Research Article

UNVEILING THE INCIDENCE AND DISTRIBUTION OF RNA VIRUSES INFECTING CUCURBIT CROPS IN DERA GHAZI KHAN DIVISION

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ABSTRACT

The *Cucurbitaceae* family holds a prominent position among the horticultural crops, comprising 800 species of herbaceous plants across 120 genera. These crops are vulnerable to several abiotic and biotic stresses, with RNA viruses being the most important biotic constraint. Viruses from the genera *Potyvirus*, *Tobamovirus*, *Polerovirus*, and *Cucumovirus* cause substantial damage and yield losses in cucurbits, potentially reducing yields by up to 70%. The precise identification and prevalence of some RNA viruses remain undetermined. This study was designed to identify and determine the prevalence of RNA viruses infecting cucurbits in Dera Ghazi Khan Division. Surveys were conducted to collect samples, which were then analysed using serological techniques. The overall disease incidence of RNA viruses in the study area was 84.37%, based on ELISA-positive samples. Precisely, the DI for potyviruses was 19.88%, for cucumoviruses 25.77%, for tobamoviruses 18.45%, and for poleroviruses 20.20%. The study provides a detailed description of the prevalence of different RNA viruses, which will serve as crucial information for the breeders to develop varieties resistant. Moreover, a proper management strategy against insect vectors is needed to stop the spread of these viruses.

Keywords: Cucurbits, RNA viruses, Disease Incidence, Cucurbits, Dera Ghazi Khan, South Punjab.

INTRODUCTION

The family Cucurbitaceae is vast, containing 120 genera and over 800 herbaceous plant species. These species are either perennial or annual and are well-established in tropical and temperate regions. The vegetables of the family Cucurbitaceae are commonly known as cucurbits. These crops are crucial because they are source of numerous vital nutrients and minerals like magnesium (Mg), Calcium (Ca), and iron (Fe). They also provide lipids, carbohydrates, and vitamins, i.e., vitamin A (retinol), B vitamin complex, and vitamin C (ascorbic acid) (Asad et al., 2022b). These crops are also important for medicinal purposes because they contain various compounds with unique pharmacological properties, such as laxative effects, anti-inflammatory substances, and notably, antioxidant properties (Ashfaq et al., 2021c; Grumet et al., 2021). Cucurbits are cultivated globally, and Türkiye is the top producer, followed by China, India, and America.

In Pakistan, these cucurbits are grown over an area of 20,433 hectares with 357,064 tons of annual production (Pakistan Bureau of Statistics, 2023). Within Pakistan, Punjab is the leading province in cucurbit production and contributes 82.8% of the country's total cucumber production (Planning Commission of Pakistan, 2020). The yield (12 tons/ha) is significantly lower than that of other countries due to various biotic and abiotic challenges (Asad et al., 2022a). Abiotic stresses include extremes in temperature and moisture, nutritional imbalances, and additional factors such as heavy metals and high salt concentrations in soils (Lecoq and Desbiez, 2012). Biotic challenges include pests, rodents, competing plants, and pathogens. Bacteria, fungi, nematodes, and viruses are the major pathogens involved in causing diseases in plants (Ahsan et al., 2020a; Arif et al.,

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2021; Iqbal et al., 2022; Mateen et al., 2022).

Viruses are responsible for substantial yield losses in vegetable crops, and their management is extremely hard. About 59 viruses are reported to infect cucurbits. Of these, major viruses belong to potyviruses, poleroviruses, cucumoviruses, and tobamoviruses are considered major (Lecoq and Desbiez, 2012). Potyvirus, the largest genus among RNA viruses, consists of 218 tentative and definite species. Potyviruses cause significant yield losses in various plant families, including cucurbitaceae, solanaceae, fabaceae, and alliaceae (Sharma et al., 2014; Ahmad et al., 2017; Hamza et al., 2018; Asad and Ashfaq, 2019; Asad et al., 2022a). Potyviruses are transmitted by multiple aphid species, while Myzus persicae is the most effective vector. Seed transmission is also reported in some species (Katis et al., 2006; Ohshima, 2012). Among the potyviruses, Zucchini yellow mosaic virus (ZYMV), Watermelon mosaic virus (WMV), and Papaya ringspot virus (PRSV) are posing a continuous threat to the cucurbits production (Desbiez and Lecoq, 1997; Ali et al., 2004; Trkulja et al., 2014; Ashfaq et al., 2015; Ashfaq and Ahsan, 2017; Hajizadeh et al., 2017; Ashfaq et al., 2021c; Amine et al., 2022).

