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INCIDENCE AND OCCURRENCE OF VIRUSES INFECTING CUCURBITS IN PUNJAB

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ABSTRACT

Incidence of viral diseases on different cucurbits (bottle gourd, sponge gourd, cucumber, muskmelon, pumpkin and watermelon) was recorded during this study. Maximum incidence of sixty per cent was observed on muskmelon crop followed by cucumber with incidence of fifty seven per cent whereas, minimum viral disease incidence of 24.1 per cent was observed on watermelon crop. The infected plants showing different types of symptoms like yellowing, mosaic, puckering, blistering, leaf deformation, leaf curl, necrosis, vein banding and interveinal chlorosis, etc. were collected and subjected for virus identification using Double Antibody Sandwich- Enzyme linked Immunnosorbent Assay (DAS-ELISA) and Polymerase chain reaction (PCR). Four viruses, namely *Cucumber mosaic virus-I* (CMV-I), *Groundnut bud necrosis virus* (GBNV), *Zucchini yellow mosaic virus* (ZYMV) and begomovirus were found to be associated with different cucurbits.

Keywords: Cucurbits, Detection, DNA viruses, Incidence, RNA viruses

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Many important vegetable crops which are commonly referred as cucurbits come under *Cucurbitaceae* Family and share about 5.6% of total vegetable production in India (Dhkal, 2018). Cucurbits are consumed as sweet (ash gourd, pointed gourd), pickles (gherkins), salad (cucumber, gherkins, long melon) and deserts (melons) as well as cooked (pumpkin, bottle gourd, bitter gourd, sponge gourd, ridge gourd) (Sharma, 2014). These crops are susceptible to a number of diseases caused by fungi, bacteria, nematodes and viruses which render their cultivation uneconomical and relatively insecure (Dhkal, 2018). However, the singular damage caused by viral diseases alone is enormous (Mansour and Almusa, 1982) as these are not easily identifiable and manageable like other plant pathogens. Yellows, mosaic, fruit malformation, puckering of leaves, blistering, yellowing of veins and veinlets, leaf narrowing, mosaic mottling, malformation of stem and leaves, appearance of chlorotic spots on leaves, vein banding, leaf filiformity, leaf rosetting and enations are some important symptoms produced as a result of viral infection on cucurbits (Sandhu and Kang, 2007; Nagendran *et al.,* 2017).

These viral diseases have been responsible for various epidemics in the world (Sharma *et al.,* 2012). In North India, *Cucumber mosaic virus* appeared in epidemic form during 1999 which resulted in more than 50 per cent reduction in yield (Thind and Singh, 2001). Cucurbits are attacked by more than 30 viruses (Zitter *et al.,* 1996; Kang and Sandhu, 2007), out of which *Begomovirus, Cucumber mosaic virus* (CMV), *Cucumber green mottle mosaic virus* (CGMMV), *Squash mosaic virus* (SqMV), *Papaya ring spot virus* (PRSV), *Watermelon mosaic virus* (WMV) and *Zucchini yellow mosaic virus* (ZYMV) are known to cause serious losses in major cucurbit growing areas (Sutic *et al.,* 1999). In Punjab cucurbits occupies 27.21 thousand hectare area with total production of 526.19 thousand tones (Anonymous, 2021). Except during severe winter, cucurbits are grown throughout the year in Punjab. Virus diseases pose a major threat to the commercial cultivation of cucurbits during active season in Punjab. Although, in past years systematic studies regarding viruses associated with different cucurbit crops have been attempted with major focus on RNA viruses with the help of several techniques *viz.* serological assays, molecular hybridization, polymerase chain reaction (PCR), reverse transcription followed by PCR (RT-PCR), loop (or RT-loop) mediated isothermal amplifications (LAMP and RT-LAMP), and microarrays (Aman *et al.,* 2020).

During this study periodical surveys were carried out during 2017 and 2018 to determine the prevalence of virus diseases, symptom variability and associated viruses with different cucurbits using serological and nucleic acid based assay in different cucurbits growing areas of Punjab.

