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Occurrence and confirmation of *Cucumber mosaic virus* infecting chilli (*Capsicum annum. L*) of Telangana

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Abstract

Chilli (*Capsicum annum. L*) is one of the most important commercial crops grown worldwide for its vegetable and spice purpose. *Cucumber mosaic virus* (CMV) is found to be a serious and destructive virus in terms of incidence and yield loss in chilli. Survey was carried out in six chilli growing districts of Telangana state to assess the incidence of CMV in chilli for 2 consecutive years from 2017-18 and 2018-19. Major symptoms observed were mosaic, mottling, shoe string, leaf deformation and stunting of plants. The survey revealed the presence of CMV in almost all the fields that were surveyed and the disease incidence was 8.44 to 16.69%. The maximum disease incidence of 16.69% was recorded in Warangal District. DAC-ELISA was performed to confirm the presence of CMV in chilli and the infected plants exhibiting shoe string symptoms showed the highest absorbance value of more than 0.7 followed by leaf deformation (0.5).

Keywords: *Cucumber mosaic virus*, chilli, survey, Telangana, DAC-ELISA

Introduction

Chilli (*Capsicum annum. L*) Belongs to the family *Solanaceae* is one of the most important commercial crops grown worldwide for its vegetable and spice purpose. Capsicum is one of the oldest domesticated and utilized crops for its high nutritional value, health benefits and medicinal properties (Saleh *et al.*, 2018) [8]. India is the world's largest producer, consumer and exporter of chilli. The two important commercial qualities *viz.*, colour and pungency levels make Indian chilli to get well recognized in the global market. In India, the green and dry chillies were cultivated in an area of 363 and 701 thousand hectares with 4027 and 1751 thousand MT production, respectively during 2019-20 (Agricultural Statistics at a Glance, 2020) [1]. The important chilli growing states in India are Andhra Pradesh, Telangana, Tamil Nadu, Karnataka and Madhya Pradesh. In Telangana, chilli was cultivated in 2.20 lakh acres with production of 3.80 lakh tonnes during 2020-21 (Chilli Outlook, 2021) [2]. The major varieties grown were Jeeva 81, Teja, Indu and Warangal Chappatta (tomato chilli).

Chilli is adversely affected by insects and diseases, which will cause comprehensive loss in production. Among the pathogens, it is known to be infected by more than 35 viruses and 11 have been reported from India (Vijeth *et al.*, 2020) [10]. *Cucumber mosaic virus* (CMV) is found to be a serious and destructive virus in terms of incidence and yield loss. It has a broader host range infecting more than 1241 host species in 101 plant families, which include monocots and dicots (Edwardson and Christie, 1991) [3]. It is transmitted by aphids in a non-persistent manner and in some cases, through seeds (Palukaitis and Garcia-Arenal, 2003) [6].

The present study was aimed to assess the occurrence and distribution of CMV in major chilli growing areas of Telangana state using Direct Antigen Coating-Enzyme Linked Immunosorbent Assay (DAC-ELISA).

Materials and Methods

Survey

A planned field survey was conducted in major chilli growing areas of Khammam, Warangal, Nalgonda, Suryapet, Ranga Reddy and Mahabubnagar districts of Telangana State during cropping seasons of 2017-18 and 2018-19 to assess the disease incidence of CMV. Thirty fields were surveyed in 2017-18 and twenty six in 2018-19. In each district, 3 Mandals and in each Mandal, 3 locations were selected randomly except Rajendra Nagar Mandal wherein 4 locations were surveyed.

Depending upon the size of the field (ranged from 1-3 acres), random survey was conducted in 5 to 10 sites in each field and in each site, total number of plants and the number of CMV infected plants based on symptoms were recorded.