Besides potyviruses, other harmful RNA viruses, such as Cucumber mosaic virus (CMV), Cucumber green mottle mosaic virus (CGMV), and Cucurbits aphid borne yellows virus (CABYV) pose significant threat to cucurbits cultivation (Ali et al., 2004; Lecoq and Desbiez, 2012). Nearly all cucurbits are susceptible to these viruses, resulting in substantial yield losses. These viruses induce symptoms on various plant parts, but symptoms on fruits can reduce quality with low market value, leading ultimately to economic losses (Sivakumaran et al., 2000; Roossinck, 2002; Knierim et al., 2010; Mochizuki and Ohki, 2012; Reingold et al., 2016; Shakeel et al., 2016, Ahsan and Ashfaq, 2018). Although plant viral infection is often confused with abiotic stresses particularly nutritional deficiencies (Kanwal et al., 2024). These viruses show specific symptoms, such as yellowing and chlorosis in cucurbits is associated with poleroviruses; leaf distortion and shoe stringing with ZYMV and CMV; green mottling with CGMMV; and ringspots with PRSV. These characteristic symptoms can serve as basic criteria for the detection of plant virus infection. However, mixed infection can limit the symptoms-based detection of viruses. Infection with these viruses not only affects the quality of produce, but yield is also compromised (Hamza et al., 2023; de Moya-Ruiz et al., 2025). Mixed viral infection makes the scenario worse, as the infected plants do not produce enough fruits, and the viruses may undergo reassortment or recombination, giving rise to new strains or species. The recombinant strains or species have enhanced virulence and often result in resistance break down (Butković and González, 2022; Sett et al., 2022; Wang et al., 2022).

An accurate and timely detection of plant diseases is necessary to avoid any epidemic situation (Rumpf et al., 2010; Oeschger et al., 2021; Ristaino et al., 2021). In Pakistan, several studies have been conducted to measure prevalence of viral diseases in the cucurbit fields of the country. In a study conducted in the Khyber Pakhtunkhwa (KPK) province of Pakistan, RNA viruses were found to be prevalent with different incidences. The most common virus was CGMMV (46.9%), and the potyviruses' combined incidence was 35.1%, with ZYMV (14.8%) being the most common (Ali et al., 2004). Researchers found co-infection of these viruses in almost all the infected plants (Ali et al., 2004). The CABYV was recently identified for the first time infecting melon fields in, Pakistan, followed by an extensive survey throughout various districts of Punjab, which indicated a recombinant isolate between Spanish and South Korean isolates (Ahsan et al., 2020b; Asad et al., 2022d). Another study identified 13 isolates from several cucurbits that were almost identical, sharing around 99.5% of their genetic material with isolates previously reported from China, Australia, USA, Greece, and France. This outcome signifies reduced diversity among the CGMMV isolates globally (Asad et al., 2022c).

In Pakistan, the RNA viruses infecting cucurbits have gained very limited attention and researchers have only focused on selected areas of the KPK and Punjab provinces of Pakistan. Although a lot of studies have been conducted in Punjab but these surveys were focused on Central Punjab, Upper Punjab and Pothowar region. While areas of Southern Punjab were neglected. Under the climate change scenario, local farmers have shifted their cropping pattern and are cultivating cucurbits in this area. These crops are facing many challenges in their production and the RNA viruses are top listed among these challenges (Hamza et al., 2023). A bunch of RNA viruses are prevalent in this area and are evolving with the passage of time, resulting in serious economic losses. To avoid losses from these viruses, periodic surveys are needed. Such periodic surveys, combined with molecular diagnosis and phylogenetic studies, provide accurate and updated knowledge regarding the viruses and their management strategies can be devised accordingly (Desbiez et al., 2011). Asad et al (2022) recommended to explore South Punjab for mapping the potential prevalence of RNA viruses in the area (Asad et al., 2022d). By keeping this in consideration, it was hypothesized that RNA viruses might be prevalent in the Dera Ghazi Khan (D. G. Khan)

Division, and the study was designed to identify the RNA viruses infecting cucurbits in D.G. Khan Division and to know their prevalence and distribution in the study area.

METHODOLOGY

During 2022 and 2023, extensive surveys were conducted in the cucurbit-producing areas of the D. G. Khan Division (Figure 1). These surveys were conducted in a random stratified design (RSD) covering all four districts within the D. G. Khan Division i.e. D. G. Khan, M. Garh, Rajanpur, and Layyah. In each Tehsil, 10 fields were surveyed, and from each field, 30 samples of cucurbit crops were collected. The samples exhibiting symptoms like mosaic, mottle, shoe stringing, dwarfing, stunted growth, deformed leaves and fruits, chlorotic leaves, ringspots, puckering, and yellowing were collected in zipper bags with proper labelling. These samples were then transported in an ice container to prevent viral RNA degradation as reported earlier (Ahsan *et al.*, 2021).