MATERIALS AND METHODS

Survey and collection of samples

Major cucurbits growing districts of Punjab *viz*. Jalandhar (Longitude-31°11'11.5'', Latitude-

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76°17'5.8"), Kapurthala (Longitude-31°37'99.18'', Latitude-75°37'99.8"), Ludhiana (Longitude-30°90'1.5'', Latitude-75°80'7.5"), Patiala (Longitude-30°25'52.8'', Latitude-76°10'47.8"), SAS Nagar (Longitude-30°70'46.42'', Latitude-76°71'78.6"), Pathankot (Longitude-32°14'45.7'', Latitude-75°34'09.1"), Gurdaspur (Longitude-32°03'12.9'', Latitude-75°25'26.8") and Sangrur (Longitude-30°32'58.8'', Latitude-75°56'9.4") were surveyed in the months of February-August during 2017 and 2018 and diseased samples were collected. The initial disease diagnosis in the field was based on characteristic symptoms exhibited by viruses. At each location number of plants infected and symptoms exhibited were recorded by observing twenty plants each from all four corners and centre of the field by roving method. The young leaf tissues from the infected plants were collected at flowering and fruit initiation stage in zip lock bags and were carried to laboratory. Samples were stored at -80°C for one week for further characterization. The observations were recorded on symptoms variability and per cent incidence was calculated by following formula:

$$\text{Per cent disease incidence} = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100$$

Serology assay

Double Antibody Sandwich and Triple Antibody Sandwich-Enzyme Linked Immunosorbent Assay were performed on samples taken from infected cucurbits showing different kind of symptoms as described by Clark and Adams (1977). The infected samples representing six cucurbit species viz. bottle gourd, sponge gourd, cucumber, muskmelon, pumpkin and watermelon were tested for the presence of seven viruses viz. *Cucumber mosaic virus-1* (CMV-I), *Cucumber mosaic virus-2* (CMV-II), *Cucumber green mottle mosaic virus* (CGMMV), *Groundnut bud necrosis virus* (GBNV), Potato virus$^{o/c}$ (PVY$^{o/c}$), *Squash mosaic virus* (SqMV) and Zucchini yellow mosaic virus (ZYMV). The antibodies of CMV-I, CMV-II, CGMMV, GBNV, PVY$^{o/c}$, SqMV and ZYMV procured from Agdia Inc (Elkhart, USA) were used for detecting viruses. The ELISA plate (Nunc, USA) was observed at A405 nm using Tecan Sunrise ELISA reader (Tecan International, Austria). Sample was considered to be positive if its A405 value was at least two times greater than the mean absorbance value of healthy control samples.

Nucleic acid assay

Samples tested for serological assay were also subjected to nucleic acid based detection method of begomovirus using PCR. Total genomic DNA from infected leaf samples was extracted using the cetyl trimethyl ammonium bromide (CTAB) method (Lodhi *et al.,* 1994). DNA of tomato leaf curl virus served as positive control. PCR was performed in a 10µl reaction volume containing 2 µl of DNA, 0.6 µl of 20pmol/ml of primer pairs PALIc (5'ACNGGNAARACNATGTGGGC3') and PARIv (5'GGNAARATHTGGATGGA3') each, 3.5 µl Master Mix (EmeralAmp®GT PCR Master Mix) and 3.3 µl nuclease free water. These primer pair is specific for the coat protein region of begomovirus and amplifies specific product of ~1280bp (Rojas *et al*., 1993).The PCR cycling profile consisted of initial cycle of denaturation at 94°C for 3 minutes followed by 35 cycles each consisting of denaturation at 94°C for 50 sec, annealing at 52°C for 45 sec followed by extension at 72°C for 1.5 min and final extension for 15 minutes at 72°C.

Gel analysis

The amplified PCR products were analyzed using 1 per cent agarose gel with ethidium bromide (10mg/ml) stain. The gel was viewed under UV light using Alpha Imager HP gel documentation system (Alpha Innotech, USA).

RESULTS AND DISCUSSION

Survey was conducted during 2017 and 2018 revealed the noteworthy incidence of viral diseases in the different cucurbit crops grown at various districts of Punjab (Table 1). In the year 2017, all the eight districts were surveyed and maximum disease incidence was recorded in Pathankot (100%) followed by Patiala (62.5%), whereas minimum incidence was recorded in district Kapurthala (33 %). In 2018, six districts were surveyed with maximum disease incidence recorded in Ludhiana (36.5%) followed by Patiala (34.2%) district (Table 1). Sharma *et al.* (2012) also observed the notable incidence of virus diseases in cucurbit crops in Trans-Gangetic plains as same was observed in this study.

