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'Prevalence of Bitter Gourd Mosaic Complex (BGMC) and Detection of Associated Viruses in Different Agro-ecological Units of Kerala

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Authors' contributions

This work was carried out in collaboration among all authors. Author DRC carried out the experiments and wrote the manuscript. Author JMJ conceived and supervised the experiments, and wrote the manuscript. Authors RNS, HG, SKB, SS and NVR provided critical comments. All authors read and approved the final manuscript.

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ABSTRACT

Bitter gourd mosaic complex (BGMC) is caused by multiple viruses in bitter gourd which seriously affects the crop performance and leads to complete crop loss. Bitter gourd is widely cultivated in Kerala as the market price is steady throughout the year. BGMC is the most important production

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constraint as most of the high yielding varieties including hybrids are severely affected by the virus complex. The study assessed the prevalence of BGMC in major bitter gourd cultivating agro-ecological units (AEUs) of Kerala and detected the viruses associated. It was found that, the disease incidence (DI) and severity in terms of Vulnerability Index (VI) of BGMC varied between AEUs as well as varieties grown. The average incidence and severity of BGMC were found to be highest i.e., 98.71 per cent and 70.2 respectively in AEU 8 with Pallichal of Thiruvananthapuram district being the highest among all locations with 100 per cent DI and 80.50 VI. Among varieties, Preethi was found to be the most susceptible with an average BGMC incidence of 99.04 per cent and severity of 65.01 per cent, whereas hybrids and local varieties were relatively tolerant. *Tomato leaf curl virus* (ToLCV), *Papaya ring spot virus* (PRSV) and *Cucumber mosaic virus* (CMV) were the major viruses associated with BGMC as these viruses were detected in all AEUs surveyed. Among the viruses, PRSV was found to be the most prevalent in all the AEUs and locations surveyed across Kerala (77.78 %). The viruses were detected singly as well as in combinations. Percentage of single virus infection (61.11 %) was more, followed by double virus infection (33.33 %) and triple virus infections (5.56 %). Combined infection of the viruses led to total crop failure without any flowering. Findings of the present study contribute to better understanding of prevalence of BGMC and offer information on its diversity among regions, varieties and viruses associated across different AEUs of Kerala.

Keywords: Bitter gourd; bitter gourd mosaic complex; viral disease; Tomato leaf curl virus; Papaya ring spot virus; Cucumber mosaic virus; prevalence; AEUs; detection.

1. INTRODUCTION

Bitter gourd cultivation in Kerala is facing a steep drop after the great Kerala Flood in 2018. In addition to the climate change, the major constraint in the cultivation of the crop is the increasing fungal, bacterial and viral diseases; of which the most challenging being the viral diseases. It was observed that the occurrence of multiple viruses with multiple transmission strategies, paved way to extensive occurrence of bitter gourd mosaic complex disease [1]. The multiple viral diseases resulting in BGMC drastically reduced the yield in bitter gourd and its cultivation became a loss to farmers. Furthermore, many of the hybrids also lost resistance against viruses. Majority of farmers abandoned the cultivation and shifted to other crops.

A number of viruses have been identified as the major cause of BGMC globally and some important viruses are from the genera *Potyvirus, Begomovirus, Cucumovirus, Tymovirus, Nepovirus, Tobamovirus, and Polerovirus* [2,3,4,5,6,7]. Globally potyviruses like *Zucchini yellow mosaic virus* (ZYMV) and *Watermelon mosaic virus* (WMV) have also been reported to infect cucurbits, including bitter gourd, and cause symptoms like mosaic patterns on leaves, stunted growth and fruit deformities [8,9]. *Cucumber mosaic virus* (CMV) was also reported to be the most prevalent virus inflicting substantial damage to cucurbitaceous plants worldwide. They produce symptoms like mosaic patterns on leaves, leaf distortion and stunted growth [5,10,11].

