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The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2021; 10(10): 2171-2173

www.thepharmajournal.com
Received: 03-07-2021
Accepted: 09-08-2021

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Correlation between Symptoms and ELISA for the detection of cucumber mosaic virus in chilli

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Abstract
Field surveys were conducted for collection of CMV virus isolates in major chilli growing areas of Telangana, depicting a wide range of symptoms ranging from mosaic, mottling, yellow discoloration, vein clearing, leaf deformation, shoe stringing or leaf narrowing, stunted growth to reduced fruit size. In an attempt to correlate the symptoms with virus concentration in different isolates, DAC-ELISA was performed on symptomatic plants and CMV was detected in majority of the isolates. It was observed that the plants with shoe stringing (typical symptom of CMV in chilli) had the highest O.D. value whereas plants with puckering had very low O.D. value. This study signifies the importance of DAC-ELISA in mass screening of chilli germplasm to determine the sources of resistance to CMV.

Keywords: CMV, distortion, ELISA, mosaic, yellowing of leaves

Introduction
Chilli (*Capsicum annuum* L.) is one of the most valued cash crops of India. It is a common and widely cultivated spices crop almost all over the world. The chilli fruits are small in size and known for their sharp acidic flavour, pungency and colour. Major chilli producing states in India are Andhra Pradesh, Telangana, Tamil Nadu, Karnataka and Madhya Pradesh. In 2019-20, Telangana occupied second position in chilli area, production and productivity *i.e.,* 2.98 lakh acres, production 3.06 lakh metric tonnes and productivity 1545 Kg per acre. The major chilli growing districts are Khammam, Mahabubabad, Gadwal, Suryapet and Warangal (Rural). During 2020-21, 1.91 lakh acres is covered under chilli crop (Chilli outlook, 2020).
Chilli is predisposed to multitude of viral, fungal, bacterial, nematode and phytoplasma diseases. Viruses are known to incite wide range of symptoms like mosaic, ring spot, curling, yellowing etc. Among them, CMV is ubiquitous and infect entire plants in the field with variable symptoms such as mosaic, mosaic mottling, yellowing, puckering and reduced size of leaves, closely set internodes and dwarfing of plants. These symptoms produce witch broom appearance and affect fruit setting (either failure or small deformed fruit formation).
In tropical and subtropical parts of India CMV is a major constraint to chilli (Dhanraj *et al.*, 1968; Chattopadhyay *et al.,* 2008). Cucumber mosaic virus is the type species in the genus *Cucumovirus* representing family *Bromoviridae* (Roossinck *et al.,* 1999). It has a wide host range including plants from approximately 365 genera and at least 85 families and is efficiently transmitted in a non-persistent manner by more than 75 species of aphids (Kaplan *et al.,* 1997) [6].

Materials and Methods
Collection of isolates
During field survey, leaf samples from infected chilli plants showing mosaic and distorted leaf symptoms and healthy plants without any symptoms were collected and brought to the laboratory for serological detection of the causal virus in the sample by DAC-ELISA using CMV antisera procured from BIOREBA, Switzerland.

ELISA detection
DAC (Double Antigen Coating) form of ELISA was used for the detection of the causal virus in the test samples. The procedure for conducting DAC-ELISA is presented hereunder.
The test samples @ 200µl/wel1 were dispensed into ELISA plates. The plates were incubated at 37 ^{0}C for 1 hr. Test plant leaf extracts were poured off and the plates were washed three

times by allowing 3 minutes between each wash with phosphate buffer saline tween (PBST), which was prepared by mixing 1.19 g Na_2 $HP0_4$, 0.2 g KH_2P0_4, 0.2 g KCl, 8.0 g NaCl and 0.5 ml tween-20 to 1 liter distilled water.

Cross absorption of antisera with healthy leaf extract was done by grinding the healthy leaves in antibody buffer, which was prepared by adding 100 ml PBST, 2.0 g polyvinyl pyrolidine of 2% final concentration and 0.2g ovalbumin with 0.2% final concentration to give 1:20 dilution and was filtered through two layers of cheese cloth and then diluted to 1: 1000 in antibody buffer. This was dispensed @ 200 µl/wel1 into ELISA plates.

The plates were incubated at 37°C for 1 hr. and washed three times with PBST, as described earlier. To each well, 200 µl of anti rabbit immunoglobulin (1gG) conjugated with alkaline phosphatase diluted to 1:500 in anti body buffer was added and the plates were incubated at 37°C for 1 hr. After washing the plates three times with PBST, 200 µl of P-nitrophenyl phosphate substrate was added to each well, which was prepared by adding 1 mg/ml. The plates were then incubated at room temperature for 30-120 min. The absorbance values were recorded at 405 nm using ECIL micro scan MS 5605A ELISA reader.