Serological Assays

Diagnosis of Viral Group Using Polyclonal Antibodies in Plate Trapped Antigen ELISA (PTA-ELISA)

PTA-ELISA was performed to confirm the presence of potyviruses in the collected samples using Potygroup test ELISA kit (Bioreba). The procedure recommended by Bioreba was used and 2087 leaf samples were crushed in extraction buffer diluted (1:50 w/v) just before starting work and the mixture was filtered using a muslin cloth. Each sample was loaded to two wells of the microtiter plate. The rest of the sap was stored at 4 °C to be used in specie specific DAS-ELISA. The controls were also diluted in extraction buffer (2.5mL). The microtiter-plate was then wrapped by a parafilm and was incubated at 4 °C for 16 hours. On the next day, the microtiter plate was washed 3-4 times by freshly prepared PBST washing buffer by mixing PBST tablet in 1 litre distilled water. The conjugate buffer was used to dilute IgG in a proportion of 1:1000 and 200 µL of diluted IgG was then poured in the wells where samples were loaded. The microtiter plates were then covered by a parafilm and were incubated for 2 hours at 37 °C. After 2 hours, the ELISA plates were washed with PBST washing buffer for 3-4 times and were dried by smashing the plates while inverted on a sterilized paper The alkaline phosphatase anti mouse IgG was diluted in conjugate buffer at 1:1000 and was then 200 µL was added in each well. These ELISA plates were covered again with the parafilm and were incubated for 2 hours at 37 °C. Then these plates were washed again with PBST for 3-4 times to remove the extra anti mouse IgG. One pNPP tablet was added to 20mL conjugate buffer to get a solution of 1mg/ mL concentration. Then each well was dispensed with 200 µL of this solution and was incubated in dark for half an hour at room temperature (Ashfaq *et al.*, 2021b). The results were then observed with naked eye and were then viewed at 405nm in ELISA plate reader (Sharma *et al.*, 2014; Nascimento *et al.*, 2017; Ashfaq *et al.*, 2021c).

Virus Specific Double Antibody Sandwich ELISA (DAS-ELISA)

The diluted (1:1000) IgG solution in the coating buffer was dispensed at the rate of 200 µL in each well of the ELISA plates. These microtiter plates were wrapped with parafilm followed by an incubation for four hours at 30 °C. The microtiter plates were then washed using PBST washing buffer for 3-4 times. The controls and samples processed during PTA-ELISA, stored at 4 °C, were used. The controls and the sap solutions were pipetted inside the wells @ 200 µL per well and were incubated overnight at 4 °C. The microtiter plates were washed by PBST washing buffer 3-4 times in the next morning. IgG was diluted in conjugate buffer @ 1:1000 and 200 µL was pipetted inside each well of microtiter plate which was wrapped and incubated for 5 hours at 30 °C followed by washing for 3-4 times with PBST washing buffer. pNPP solution with concentration 1mg/ml was prepared by the same method as followed in PTA-ELISA and in each well 200 µL was dispensed. The plates were then incubated in the dark at room temperature for half an hour. The microtiter plates were then observed at 405nm in an ELISA Reader (HER-480 HT Company (Illford) Ltd. UK) (Riaz *et al.*, 2021). The absorbance value of the samples was compared to that of control. The samples whose absorbance value was double than the negative control were considered positive (Abd El-Aziz, 2019; Asad and Ashfaq, 2019).

RESULTS

Extensive surveys were conducted in all four districts of D. G. Khan division including D. G. Khan, Rajanpur, Layyah, and M. Garh (Figure 1). A total of 2,087 leaf samples were collected from infected fields across all four districts during flowering and fruiting stages. The samples exhibiting viral or virus-like symptoms, including yellowing, blistering, mosaic, mottling, shoe stringing, malformation, dwarfing, leaf thickening, curling, and vein thickening were collected. Some symptoms were classified as miscellaneous because they overlapped and were likely due to mixed infections. Nevertheless, yellowing was noted in cases of polerovirus infections, shoe stringing was prevalent in CMV infections, and blistering was commonly observed in plants infected with ZYMV (Figure 2). Out of the 2,087 samples collected during 2022-2023 from cucurbit fields in the D. G. Khan Division, 1,758

(63.58%) tested positive in serological assays. The serological assays yielded positive results for 1758 samples using genus-specific PTA-ELISA for potyviruses and species-specific DAS-ELISA (Table 1). Of these 1,758 positive samples, potyviruses were identified in 415 samples via PTA-ELISA, of which 361 samples tested positive in DAS-ELISA for ZYMV, 3 for PRSV, and 51 for WMV. Additionally, 537 samples were positive in DAS-ELISA for CMV, while 385 and 421 samples tested positive for CGMMV and CABYV, respectively (Table 2). Although RNA viruses were detected in cucurbits across all surveyed districts, their incidence varied among different localities.