During survey, chilli fields were observed for the Occurrence of CMV suspected symptoms such as mosaic, dark green patches, leaf deformation, shoe-string and mottling. The percent disease incidence was recorded by counting the total number of plants and number of CMV infected plants based on apparent symptoms. In each field, the leaves showing characteristic CMV symptoms were collected in Ziplock polyethylene bags, labelled and stored at 4 °C for further use. The percent disease incidence was calculated by using the following formula:

$$\text{Per cent Disease Incidence (PDI)} = \frac{\text{No. of plants suspected to be infected}}{\text{Total no. of plants}} \times 100$$

Direct antigen coating – enzyme linked immunosorbent assay (DAC-ELISA)

The CMV suspected samples were collected from 55 fields of 6 districts and 120 samples were subjected to DAC-ELISA as described by Hobbs *et al.*, (1987) [4] using 96-well Tarson microtitre plates. Out of 120 samples, 30 samples each were taken from the districts of Khammam and Warangal, while 15 samples each were taken from the remaining 4 districts. Each sample was technically replicated thrice and tested. The polyclonal antibody against CMV was obtained from DSMZ, Germany. Five hundred milligram sample was homogenized in one millilitre of coating buffer using pestle and mortar and 100 µl was loaded in each well of ELISA plate. The plate was covered with aluminium foil and incubated at 4 °C overnight in a refrigerator and washed three times with PBS-T buffer. Then, blocking solution of PBST with 5% skimmed milk was added to the plate and incubated at 37°C for 2 hours. After washing three times with PBS-T, CMV polyclonal antibody diluted in antibody buffer @ 1:200 dilution was added to the wells and kept at 37°C for 2 h for incubation. Then, the plate was washed three times with PBS-T and antirabbit IgG (produced in goat) conjugated with alkaline phosphatase enzyme (1:7000 dilution) was added @ 100 µl per well and kept for 2 h at 37°C. Finally, the wells were washed three times with PBS-T and 100 µl of 0.5 mg substrate *p*-nitrophenyl phosphate dissolved in one ml of diethanolamine substrate buffer (pH 9.8) was added in each well and the plate

was kept under dark for 30 minutes for colour development. The reaction was stopped by addition of 50 µl of 3 M NaOH to each well and the absorbance was read at 405 nm in Biotek - ELISA micro plate reader. Positive, negative and healthy control were added to each plate to serve as control.

Results and Discussion

Survey

Survey was conducted in six major chilli growing districts of Telangana state to assess the incidence of CMV infecting chilli from 2017-18 to 2018-19. Chilli cultivars such as Teja, Wonder Hot, US 341, Fatki, Byadgi, Warangal Chappatta, Jwala, Jeeva 81 and Indu were commonly grown by farmers in these areas. Chilli plants showing CMV suspected symptoms were selected randomly in the field and the symptomatic leaf samples were collected. During survey, variable symptoms such as mosaic, dark green patches, mottling, shoe string, leaf deformation and stunting were observed in 3-4 month old plants. The symptoms such as mosaic, shoe string, severe leaf deformation were commonly observed on the top leaves of the plant and in some field, stunted growth of the plant was observed. The symptoms observed were varied from one location to another and one variety to another. In Warangal, only in Chappatta variety, mosaic symptom was observed, where as in other varieties shoe string, leaf deformation and mottling were also expressed along with mosaic (Fig.1).

The survey revealed the presence of CMV incidence in almost all 55 fields of 6 districts that were surveyed. All the varieties grown under field conditions were naturally infected with CMV. As per visual observations, the average disease incidence was 8.44% and it varied among the mandals which ranged from 3.54 to 18.13%. Maximum disease incidence of 18.13% was recorded in Nallabelly mandal of Warangal district and minimum of 3.54% recorded in Gundlapalle mandal of Nalgonda district. The districtwise average incidence of CMV in Warangal, Khammam, Mahabubnagar, Ranga Reddy, Suryapet and Nalgonda was 16.69%, 8.4%, 8.17%, 7.24%, 6.18% and 3.92%, respectively (Table 1; Fig. 2). Maximum disease incidence was recorded in Warangal district. Increased incidence of the disease in some areas might be due to factors such as age of the crop, vector population, season and susceptibility of the crop (Jagadeeshwar, 2004) [5].