Results and Discussion

Survey and symptomatology

In the present study, cucumber mosaic virus infecting chilli at different fields belonging to various chilli growing areas of Telangana were surveyed to record the incidence of CMV and also to collect different virus isolates on the basis of different type of symptoms observed during field surveys. The incidence of virus diseases in chilli grown at different locations ranged from 15.80 – 36.55 per cent during the period of survey. Since the symptoms on chilli were observed to be of complex nature, the incidence of CMV could not be recorded specifically. However, to confirm the positive association of CMV with chilli crop surveyed, DAC-ELISA was performed to identify CMV in symptomatic plants.

The most prominent and striking symptoms of CMV in infected bell pepper plants were in the form of mosaic, mottling, vein clearing, leaf deformation, shoe stringing or leaf narrowing, stunted growth and reduced fruit size (Fig. 1–4). Studies conducted on CMV in bell pepper from India and many other countries have observed mosaic, mottling, shoe stringing, leaf narrowing and stunted growth to be associated with CMV infection in chilli (Sevik *et al.*, 2003; Korbin and Kaminska, 1998; Khan *et al.*, 2006) [11, 8, 7].

DAC-ELISA

Positively marked bell pepper plants growing at different experimental farms were screened by DAS-ELISA to confirm the presence of CMV. Individual symptomatic plant was considered to be an independent sample. Since varied symptoms were observed in the field, each plant exhibiting different symptom was considered to be representing different virus isolate. On the basis of these symptoms, a large number of virus isolates were collected from different experimental farms and loaded into ELISA plate as an individual sample.

It is evident from the data presented in Table 1 that out of a total of 15 virus isolates collected from 6 different districts of Telangana, 11 virus isolated tested positive in DAC- ELISA tests as evident from the O.D. values recorded in microprocessor based ELISA plate reader. The highest O.D value of 0.613 was recorded from virus isolate 15 (collected from Warangal) exhibiting shoe stringing symptoms followed by virus isolate 5 (collected from Khammam) also exhibiting shoe stringing symptoms. Many workers have also reported shoe stringing to be the major symptom of CMV on chilli (Kamiska *et al.*, 2005; Kumari *et al.*, 2013; Iqbal *et al.*, 2011) [5, 9, 4].

Virus isolate with puckering, blistering, yellowing and leaf curling type of symptoms were found to be free from CMV as indicated by negative ELISA results. A critical scanning of the literature on symptomatology of CMV in bell pepper reveals that none of these symptoms are associated with CMV anywhere in the world.

The present studies have clearly established the importance of ELISA for detecting CMV in chilli as the virus was found in detectable limits in all virus isolates exhibiting typical symptoms of CMV infection. These findings further highlight the importance of ELISA for large scale screening of germplasm to ascertain the sources of resistance to this virus. Many reports available in the literature also emphasise the need of ELISA in the detection of CMV in symptomatic plants and also stresses upon its utility in mass screening of germplasm (Soleimani *et al.*, 2014; Ashfaq *et al.*, 2014; Xu *et al.*, 2006; Perry *et al.*, 1993) [12, 2, 13, 10].

Table 1: Reaction of virus isolates to CMV in DAS- ELISA

District	Isolate	Symptoms	Reaction in ELISA	O.D. Value (A_{450}nm)
Warangal	W-3	Mottling	+	0.265
	W-6	Mosaic	+	0.424
	W-13	Vein clearing	+	0.283
Khammam	K-3	Puckering	-	0.091
	K-6	Shoe stringing	+	0.617
	K-9	Blistering	-	0.115
Mahabubabad	MB-4	Leaf distortion	+	0.279
	MB-7	Yellowing	-	0.104
	MB-13	Stunted growth	+	0.238
Jayshanker Bhupalpally	J-4	Mottling	+	0.355
	J-8	Leaf narrowing	+	0.411
Mulugu	M-5	Leaf curling	-	0.089
	M-10	Vein clearing	+	0.290
Nagarkurnool	N-4	Reduced fruit size	-	0.250
	N- 7	Mosaic mottling	+	0.249

Positive control: 0.340 (+)
Negative control: 0.110(-)

Fig 1: Vein clearing

Fig 2: Puckering

Fig 3: Shoe stringing

Fig 4: Mosaic

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