Incidence of viral disease varied largely among the interspecies. Maximum disease incidence of 60 % was recorded in muskmelon followed by cucumber (57%), sponge gourd (52%) and bottle gourd (48.75%), whereas minimum disease incidence was observed in watermelon (24.1) followed by pumpkin (40%) (Table 1). Sharma *et al*. (2012) also recorded viral disease incidence on various cucurbits grown in different districts of Punjab. They observed viral disease incidence of 100% in muskmelon and cucumber, whereas minimum disease incidence was reported in sponge gourd (10%), followed by bottle gourd (15%), snap melon (25%) and bitter gourd (25%).

Serological assay was carried out on randomly collected infected samples using DAS/TAS ELISA for seven major viruses' revealed that CMV-I, ZYMV

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Table 1. Year-wise comparison of incidence of viral diseases on cucurbits in Punjab

S.No	District	2017	2018
		Disease incidence	Disease incidence
1	Sangrur	55	25
2	Patiala	62.5	34.2
3	Ludhiana	56.7	36.5
4	Jalandhar	40	24.1
5	Kapurthala	33	13.8
6	SAS Nagar	40	19.3
7	Gurdaspur	50	-
8	Pathankot	100	-

and GBNV were major RNA viruses associated with cucurbits. About 53 per cent samples that were tested showed association with either RNA or DNA virus. *Cucumber mosaic virus-I* (CMV-I) was associated with bottle gourd and muskmelon whereas; *Zucchini yellow mosaic virus* (ZYMV) was associated with sponge gourd, muskmelon and pumpkin. Besides CMV-I and ZYMV, *Groundnut bud necrosis virus* (GBNV) was observed to infect bottle gourd and watermelon crop (Table 2). Unlike the findings of Sharma *et al.* (2012) in this study infection of *Cucurbit green mottle mosaic virus* (CGMMV) and *Squash mosaic virus* (SqMV) was not observed in the cucurbits. Nagender *et al.* (2017) also observed that infection of CMV and ZYMV in various cucurbit samples that were collected from different zone of Tamil Nadu state of India. Besides RNA viruses, whitefly transmitted begomoviruses was also reported to infect muskmelon and cucumber crop during this study.

Wide varieties of symptoms were observed on infected cucurbits during this study. Highest variability in symptom was observed on muskmelon crop with yellows, mosaic, leaf curl, puckering, leaf deformation and blistering types of symptoms followed by pumpkin crop with blistering, vein banding, mosaic, yellows and intervenial chlorosis type of symptoms. In bottle gourd and sponge gourd common symptoms of yellows, mosaic and puckering were observed. In cucumber leaf curl, yellows, blistering and mosaic type of symptoms were observed whereas, in watermelon necrosis, puckering, blistering, stunting and leaf deformation type of symptom were observed (Table 2). In cucumber and muskmelon crop heavy infestation of whitefly was observed. Earlier these types of symptoms on cucurbits due to virus infection were also observed by other workers in different part of India (Sharma *et al.,* 2012; Nagender *et al.,* 2017).

Table 2. Virus disease symptoms and associated viruses infecting cucurbits in Punjab

Crop	Incidence (%)	Symptoms	DAS/TAS-ELISA Positive samples (%)	Begomovirus Positive samples (%)	Associated viruses (% of infected samples)
Bottle Gourd	48.75	Yellows, mosaic, necrosis, puckering	15	0	CMV-I (66.67), GBNV (33.33)
Sponge Gourd	52	Yellows, mosaic, puckering, leaf deformation	37.5	0	ZYMV (100)
Cucumber	57	Leaf curl, yellows, blistering, mosaic	0	41.7	Begomovirus (100)
Muskmelon	60	Yellows, mosaic, leaf curl, puckering, leaf deformation, blistering,	10	63.2	CMV-I (1.56), ZYMV (12.5), Begomovirus(85.9)
Pumpkin	40	Blistering, vein banding, mosaic, yellows, intervenial chlorosis	25	0	ZYMV (100)
Watermelon	24.1	Necrosis, puckering, blistering, stunting, leaf deformation	15.4	0	GBNV (100)