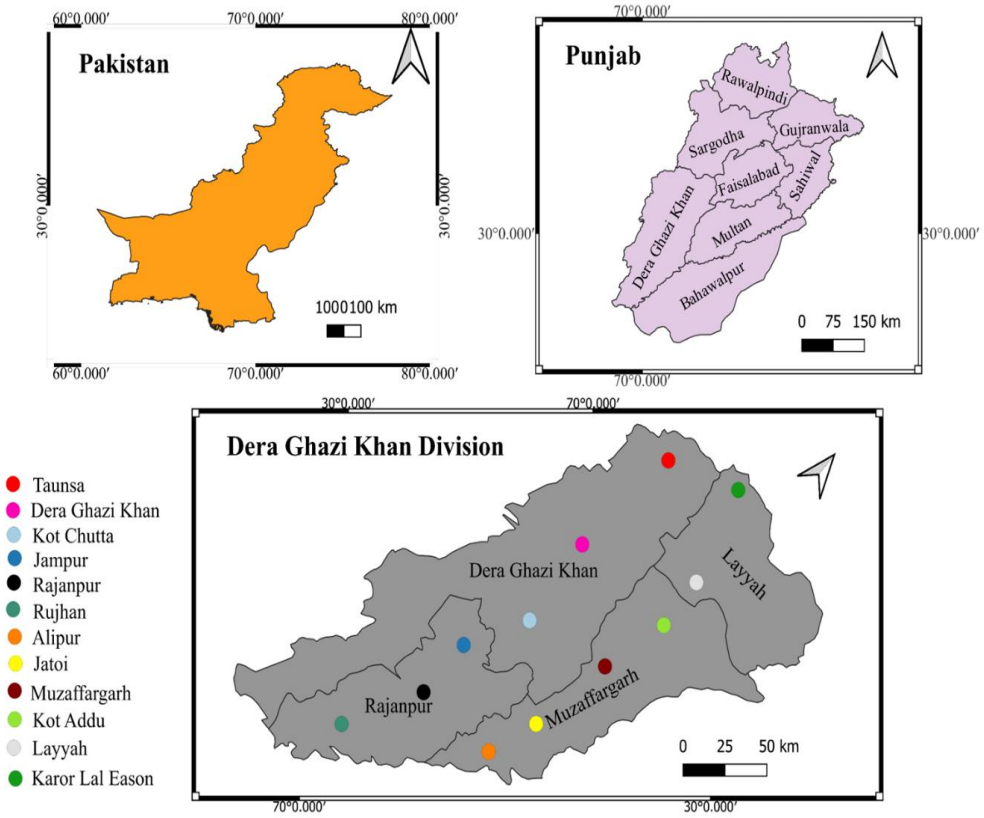

Figure 1. Map of the D. G. Khan Division

Table 1. Disease incidence of RNA viruses in D. G. Khan Division during 2022-2023

Year	Total	Infected	Potygroup	ZYMV	PRSV	WMV	CMV	CGMMV	CABYV
2022	1020	852	214	176	1	37	245	192	201
2023	1067	906	201	185	2	14	292	193	220
Total	2087	1758	415	361	3	51	537	385	421
Disease Incidence		84.23%	19.89%	17.29%	0.14%	2.44%	25.73%	18.45%	20.17%

