The Pharma Innovation Journal 2021; SP-10(7): 406-409

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2021; SP-10(7): 406-409

www.thepharmajournal.com
Received: 13-05-2021
Accepted: 15-06-2021

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Serological detection of cucumber mosaic virus affecting cucumber from Jammu region

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Abstract
Cucumber (*Cucumis sativus* L.) is one of the most important vegetable crops of the family Cucurbitaceae, grown extensively in tropical and sub-tropical parts of the country. Cucumber production is greatly hampered by cucumber mosaic virus (CMV). A survey was conducted in three districts (Jammu, Samba and Kathua) of Jammu division during 2019 and 2020 and it was found that cucumber mosaic disease was prevalent in all locations and the overall incidence of disease in Jammu division was found maximum (34.66%) in Bishnah of Jammu district and minimum (21.33%) in Hirangar of Kathua district during 2019 while during 2020, maximum disease incidence of 36.00% was recorded from Akhnoor of Jammu district and minimum (20.00**%**) was recorded from Hirangar of Kathua district. Infected samples collected during the survey were brought under laboratory conditions for detection of the virus by serological means through DAS-ELISA and the overall result showed positive reaction with CMV specific antibody thus confirming the presence of cucumber mosaic virus.

Keywords: cucumber mosaic virus, DAS-ELISA, serology, cucumber

Introduction
Cucumber (*Cucumis sativus* L.) is a member of the cucurbitaceous family and is one of the most popular cash crops. It is the second most widely cultivated cucurbit after watermelon. Cucumber has tremendous economic and dietic importance. It is rich source of vitamin A and C and contains carotenoids when consumed with skin. In India area under cultivation of cucumber is 82 thousand hectare with the production 1260 thousand MT (Anonymous, 2017).
Cucumber is susceptible to various diseases such as mosaic, wilt, anthracnose, seedling blight, leaf spot, root rot, downy and powdery mildews. Among these, mosaic caused by cucumber mosaic virus (CMV) is an economically important disease. The virus was first reported in 1916 by Doolittle and since then reported to cause disease in a variety of economically important agricultural and ornamental crops. The virus has widest host range infecting over 1,200 species from 100 plant families. CMV is a positive sense tripartite virus having single stranded RNA, which is en-capsidated in a 28nm icosahedral particle (Nault, 1997) [14] and belonging to genus *Cucumovirus* and family Bromoviridae. Cucumber plants may become infected at any stage of growth, from emergence of the seedling to crop maturity (Takanami, 1981) [21] and estimated to cause severe yield losses up to 40-60 per cent (Varma and Giri, 1998) [22]. CMV infected cucumber plant displays a mottled leaf-pattern, with yellow and green areas (Davis and Whitaker, 1962) [9]. CMV is easily transmitted by mechanical inoculation of plant sap and naturally transmitted by more than 80 species of aphids in non-persistent manner (Palukaitis and Garcia-Arenal, 2003) [15]. It was reported that *Myzus persicae* and *Aphis gossypii* are among the more efficient vectors for this virus (Edwardson and Christie, 1991) [11]. The detection of the virus at early stages is very important for developing management strategies and the serological approaches are very sensitive and accurate for their detection. Therefore, keeping in view the economic importance of the crop and the magnitude of the damage caused by the disease, the present study was undertaken to ascertain the status and serological detection of CMV in cucumber in Jammu region.

Materials and Methods
Sample collection
A survey of different cucumber growing areas of Jammu division particularly, Jammu, Samba and Kathua districts was conducted during cropping season of 2019 and 2020 to determine the status of cucumber mosaic disease.

A minimum of five locations from each district were selected and from each location five fields were randomly surveyed. Observations were recorded regularly by counting the number of healthy and diseased plants exhibiting characteristics symptoms of cucumber mosaic disease.

Percent disease incidence was calculated by using the following formula:

$$\text{Per cent Disease incidence} = \frac{\text{No. of diseased plants}}{\text{Total no. of Plants observed}} \times 100$$

Detection of virus through serological methods (DAS-ELISA)

Samples of cucumber plant showing characteristics symptoms of mosaic disease such as yellowing and pale green patches, mottling, mosaic and distortion of infected leaves were collected from different locations of Jammu region during the survey. Serological detection of these samples through DAS-ELISA (Double Antibody Sandwich- Enzyme Linked Immuno-sorbant Assay) described by Clark and Adams (1977) [8] was carried out under laboratory conditions of Division of Plant Pathology, Sher-e-Kashmir University of Sciences and Technology, Jammu. The samples were exposed against antisera of cucumber mosaic virus.