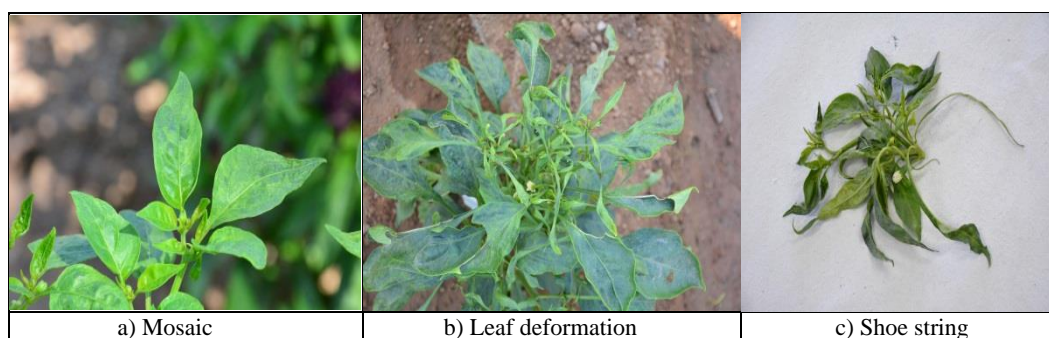


Fig 1: Chilli plants infected with CMV showing different types of symptoms

Table 1: Documentation of CMV incidence in chilli crop of Telangana state

District/Mandal	Location	Variety*	Latitude (° N)	Longitude (°E)	No. of CMV suspected samples based on symptoms/ Total plants	Disease Incidence (%) based on symptoms	Mean disease incidence/ Mandal
Khammam-Nelakondapalle	Appale Narsimhapuram	Teja, Wonder Hot,	17.100014	80.050586	24/358	6.7	7.7
	Konaigudem	US 341 Byadgi,	17.320012	80.150786	32/350	9.1	
	Ammugudem	Jwala, LCA -334	17.210013	80.120576	27/366	7.3	
Khammam-Kamepalli	Kamepalli	Teja, Byadgi,	16.125491	79.906676	34/364	9.3	8.4
	Thallagudem	Jwala, Wonder Hot,	16.025492	79.816673	22/286	7.6	
	Garidepalli	Sannam	16.125492	79.906671	40/479	8.3	
Khammam-Mudigonda	Pedamandava	Teja, Byadgi,	17.183243	80.091272	20/254	7.8	9.1
	Venkatapuram	Jwala,	17.143265	80.091298	27/260	10.3	
	Mallannapalem	No. 273, Jeeva 81	17.183234	80.091221	32/340	9.4	
Warangal - Nallabelly	Medapalle	Teja,	18.040312	79.861989	48/290	16.55	18.13
	Asaravelli	US 341 Warangal	18.052043	79.684321	29/150	19.33	
	Rangapuram	Chappatta,	18.045231	79.861236	38/205	18.53	
Warangal - Narsampet	Nagurlapalle	Teja, US 341,	17.928134	79.894576	45/320	14.06	15.23
	Kammepalle	Byadgi, Warangal	17.9438154	79.452178	35/194	18.04	
	Dasaripalle	Chappatta,	17.348621	79.764382	23/169	13.60	
Warangal - Duggondi	Maripally	Teja, Warangal	18.021034	79.801754	31/215	14.41	16.73
	Duggondi	Chappatta, Wonder	18.432194	79.549320	28/182	15.38	
	Keshwapur	Hot, Jwala	18.035127	79.826543	18/108	17.14	
Nalgonda - Dameracherla	AdaviDevulapalli	Teja, Jwala, Jeeva	17.595011	81.063043	22/458	4.80	4.25
	Nadigadda	81	17.459509	81.566304	25/620	4.03	
	Veerlapalem		17.231190	81.323856	16/408	3.92	
Nalgonda - Peddavoora	Pinnavura	Teja, Jwala, Jeeva	16.728054	79.209234	18/410	4.39	3.99
	Pulicherla	81	16.672802	79.320925	15/532	2.81	
	Sangaram		16.723801	79.219224	19/396	4.79	
Nalgonda - Gundlapalle	Kandukur	Teja,	16.715212	79.857723	12/298	4.02	3.54
	Bollanapalle	US 341, Indu	16.871256	79.237452	19/590	3.22	
	Cherkupalle		16.329457	79.286512	22/648	3.39	
Suryapet -Suryapet	Suryapet	Teja, Jwala, Jeeva	17.1353	79.6334	41/588	6.97	6.69
	Pinnaipalem	81, Indu	17.22045	79.52305	26/370	7.02	
	Solipet		17.2800	79.4985	35/575	6.08	
Suryapet - Chivvemla	Gunjaluru	Teja, Jeeva 81,	17.080864	79.752579	31/582	5.32	6.13
	Gumpula	Indu	17.10521	79.72770	37/574	6.45	
	Beebiguda		17.16832	79.63540	25/378	6.61	
Suryapet - Nuthankal	Yadavally	Teja, Jwala, Jeeva	17.34167	79.6204	15/253	5.93	5.74
	Miryala	81, Indu	17.34169	79.73152	22/340	6.47	
	Tallasingaram		17.32183	79.63152	29/600	4.83	
Ranga Reddy - Rajendra Nagar	Budvel Rajendranagar	Teja	17.340077	78.4119494	12/98	12.24	13.17
	Experimental field, College of Agriculture, Skltshu, Rajendranagar	Germplasm accessions	17.316204	78.415501	8/178	4.49	
	Field, ARI, PJTSAU, Rajendranagar	Local variety	17.326713	78.417559	10/156	6.41	
	NBPGR- RS, Rajendranagar	Germplasm accessions	17.331718	78.410625	52/176	29.54	
Ranga Reddy - Moinabad	Aziznagar	Teja, Jwala, Jeeva	17.417545	78.446965	18/478	3.76	4.69
	Amdapur	81, Indu	17.273646	78.292329	21/420	5.00	
	Chilkur		17.360224	78.298615	32/602	5.31	
Ranga Reddy-Chevalla	Chevalla	Teja, Jwala, Jeeva	17.312476	78.138534	12/476	2.52	3.88
	Chanvelli	81, Indu	17.2707	78.1001	24/534	4.49	
	Mirjaguda		17.3079	78.0795	17/366	4.64	
Mahabubnagar - Midjil	Boinpalle	Teja, Wonder Hot,	16.73908	78.34477	29/324	8.95	8.17
	Wadiyal	US 341, Fatki,	16.76306	78.29732	16/191	8.376	
	Chiluveru	Byadgi,	16.72959	78.26260	24/275	7.169	
Mahabub Nagar - Rural	Allipur	Teja, Wonder Hot,	16.45888	78.17159	36/402	8.955	8.22
	Machanpalle	US 341, Byadgi,	17.19820	78.17213	19/278	6.834	
	Ippalpalle	Indu	16.67874	77.98033	19/214	8.878	
Mahabub Nagar - Urban	Boyapalle	Teja, Wonder Hot,	16.78199	77.99155	21/298	7.046	8.13
	Yedira	US 341, Indu	16.74289	78.06331	17/208	8.17	
	Yenugonda		16.75437	78.04559	13/98	9.167	