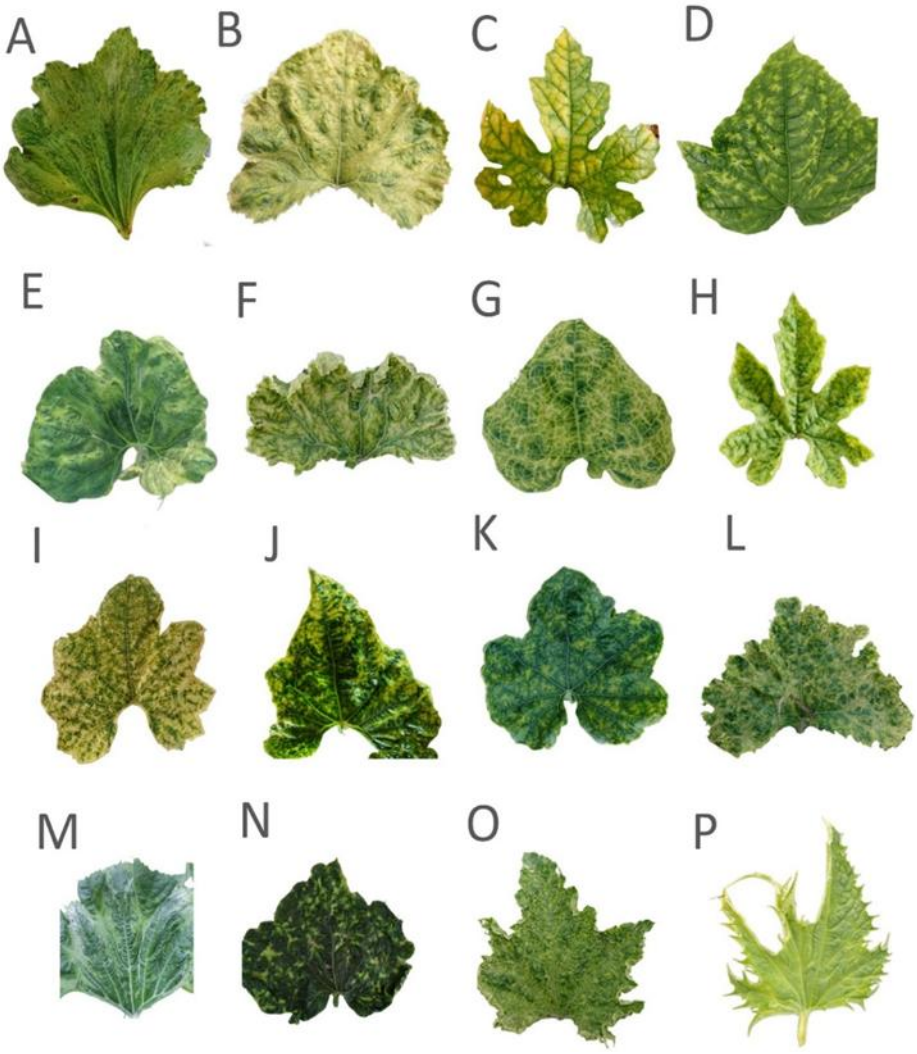

Figure 2. Symptoms of RNA viruses on cucurbit crops

A: Round gourd leaf shows distinct green mosaic and mottling caused by CGMMV; B: Pumpkin leaf with notable yellowing due to CABYV; C: Bitter gourd leaf displaying mosaic symptoms resulting from CMV infection. D and N: Cucumber leaves demonstrating CGMMV symptoms; E: Mild mosaic on round gourd leaves by WMV infection. F and I: Yellowing of ridge gourd leaves due to CABYV infection; G- mosaic symptoms on Melon leaf due to CMV infection; H:- Chlorosis in bitter gourd leaves infected with CABYV; J- Deformed leaves with mosaic symptoms due to ZYMV infection; K: Mosaic on ridge gourd leaves due to CMV infection; L:- Interveinal chlorosis due to CABYV infection in round gourd; M- Characteristic green mottling of bottle gourd leaves due to CGMMV infection; O:- cucumber leaf exhibiting mosaic and shoe stringing; P: Ridge gourd leaf displaying shoe stringing symptoms due to ZYMV infection

Incidence of RNA viruses in different Districts

Although all the viruses in this study were present throughout the area but their distribution was variable

Incidence of Potyviruses

A total of 2087 samples were examined throughout the 2022–2023 period to collect samples displaying viral symptoms, and 415 samples were detected positive using PTA-ELISA. In 2022 and 2023, 214 and 201 samples out of 1020 and 1067 samples were reacted positive to genus specific ELISA, respectively. The positive samples were further analyzed using DAS-ELISA to detect ZYMV, PRSV, and WMV. Out of total 415 positive samples, ZYMV was found in 361 samples (176 samples in 2022, while 185 samples in 2023). On the other hand, three samples were found infected with PRSV in both years and WMV was found in 51 samples (37 samples were found infected in 2022 and 14 samples in 2023). The overall disease

incidence was 19.89%, with the highest incidence of potyviruses was found in the D. G. Khan district at 21.83%, followed by Rajanpur with 20.47%, Layyah with 19.50%, and the lowest rate i.e. 18.08% in the M. Garh district (Table 2 and Figure 3). In the D. G. Khan district, the highest D.I. was observed in Tehsil Taunsa (22.22%), followed by Tehsil D.G. Khan (21.98%), while the lowest incidence was recorded in Tehsil Kot Chutta (21.35%). In District Rajanpur, the highest incidence of potyvirus was detected in Tehsil Rujhan (28.57%), followed by Tehsil Rajanpur (18.28%), with the lowest incidence recorded in Jampur Tehsil (17.39%). Tehsil Layyah of District Layyah exhibited the highest disease incidence at 20.44%, while Tehsil Karor Lal Eisan had the lowest incidence of 18.58%. In M. Garh District, the highest prevalence of disease was observed in Tehsil Alipur (22.28%), followed by Tehsil Jatoi (19.38%) and Tehsil M. Garh (16.43%), while the lowest incidence was observed in Tehsil Kot Adu (13.92%) (Table 3).