Results and Discussions

Extensive survey was undertaken in selected locations of Jammu (R.S.Pura, Bishnah, Udheywala, Marh and Akhnoor), Samba (Vijaypur, Rajpura, Samba, Jatwal and Nud) and Kathua (Nagari, Kathua, Keerian Gandyal, Barnoti and Hiranagar) districts of Jammu division during 2019 and 2020, to assess the incidence of mosaic disease on cucumber (*Cucumis sativus* L.). The disease incidence was recorded on the basis of visual symptoms and was then confirmed serologically by using DAS-ELISA method.

The overall range of disease incidence observed in Jammu division ranged between 21.33- 34.66 per cent with an overall mean of 27.44 per cent during 2019. In Jammu district, the maximum incidence of cucumber mosaic disease of 34.66 per cent was recorded from Bishnah followed by Akhnoor (33.33%), R.S.Pura (30.66%), Marh (28.00%) and Udheywala (26.66%) with the mean of 30.66 per cent. In Samba district, the maximum disease incidence was recorded from Vijaypur (30.66) followed by Samba (28.00%), Jatwal (26.66%), Nud (24.00%) and Rajpura (22.66%). with the mean of 28.39 per cent. In Kathua district, the maximum disease incidence of 32.00 per cent was recorded from Nagari followed by Kathua (25.33%), Keerian Gandyal (25.33%), Barnoti (24.00%) and Hirangar (21.33%). However, the overall mean of the disease was 25.59 per cent (Table 1).

During the cropping season of 2020, the overall range of disease incidence in Jammu division was 20.00-36.00 per cent while overall mean of disease incidence was 27.81 per cent. However, in Jammu district, the maximum disease incidence was recorded in Akhnoor (36.00%) followed by R.S. Pura (32.00%), Bishna (29.33%), Udheywala (28.00%) and Marh (26.66%) having the mean disease incidence of 30.39 per cent. In Samba district the maximum disease incidence of 30.66 per cent was observed in Samba followed by Vijaypur (29.33%), Rajpura (25.33%), Jatwal (25.33%) and Nud (21.33%). However, in Kathua district, the maximum disease incidence recorded was in Nagari (34.66%) followed by Barnoti (29.33%), Kathua (26.66%), Keerian Gandyal (22.66%) and Hirangar (20.00%). The mean disease incidence recorded was 26.66 per cent (Table 1).

Table 1: Status of mosaic disease of cucumber in different districts of Jammu during 2019 and 2020

District	Location	Per cent Disease Incidence		Pooled (%)
		2019	2020	
Jammu	R. S. Pura	30.66	32.00	31.33
	Bishna	34.66	29.33	31.99
	Udheywala	26.66	28.00	27.33
	Marh	28.00	26.66	27.33
	Akhnoor	33.33	36.00	34.66
	Mean	30.66	30.39	30.53
	Range	26.66-34.66	26.66-36.00	27.33-34.66
Samba	Vijaypur	30.66	29.33	29.99
	Rajpura	22.66	25.33	23.99
	Samba	28.00	30.66	29.33
	Jatwal	26.66	25.33	25.99
	Nud	24.00	21.33	22.66
	Mean	28.39	26.39	27.39
	Range	22.66-30.66	21.33-30.66	22.66-29.99
Kathua	Kathua	25.33	26.66	25.99
	Nagari	32.00	34.66	33.33
	Keerian Gandyal	25.33	22.66	23.99
	Barnoti	24.00	29.33	26.66
	Hirangar	21.33	20.00	20.66
	Mean	25.59	26.66	26.13
	Range	21.33-32.00	20.00-34.66	20.66-33.33
Overall Range		21.33-34.66	20.00-36.00	20.66-34.66
Overall Mean		27.44	27.81	27.68