Average Disease Incidence 8.44

* Information collected from farmers during survey

In the present study, major symptoms observed were mosaic, shoe string, leaf deformation, mottling and stunting of plants. In Himachal Pradesh, Shelly Kapoor *et al.*, (2018)^[9] observed that mosaic, mottling, vein clearing, leaf deformation, shoe stringing or leaf narrowing, stunted growth and reduced fruit size as the most prominent and striking symptoms of CMV in infected bell pepper plants. From Tamil Nadu, Rajamanickam and Nakkeeran (2020)^[7] reported the occurrence of CMV in chilli with characteristic symptoms of necrosis, leaf malformation, stunting and reduction in plant size in Coimbatore district, while Vinodhini *et al* (2020)^[11] described the CMV infected chilli symptoms as mosaic with leaf filiformity, mosaic mottling and stunted growth in major chilli growing regions of Tamil Nadu. Many factors influence

the symptom expression and the variation in symptoms might be due to age of plant at the time of infection, virus strain, vectors and environmental conditions. In the present study, the symptoms caused by CMV infection in chilli depends on the variety and the time of infection.

DAC-ELISA

The collected CMV suspected samples (120) from six districts were subjected to DAC-ELISA. The absorbance values of the samples tested, including positive, negative and healthy control were presented in Fig. 2. The value twice that of negative control is considered as positive and among the samples tested, 59 were positive for CMV.