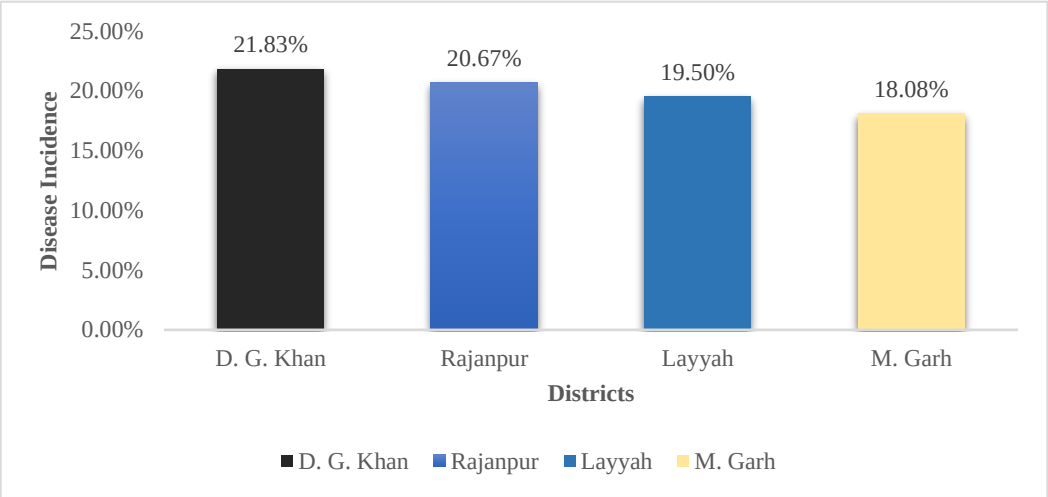

Figure 3. Overall disease incidence of potyviruses in Dera Ghazi Khan Division

Table 2. Disease incidence of potyviruses in Dera Ghazi Khan Division

District	Tehsil	Location	Total Samples Tested	ZYMV	PRSV	WMV	D.I. Tehsil	D.I. District
D. G. Khan	D. G. Khan	Kala	64	11	0	3	21.97802	21.83908
		Ghazi Ghat	55	9	0	1		
		Vador	63	11	0	5		
	Taunsa	Taunsa	42	11	0	2		
		Ratra	62	11	0	4		
		Hairo	58	7	0	1	22.22222	
	Kot chutta	Jhok	58	7	0	3		
		Kot chutta	58	11	1	2		
		Khan pur	62	11	0	3	21.34831	
Rajanpur	Rajanpur	Rajanpur	45	7	0	2		20.47244
		Fazilpur	72	9	0	2		
		Kot Mithan	58	10	0	2	18.28571	
	Jampur	Jampur	71	5	0	1		
		Muhammad Pur	72	12	2	2		
		Kotla Mughlan	64	12	0	2	17.3913	

	Rujhan	Umarkot	28	12	0	1		
		Rujhan	25	8	0	2		
		Uzman	73	12	0	1	28.57143	
M.Garh	Jatoi	Jatoi	70	12	0	0		18.18182
		Bet Meer Hazar	62	13	0	0		
		Shehr Sultan	64	13	0	0	19.38776	
	Alipur	Alipur	70	18	0	1		
		Seetpur	60	12	0	2		
		Khairpur Sadat	45	6	0	0	22.28571	
	Kot Addu	Kot Addu	48	5	0	2		
		Sinawan	50	4	0	1		
		Qasba Gujjrat	60	7	0	3	13.92405	
	M. Garh	Chok qureshi	60	11	0	0		
		Khan Garh	52	6	0	0		
		Shah jamal	52	10	0	0	16.46341	
Layyah	Layyah	Lala Zaar	54	8	0	0		19.50549
		Kot Sultan	59	12	0	1		
		Layyah	68	16	0	0	20.44199	
	Karor Lal Eisan	Karor lal eisan	71	12	0	1		
		Chak 94 TDA	62	10	0	1		
		Fateh Pur	50	10	0	0	18.57923	
		Total	2087	361	3	51	415	
		D.I.		17.29	0.14	2.44	19.885	

2.3.1.2. Incidence of CGMMV

The DAS-ELISA test for CGMMV identified 385 infected samples resulting in an overall disease incidence of 18.45%. In 2022-23, the highest disease incidence i.e. 22.80% was observed in D. G. Khan followed by M. Garh, Layyah, and Rajanpur with 19.5%, 18.13%, and 12.99%, respectively (Figure 4). In the D. G. Khan district, the highest incidence of CGMMV was recorded in tehsil Taunsa (24.69%), followed by Kot Chutta and D. G. Khan tehsils, with 23.59% and 20.00% incidences, respectively. In the M. Garh district, the highest CGMMV incidence of 25.61% was observed in the M. Garh tehsil, followed by Kot Addu with 18.98%, Alipur with 17.14%, and Jatoi with 16.33%. In the Layyah district, the highest incidence of CGMMV was observed in tehsil Karor Lal Eisan at 18.58%, while the lowest incidence i.e. 17.68% was recorded in tehsil Layyah. In the Rajanpur district, the highest incidence of disease was recorded in tehsil Rajanpur (17.14%), followed by Rujhan (11.11%) and Jampur (10.62%) (Table 3).

Incidence of CABYV

To estimate the prevalence of CABYV, DAS-ELISA was used, and 421 samples out of 2087 were found positive, making an overall disease incidence of 20.17% during 2022-23. CABYV was most frequently found in the samples from the D. G. Khan district and had DI of 21.65%, followed by M. Garh, Rajanpur, and Layyah with incidences of 20.20%, 19.48%, and 18.96%, respectively (Table 3).

Within the D.G, Khan district, the maximum disease incidence was found in tehsil Taunsa (24.69%), followed by tehsil D. G. Khan (21.43%) and tehsil Kot Chutta (19.10%). In the Muzaffargarh district, the highest disease incidence was observed in tehsil Kot Addu (24.05%), followed by M. Garh (20.12%), Jatoi (18.88%), and Alipur (18.28%). In district Rajanpur, highest incidence of CABYV was observed in tehsil Rajanpur (21.71%), followed by Jampur (18.35%) and

Rujhan (18.25%). The highest disease incidence in Layyah was observed in Karor Lal Eisan at 19.12%, while the lowest was in Layyah tehsil at 18.78% (Table 3).