Similarly, viruses like CMV [12], PRSV-W, *Indian cassava mosaic virus*, *Bitter gourd yellow mosaic virus* [13], *Pepper leaf curl Bangladesh virus* [14], *Tomato leaf curl New Delhi virus* [15], *Cucumber green mottle mosaic virus* and *Zucchini yellow mosaic virus* [16] have been reported from different parts of India. In India, CMV infection of bitter gourd was reported for the first time from Coimbatore, Tamil Nadu [12]. The association of *Bitter gourd distortion mosaic virus* was first reported from Kerala [17]. The infection of *Papaya ring spot virus* was also reported with variable symptoms like vein clearing, blistering and filimorphism in bitter gourd [18]. Interestingly, the association of *Indian cassava mosaic virus* (*begomovirus*) with yellow mosaic disease of bitter gourd has been reported from Tamil Nadu, South India [19]. In Kerala, the major viruses reported to cause bitter gourd mosaic complex disease included viruses in the family *Potyvirus*, *Begomovirus* and *Cucumber mosaic virus* [1].

Mixed infections of two or three of these viruses were observed in more than 50 per cent of the samples analyzed by Nagendran et al. [16] while studying the occurrence and distribution of major viruses infecting cucurbits in Tamil Nadu. Mixed infection of CMV, PRSV and ToLCV resulted in disease incidence as high as 100 per cent and severity in terms of VI of 82 in Kerala [1]. As per

the findings of Naik et al. [20], the symptoms of mixed virus infections are complex resulting in severe damage. The present study was conducted to assess the prevalence of bitter gourd mosaic complex in major bitter gourd cultivating AEUs of Kerala, detect the major viruses associated with the disease complex, and assess and characterize the symptoms produced by the viruses.

2. MATERIALS AND METHODS

2.1 Assessment of Disease Incidence and Severity

Roving survey was conducted during summer (Feb – May, 2021) in 18 locations across six major bitter gourd cultivating AEUs of Kerala. Three locations were selected from each AEU which are geographically separated *viz.,* AEU 1 - Southern coastal plain (Ayyappancheri, Kuthiathode and Kanjikkuzhy of Alappuzha district), AEU 6 - Kole land (Vellanikkara, Cherpu and Anthikkad of Thrissur district), AEU 8 - Southern laterite (Vellayani, Pallichal and Kalliyoor of Thiruvananthapuram district), AEU 10 - North Central laterite (Vaniyamkulam, Pattambi and Thrithala of Palakkad district), AEU 11 - Northern laterite (Kanhangad, Periya and Cheruvathur of Kasaragod district); and AEU 12 - South and Central foot hills (Alakode, Kodikulam and Keerikode of Idukki district).

Fields covering a minimum area of 25 cents were chosen for the study. The disease incidence was assessed by randomly selecting 50 bitter gourd plants from the field and number of plants showing the symptoms of mosaic complex disease was recorded.

$$Disease\ incidence\ (DI) = \frac{Number\ of\ plants\ infected}{Total\ number\ of\ plants} \times 100$$

Whereas, the disease severity in terms of Vulnerability Index (VI) was estimated as per the score chart prepared for bitter gourd mosaic complex disease after Bos [21]. The calculations were made in accordance with the scale of 0 to 6 (Plate 1.) as described below,

0 - no symptom
1 - yellow spots in green leaves of normal size
2 - mottling of leaves with light and dark green colour
3 - yellowing of leaves with vein banding
4 - blisters and puckering on leaves
5 - distortion of leaves, reduction in leaf size, papery leaves
6 - stunting, rosetting, hairiness, no fruits/malformed fruits

Based on the above-mentioned scale, VI was calculated using the following formula:

$$Vulnerability\ Index\ (VI) = \frac{0n_0+1n_1+2n_2+3n_3+4n_4+5n_5+6n_6}{n_1(n_c-1)} \times 100$$

$n_0, n_1 \ldots n_6$ - number of plants in category of 0, 1, 2, 3, 4, 5 and 6
n_t - total number of plants
n_c - number of categories