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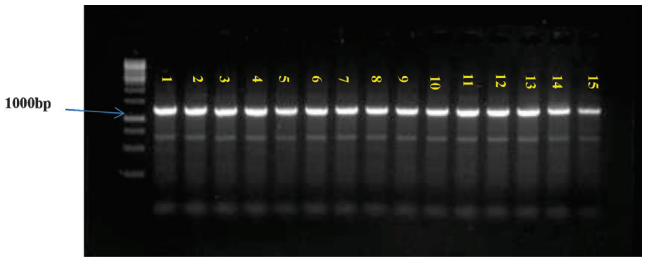


Fig. 1. Agarose gel analysis of begomovirus (~1280bp) obtained using PALIc and PARIv primers (Rojas *et al*., 1993) in muskmelon samples. Lane 1 in the gel contain 1kbp DNA ladder (SimBio)

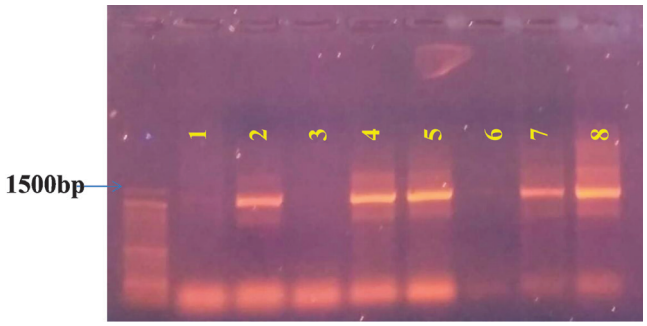


Fig. 2. Agarose gel analysis of begomovirus (~1280bp) obtained using PALIc and PARIv primers in cucumber samples. Lane 1 in the gel contain 50bp DNA ladder (NextGen Life Sciences)

PCR amplification of DNA isolated from various symptomatic samples using begomovirus specific primers resulted in generation of 1280 bp product (Figs. 1 and 2). Out of total samples that were analyzed for the infection of begomovirus, cucumber and muskmelon that contributes about 38.4 per cent of total samples were found to be infected with begomovirus. Begomovirus infected cucumber leaf exhibited leaf curl, yellows, blistering and mosaic type of symptoms and muskmelon samples exhibited yellows, leaf curl and leaf deformation type of symptoms. Suresh *et al.* (2013) reported severe yellows and leaf crumpling type of symptoms on cucumber crop due to the infection of begomoviruse. These viruses are readily transmitted by whitefly, *Bemisia tabaci* in persistant manner. In North India infection of *tomato leaf curl New Delhi Virus* on cucumber was reported by Jythosna *et al.* (2013). Unlike cucumber severe incidence of begomovirus was not observed on the muskmelon crop in India till now. However, in some parts of the world yellows and leaf curl type of symptoms were observed on muskmelon crop due to the infection of begomovirus (Malik *et al.,* 2011; Mnari-Hattab *et al.,* 2015; Trisciuzzi *et al.,* 2018). During this study begomovirus infection was not observed in pumpkin and sponge gourd crop that could be due to the sampling of this crop was done in the month of March-April so either due to the absence or low titer of begomovirus it could not be amplified in the infected samples. Generally, the incidence of begomovirus on sponge gourd and pumpkin was more during the month of June-July in Punjab.

During this study whitefly transmitted begomovirus was observed to be most prevalent on muskmelon and cucumber. Recently whitefly transmitted begomoviruses were also reported from muskmelon under Punjab conditions (Dhkal *et al.,* 2020a; Dhkal *et al.,* 2020b). Earlier aphid transmitted viruses were more prevalent in cucurbits as compared to the whitefly transmitted begomovirus (Sharma *et al.,* 2012). This shift in the virus population either could be due to the change in climatic conditions or due to the availability of host crop for whitefly and begomovirus throughout the year. As begomovirus is infecting more hosts with the time, it can pose a serious threat in future to the vegetable cultivation under Punjab condition. However, predication of a particular virus infecting cucurbits on the basis of symptoms was also very difficult, as most of the time symptoms caused by different viruses on cucurbits may overlap each other. Moreover, direct introduction of new varieties for cultivation is also a serious concern, as most of these are either susceptible to the viruses present in the region or may harbour seed transmitted virus (Sharma *et al.,* 2012). Therefore, a better understanding of virus and vector reservoirs, vector population behavior, and vector transmission efficiency are necessary for the development of effective management strategies.

Authors' contribution

Conceptualization and designing of the research work (MD, AS); Execution of field/lab experiments and data collection (MD, AS); Analysis of data and interpretation (MD, AS); Preparation of manuscript (MD, AS).

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