Incidence of CMV

Out of the total samples tested, 537 reacted positive to *Cucumber mosaic virus*, as determined by DAS-ELISA. The overall disease incidence of CMV was 25.73%, with the maximum disease incidence in Rajanpur district i.e. 30.90%, followed by D. G. Khan, M. Garh and Layyah with disease incidences of 28.35%, 25.39%, 24.72% respectively (Figure 4). Within the district Rajanpur, tehsil Rujhan had the highest incidence of 34.92%, followed by tehsil Rajanpur with 30.85% and Jampur with 28.50%. In the D. G. Khan district, the highest D.I. of 29.21% was recorded in tehsil Kot Chutta, followed by Taunsa and D.G. Khan tehsils, with D.I. of 29.01% and 26.92%, respectively. In the M. Garh district, the highest incidence of CMV was noted in tehsil Alipur at 27.42%, followed by tehsils Jatoi, Kot Addu, and M. Garh, with recorded CMV incidences of 26.53%, 24.05%, and 23.17%, respectively. In the Layyah district, the highest D.I. was observed in Karor Lal Eisan with 25.14%, followed by tehsil Layyah with 24.31% (Table 3).

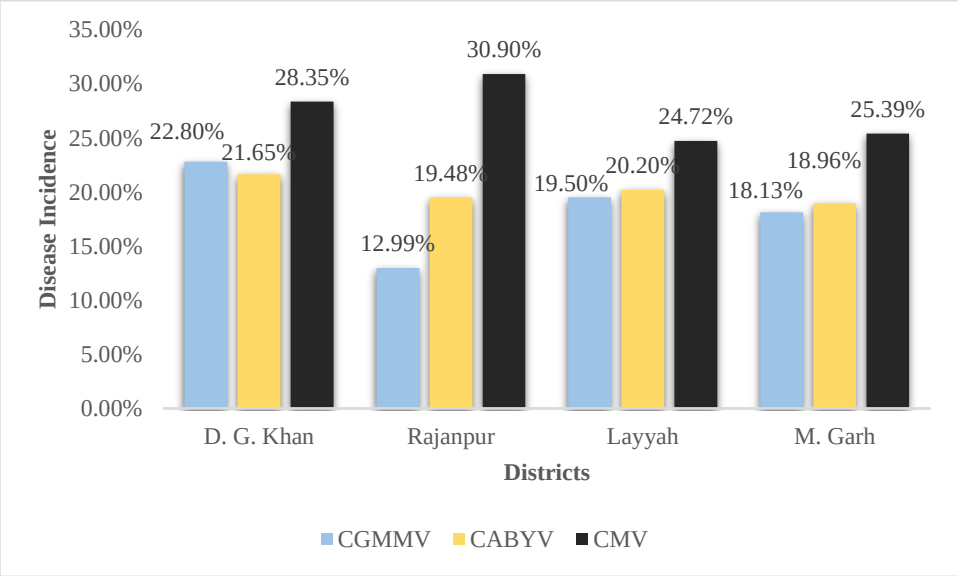

Figure 4. Disease incidence of CGMMV, CABYV and CMV in study area

Table 3. Disease incidence of CGMMV, CABYV and CMV in Dera Ghazi Khan Division

District	Tehsil	Location	Total Samples Tested	CGMMV	CABYV	CMV
D. G. Khan	D.G Khan	Kala	64	15	13	18
		Ghazi Ghat	55	11	10	16
		Vador	63	11	16	15
	Taunsa	Taunsa	42	13	16	15
		Ratra	62	10	10	15
		Hairo	58	17	14	17
	Kot Chutta	Jhok	58	13	10	16
		Kot chutta	58	13	10	20
		Khan pur	62	16	14	16
Rajanpur	Rajanpur	Rajanpur	45	10	16	17
		Fazilpur	72	12	10	22
		Kot Mithan	58	8	12	15
	Jampur	Jampur	71	10	16	22

		Muhammad Pur	72	8	12	20
		Kotla Mughlan	64	4	10	17
	Rujhan	Umarkot	28	5	8	10
		Rujhan	25	3	3	10
		Uzman	73	6	12	24
M. Garh	Jatoi	Jatoi	70	10	14	18
		Bet Meer	62	14	10	18
		Hazar Shehr Sultan	64	8	13	16
	Alipur	Alipur	70	12	10	18
		Seetpur	60	12	12	16
		Khairpur Sadat	45	6	10	14
	Kot Addu	Kot Addu	48	12	10	14
		Sinawan	50	8	12	12
		Qasba Gujjrat	60	10	16	12
	M. Garh	Chok qureshi	60	17	12	14
		Khan Garh	52	11	11	12
		Shah jamal	52	14	10	12
Layyah	Layyah	Lala Zaar	54	9	7	14
		Kot Sultan	59	12	11	15
		Layyah	68	11	16	15
	Karor Lal Eisan	Karor lal eisan	71	10	10	18
		Chak 94 TDA	62	13	14	13
		Fateh Pur	50	11	11	15
		Total	2087	385	421	537
		DI		18.44753	20.1725	25.73071

DISCUSSION

Pakistan is a major cucurbit producing country in Asia, with 357,064 tons of production. Cucurbits are cultivated over an area of 20,433 ha in Pakistan (Pakistan Bureau of Statistics, 2023). However, cucurbits production faces many calamities, including abiotic stresses and biotic factors. Among the biotic factors, plant viruses are continuous threat to various vegetables and a major hindrance in their production around the globe (Amari *et al.*, 2017; Moriones *et al.*, 2017). Several studies from Pakistan have already reported high incidences of different plant viruses. These viruses infect every single cucurbit crop and cause a variety of symptoms based on the host and the viral species as well as the strain of a particular virus (Mandal *et al.*, 2008).