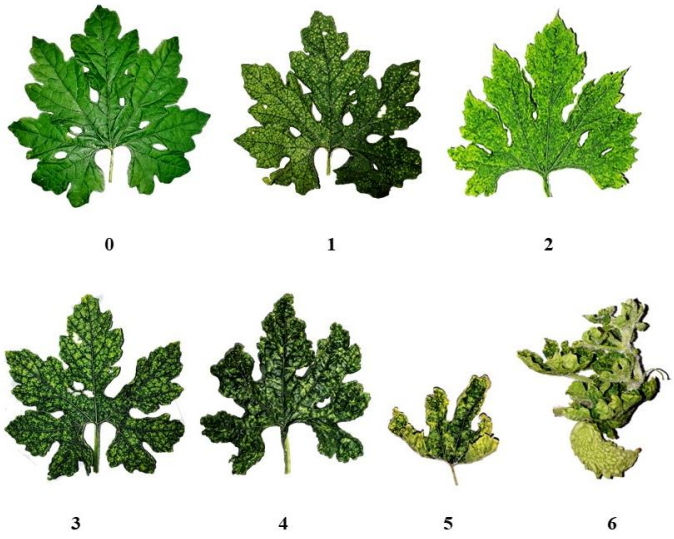

Plate 1. Score chart developed for bitter gourd mosaic complex with score 0 – 6

2.2 Assessment of Symptoms Produced by Bitter Gourd Mosaic Complex

The nature and type of symptoms produced by the viral disease in different locations were thoroughly investigated and documented. General as well as specific symptoms of mosaic complex in bitter gourd plants were recorded during the roving survey. The prevalence of different symptoms in respective locations was also recorded.

2.3 Detection of Viruses in Bitter Gourd Mosaic Complex

The diseased specimens were collected from the plants showing symptoms of viral diseases through diagonal survey of field. A total of 18 samples were collected from six AEUs (three locations each). For every sample, three biological replications were maintained. The samples were tested for the presence of PRSV, ToLCV and CMV using immunological (DAS-ELISA and DIBA) and molecular methods.

Double antibody sandwich ELISA (DAS-ELISA): The leaf samples were ground extracted using extraction buffer (1 g sample in 10 ml buffer) and centrifuged at 12000 rpm for 2 min at 4ºC. The supernatant was used as aliquot. The samples were then tested for the presence of PRSV, ToLCV and CMV using virus-specific polyclonal antibodies (DSMZ, Germany) as per the protocols described by Clark and Adams [22]. The results were assessed by spectrophotometric measurement of absorbance (optical density at 405 nm) using Automatic ELISA Reader (HER - 480HT Company (Ilford) Ltd, UK). Samples were considered positive for the respective virus(es) when the ELISA absorbance value was two times higher than the average of the absorbance value of the healthy sample [23].

Dot-immunobinding assay (DIBA): In this technique, the extracts of the diseased as well as healthy bitter gourd leaf samples were spotted directly onto nitrocellulose membrane (NCM). NCM strips of 5 cm x 1 cm were cut and 5 square blocks each of one cm^2 area were demarcated. The strips were soaked in tris buffer saline (TBS) and air dried. Then, 1 g of leaf sample was extracted using 10 ml of antigen extraction buffer and centrifuged at 12000 g for 2 min. 200 µl of the supernatant was mixed with 800 µl of antigen extraction buffer in 2 ml centrifuge tube and vortexed. 10 µl of this mix was spot blot onto respective blocks on the NCM strip. First block was blotted with healthy leaf extract; second, third and fourth block with three different diseased leaf samples from the same AEU and last block with buffer/blank. The NCM was allowed to air dry and soaked in blocking solution (TBS-SDM) for 1 h with moderate oscillation at room temperature. After incubation, the strip was rinsed in TBS for 10 min. The strip was then immersed in primary antibody diluted in TBS-SDM and incubated for 1 h at room temperature. The strip was rinsed thrice in TBS for 10 min each after the incubation. The strip was then incubated in secondary antibody diluted in TBS-SDM for 1 h at room temperature. After incubation, the rinsing was repeated as above. Finally, the strip was incubated in substrate solution and kept in dark at room temperature for 5 to 10 min. The intensity of the colour developed was measured using the gel documentation system. The buffers used in the experiment were prepared as per the protocol of Bhat and Rao [24].

