CD at (P=0.05)	SEm±	CD at (P=0.05)	SEm±	CD at (P=0.05)
1	V	0.006	0.016	0.016	0.046	0.028	0.081
2	H	0.012	0.034	0.008	0.022	0.013	0.038
3	VxH	0.017	0.048	0.023	0.065	0.040	0.115

Table 3: Estimation of total phenol (mg/g leaf) in healthy and leaf curl virus infected genotypes of chilli

S. No.	Varieties	Total phenols		Average
		Healthy leaf	Infected leaf	
1	Bydagikaddi	9.72	6.94	8.328
2	Musa badi	3.80	1.74	2.772
3	Phuleyjoyti	4.51	2.84	3.675
4	CA-960	6.17	4.67	5.420
5	Gucchedar	10.35	9.46	9.905
6	POL-75	10.06	8.90	9.480
7	NPKT-2	7.91	5.44	6.675
8	PDC-54	6.88	4.34	5.610
9	Pusasadabhar	7.96	5.91	6.935
	Average	7.544	5.582	6.533
Interaction				
		SEm±	CD at (P=0.05)	
1	V	0.070	0.203	
2	H	0.033	0.095	
3	VxH	0.100	0.286	

Table 4: Estimation of total tannin (mg/g leaf) in healthy and chilli leaf curl virus infected genotypes of chilli.

S. No.	Variety	Total tannin		Average
		Healthy leaf	Infected leaf	
1	Bydagi kaddi	4.30	3.14	3.72
2	Musa badi	1.57	1.18	1.37
3	Phuley Joyti	4.56	3.67	4.11
4	CA-960	4.50	3.86	4.18
5	Gucchedar	5.80	4.74	5.27
6	POL-75	3.75	2.74	3.24
7	NPKT-2	3.47	2.74	3.10
8	PDC-54	3.72	2.52	3.12
9	Pusa Sadabhar	4.59	3.57	4.08
	Average	4.028	3.128	3.576
Interaction				
		SEm±	CD at (P=0.05)	
1	V	0.086	0.246	
2	H	0.040	0.116	
3	VxH	0.121	0.349	

Discussion

This biochemical might be phenol, tannins, flavonoids, saponnins etc. which suppresses the incidence of both whitefly and viruses. The biochemical study of infected and healthy plants showed the availability lower amount of total phenol and tannin content in virus infected plants. The accumulation of phenol and tannin were highest in resistant variety and lowest in susceptible variety. Chatterjee and Ghosh (2008) [6] observed lower amounts of phenolics in diseased plants after 110 days of virus inoculation in Mesta plants. Rathi *et al.*, (1986) [17] also found high accumulation of phenolic compounds in resistant varieties and low amount in susceptible varieties of virus infections. Finding are supported with Mishra and Mohanty, (2007) [15], Mishra *et al.*, (2010) [16] and Mahjbeen (2011). Tannin, starch, Polyphenol oxidase and peroxidase were observed an enhanced quantity in leaf curl infected chilli leaf as compared to healthy leaf, it is due to localization of metabolites and enzymes activity in infected leaves. Singh *et al.*, (2013) [20] observed enhanced amount of tannic acid in healthy roots than root knot infected roots. It is concluded that resistant cultivars have higher amount of chlorophyll, tannin and phenol contents as compared to susceptible cultivars.

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