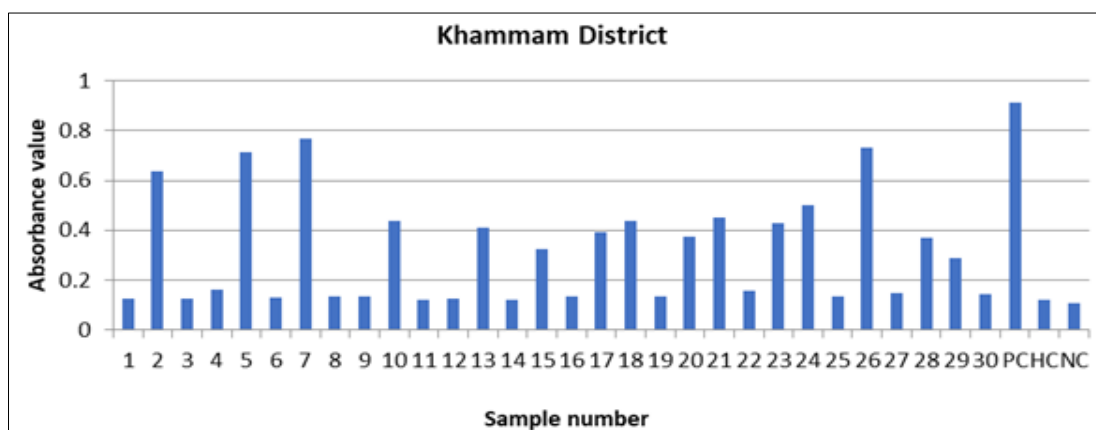


Fig 2a: Detection of *Cucumber mosaic virus* (CMV) in chilli plants from Khammam District

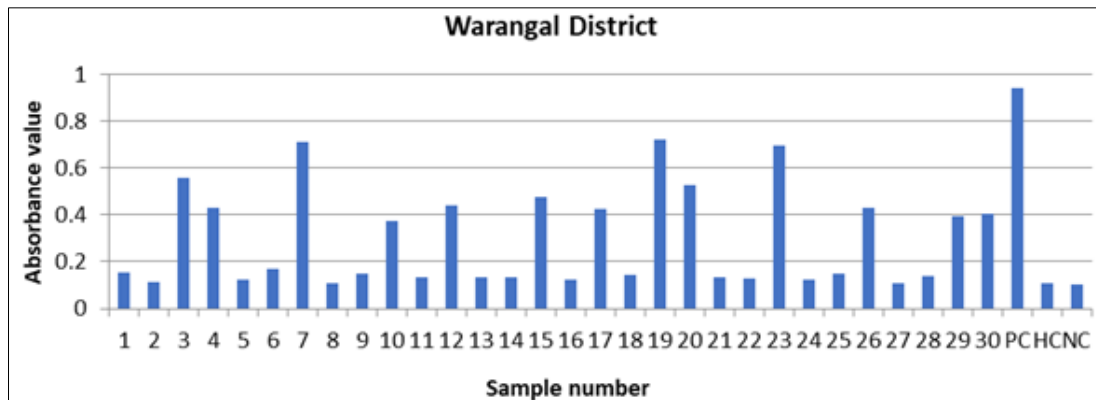


Fig 2b: Detection of *Cucumber mosaic virus* (CMV) in chilli plants from Warangal District