It is suggested that variation in disease incidence over locations might be due to survival of virus in different hosts and various environmental conditions congenial for disease development. Earlier workers have also found variable incidence of viral diseases in Cucumber (*Cucumis sativus* L.) at different places. Andotra (1990) [3] recorded 0-46 and 0-32 per cent incidence of mosaic diseases of cucumber in Himachal during cropping seasons of 1988 and 1989,

respectively. However, 60-80 per cent incidence of CMV and WMV was reported in cucumber from Gorakhpur (Bhargava and Bhargava, 1977) [6]. Bananej and Vahdat (2008) [4] also recorded the highest mean incidence of CMV at harvesting (51.76%), followed by flowering (44.78%) and vegetative stage (24.13%) in Iran. They reported that the disease incidence increased with age of crop because the infected plants served as source for further spread. Similarly, variable incidence of mosaic disease has been observed at different places by a number of other workers (Li *et al*., 2004; Massumi *et al*., 2007; Raj *et al*., 2008; Revadi and Patil, 2017) [12, 13, 17, 18].

All the samples showing the infection of CMV were collected during the cropping season of year 2019 and 2020 from different locations of Jammu region. The infected samples were then brought under laboratory conditions for confirmation of the virus by DAS-ELISA. Specific CMV antibody was used to test the presence and absence of the respective causal virus and the data recorded on optimal density (O.D.) i.e. absorbance value at 405 nm wavelength in both the year are presented in Table 2.

The overall O.D. value of CMV infected samples in Jammu, Samba and Kathua district ranged from 0.0515–0.3201, 0.0420-0.2031 and 0.1196-0.2611, respectively during 2019 while during 2020 the overall range of O.D. value recorded in Jammu, Samba and Kathua districts were 0.1350-0.2912, 0.0925-0.2516 and 0.0165-0.2960, respectively. However, the wells which were charged with healthy tissue and buffer showed absorbance value in the range of 0.0180-0.1055 and 0.0132-0.0500, respectively during 2019 while O.D value of 0.0120-0.1026 (healthy tissue) and 0.0103-0.0118 (buffer) during 2020.

Table 2: Serological detection of cucumber mosaic virus (CMV) from different locations of Jammu region during 2019 and 2020

District	Location	No. of wells charged	OD value of CMV at 405 nm		Presence (+) or Absence (-) of virus
			2019	2020	
Jammu	R. S. Pura	6	0.2412- 0.3201	0.2460- 0.2912	+
	Bishna	6	0.1302-0.2196	0.2051-0.2265	+
	Udhawala	6	0.2031-0.2815	0.1803-0.2350	+
	Marh	6	0.0759-0.0850	0.1706-0.1801	+
	Akhnoor	6	0.0515-0.1054	0.1350-0.1720	+
Samba	Vijaypur	6	0.1695- 0.17185	0.2210- 0.2516	+
	Rajpura	6	0.0639-0.0690	0.1860-0.2130	+
	Samba	6	0.2010-0.2031	0.0925-0.1375	+
	Jatwal	6	0.1400-0.1480	0.1606-0.1820	+
	Nud	6	0.0420-0.0521	0.2173-0.2501	+
Kathua	Nagari	6	0.1701- 0.1800	0.0403- 0.0860	+
	Kathua	6	0.1196- 0.1206	0.0165- 0.1208	+
	Keerian Gandyal	6	0.2054-0.2148	0.2104-0.2690	+
	Barnoti	6	0.1362-0.2540	0.1822-0.2210	+
	Heranagar	6	0.2320-0.2611	0.2541-0.2960	+
Healthy tissue		2	0.0180-0.1055	0.0120-0.1026	-
Buffer		2	0.0132-0.0500	0.0103-0.0118	-

The overall result thus revealed that samples collected from Jammu, Samba and Kathua districts were found infected with cucumber mosaic virus (CMV) and showed positive reaction with CMV specific antibody as the O.D values were more than twice the value of negative control. Akanada *et al*. (1991) [2] collected 92 samples from different hosts of 15 families showing virus like symptoms from various locations in Bangladesh and found that two cucumber samples were positive against CMV. Bashir *et al*. (2006) [5] also detected one hundred and twenty three cucurbit samples showing symptoms of leaf mosaic, leaf distortion, fruit mosaic, stunting, mottling and yellowing from different locations of northwest region of Iran through DAS-ELISA and found positive reactions from 13 samples with cucumber mosaic virus (CMV) polyclonal antibody. DAS-ELISA was performed for the detection of CMV in cucumber by many other workers (Sako *et al*., 1980; Perez *et al*., 2004; Shetti *et al*., 2012; Biswas *et al*., 2013) [19, 6, 20, 7].

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