A variety of symptoms were observed during the survey, and serological and molecular analyses confirmed that different viruses were responsible for these varying symptoms. The observed symptoms, such as mosaic, mottling, malformation, dwarfing, leaf thickening, curling, and vein thickening, were common in plants infected with RNA viruses. However, complete yellowing was noted in plants infected by CABYV, while green mosaic was prominent in plants infected by CGMMV. Shoe stringing was frequently observed in CMV-infected plants, and blistering occurred often in plants infected by ZYMV. Our study also observed these symptoms

associated with different viruses in the surveyed area of D.G. Khan division and these findings are consistent with previous research (Reingold *et al.*, 2016; Constable *et al.*, 2018; Ahsan *et al.*, 2023).

This study has detected prevalence of RNA viruses in the cucurbit fields of Southern Punjab. Potyvirus is the largest genus of plant RNA viruses, and they infect all crops, including legumes, cereals, fodder, fruit, and vegetable crops. Three plant virus species, viz., ZYMV, PRSV, and WMV, are already reported in Pakistan. Previous studies have reported a higher incidence of ZYMV as compared to PRSV and WMV. In a recent study, the overall D.I. of ZYMV, PRSV, and WMV was calculated as 37.52%, 2.3%, and 0.38%, respectively. Multiple studies align with our findings about the relative disease incidences and prevalence of the potyviruses (Asad and Ashfaq, 2019; Ashfaq *et al.*, 2021a; c) In another study, the recorded incidence of WMV was higher than PRSV, which contradicts our findings. However, ZYMV was still the most prevalent virus, as per their report, which aligns with our study (Asad *et al.*, 2022a).

In the case of CGMMV-infected cucumber plants, the characteristic mosaic and mottling symptoms was observed on the leaves as well as on the fruits, and gradually plant collapsed. Mottling and mosaic symptoms were visible on every cucurbit; however, in the case of round gourd, only green-coloured mottling was visible. All the symptoms observed during the study were similar to the symptoms reported in the previous studies (Reingold *et al.*, 2013; Dombrovsky *et al.*, 2017; Asad *et al.*, 2022c). It was also noted that the growers do not cultivate resistant varieties and are unaware of the seed-borne nature of viruses; therefore, they end up buying seeds from the local market, which is an alarming factor, as CGMMV, being a *Tobamovirus*, spreads with seeds. This causes the growth of an infected crop right from the germination of the seed.

CABYV is a *Polerovirus* and is transmitted in a persistent manner by the aphid species, *Myzus persicae*. The virus is reported to have a great impact on the feeding behaviour of the vector, as the non-viruliferous aphids are attracted to the infected plants due to their visual appearance. After acquisition, the vector becomes irritated and tends to get rid of the virus; hence, it is attracted by the healthy plant, and as a result, it transmits the disease to the healthy plant. This interaction changes the overall feeding behaviour of the insect. The symptoms of CABYV were confused with the nutritional imbalance, as there was interveinal yellowing of leaves while the veins were green, as observed in the case of magnesium deficiency. The symptoms observed during the present study are in accordance with the previously reported symptoms, and the incidence of the virus was not much higher, as it remained under 30% (Shang *et al.*, 2009; Asad *et al.*, 2022d).

We carried out the study during the early fruiting season, when the market rate of the cucurbits was skyrocketing, while the contrasting studies were carried out throughout the growing season and an average incidence was calculated. The symptoms observed during the study ranged from mild mosaic to severe mosaic on leaves and fruits, with mottling on some plants. The infected plants were stunted and borne less fruit. All of the symptoms observed during this study are in accordance with the symptoms reported in previous studies (Iqbal *et al.*, 2011, 2017; Asad *et al.*, 2022d; Ahsan *et al.*, 2023). The disease incidence was observed between 25% and 30% in various areas, depending on the climate of the locality, variety, and crop age. The incidence was much greater on older plants compared to the young ones. The previous studies (Radouane *et al.*, 2021) support our findings. Interestingly, all the studies mentioned above recorded similar patterns of increasing disease incidence in the successive years and hence support our findings.

CONCLUSIONS

Cucurbits are crucial due to their medicinal and culinary uses, and they serve as a source of income for small and large-scale farmers. These crops are experiencing significant losses due to prevalent viral diseases in Pakistan. This study revealed that RNA viruses are present with varying incidences. The combined incidence, exceeding 80%, is alarming and requires mitigation. Therefore, a comprehensive study is needed related to genome-based identification of viruses prevalent in this area. Additionally, designing effective management strategies against these viruses is need of the hour to avoid epidemics, and a proper initiative for breeding resistant varieties is needed to prevent yield losses.

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