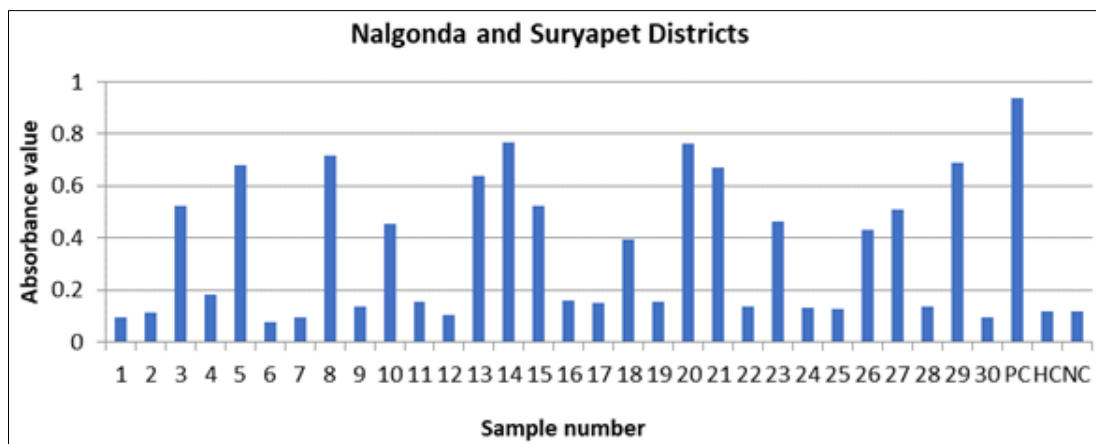
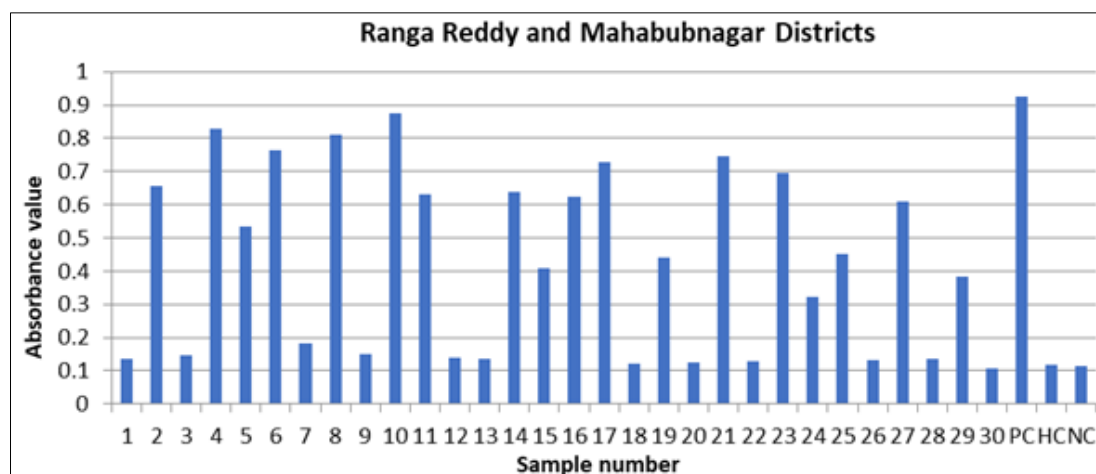


Fig 2c: Detection of *Cucumber mosaic virus* (CMV) in chilli plants from Nalgonda and Suryapet Districts



PC: Positive Control; HC: Healthy Control; NC: Negative Control

*A405 absorbance values recorded half an hour after the addition of substrate. Value represents average of three replications.

Fig 2d: Detection of *Cucumber mosaic virus* (CMV) in chilli plants from Ranga Reddy and Mahabubnagar Districts

Fig 2: Detection of *Cucumber mosaic virus* (CMV) in chilli samples from six districts of Telangana through DAC- ELISA

The highest absorbance value of more than 0.7 and 0.5 were recorded from infected leaf exhibiting shoe string as well as leaf deformation symptoms respectively. This is in line with the findings of Shelly Kapoor *et al* (2018) ^[9] in which they detected the highest concentration of CMV in bell pepper plants with shoe string symptoms using Double Antibody Sandwich (DAS) ELISA.

Conclusion

The findings of the present study demonstrated the occurrence of *Cucumber mosaic virus* in six chilli growing districts of Telangana State. The major symptoms observed were mosaic, mottling, shoe string, leaf deformation and stunting of plants and the average disease incidence was 8.44%. The maximum disease incidence of 16.69% was recorded in Warangal District. DAC – ELISA was performed to confirm the presence of CMV and the infected plant exhibiting shoe string symptoms showed the highest absorbance value followed by leaf deformation. DAC-ELISA was found as the suitable test for the detection of CMV in Chilli.

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