Detection of DNA viruses: 100 mg of leaf samples were used for total DNA extraction using the DNeasy® Plant Mini extraction kit (Cat. No. 69104, Qiagen Inc., Germany) as per the manufacturer's Quick-Start protocol. The DNA was finally eluted in 50 µl of DNase-free water and stored at -20°C in deep freezer. The yield and quality of the extracted DNA was assayed using UV/Vis spectrophotometer (BioSpectrophotometer® Basic, Eppendorf AG, Germany) and also confirmed using gel electrophoresis in 1 per cent agarose gel in 1X TAE buffer (Tris-acetate-EDTA buffer). DNA extracted was subjected to PCR amplification to detect the presence of *Begomovirus* using primer pairs *viz.* Deng 540F/541R and AV-494F/ AC-1048R primers (Table 1). The PCR was performed in 25 µl reaction mix consisting of 12.5 µl master mix (Takara EmeraldAmp® GT PCR master mix, Cat. No. RR310A, Japan), 2.5 µl each of forward and reverse primers, 3 µl of template DNA (0.5 µg) and 4.5 µl double distilled water. The PCR protocol was set with an initial cycle of denaturation at 94°C for 4 min followed by 30 cycles of, denaturation at 94°C for 1 min, annealing for 1 min at 50°C for Deng primers and 55°C for AV/AC primers, and extension for 72°C for 1 min; and a final extension for 72°C for 10 min. 5 µl each of the PCR products were resolved on 1.2 per cent agarose gel electrophoresis after staining the gel with ethidium bromide @ 2 µl per 50 ml of gel (0.5 µg/ml). After electrophoresis, the gel was documented (Gel Doc ™ XR+, BIO-RAD, USA).

Table 1. Details of primers used in the study

Virus	Primer name	Sequences	Size (bp)	Reference
Begomovirus	Deng 541 F	5' TAATATTACCKGWKGVCCSC 3'	520	[25]
	Deng 540 R	5' TGGACYTTRCAWGGBCCTTCACA 3'		
	AV 494 F	5' GCCHATRTAYAGRAAGCCMAGRAT 3'	575	[26]
	AC 1048 R	5' GGRTTDGARGCATGHGTACANGCC		
PRSV (*Potyvirus*)	GK PRSV F	5' GCAATGATAGARTC ATGGGG 3'	1267	[16]
	GK PRSV R	5' AAGCGGTGGCGCAGCCACACT 3'		
CMV	GK CMV F	5' GAGTTCTTCCGCGTCCCGCT 3'	1218	[16]
	GK CMV R	5' AAACCTAGGAGATGGTTTCA 3'		

Detection of RNA viruses: Total RNA was extracted from the diseased leaf samples using the RPD Trio TM Reagent (Himedia, Cat No. MB566, India), as per the manufacturer's protocol. The yield and quality of the total isolated RNAs were assayed using UV/Vis spectro-photometer (BioSpectrophotometer® Basic, Eppendorf AG, Germany) and confirmed using gel electrophoresis in 1 per cent agarose gel in RNase free-1X TAE buffer. In this study, two step RT-PCR was performed. The cDNA library synthesis from extracted total RNA was carried out in 20 µl mixture using Verso cDNA synthesis kit (Cat No. AB-1453A, Thermo Fisher Scientific Inc, USA) in accordance with the manufacturer's protocol. PCR amplifications were performed using specific primers (Table 1) for detecting RNA viruses. The RT-PCR for the detection of PRSV as well as CMV was carried out in 25 µl mixture containing 12.5 µl master mix, 0.25 µl each of forward and reverse primers, 2.5 µl of template cDNA and 9.5 µl double distilled water. The coat protein gene of PRSV was amplified using the conditions: 4 min at 94°C; 30 cycles of 1 min at 94°C, 1 min at 52°C, and 1 min at 72°C; with a final elongation at 72 °C for 7 min. The coat protein gene of CMV was amplified by performing PCR run at 94°C for 2 min followed by 30 cycles of 1 min at 94°C, 1 min at 54°C, and 1 min at 72°C; with a final elongation at 72°C for 7 min. The PCR products (5 µl) were resolved on 1.2 per cent agarose gel in 1X TAE buffer. Gels were stained with ethidium bromide @ 2 µl per 50 ml of gel (0.5 µg/ml) and visualized both in UV transilluminator and gel documentation system (Gel Doc ™ XR+, BIO-RAD, USA).

3. RESULTS AND DISCUSSION

3.1 Disease Incidence and Severity Varied in Agro-Ecological Units and Varieties

All the fields surveyed in different AEUs reported occurrence of BGMC with varied incidence and severity. The incidence of BGMC in the fields was recorded based on the number of plants infected among the total number of the plants observed randomly in the field (50 numbers). Out of the 18 locations, 14 locations spread across the state recorded higher incidence of BGMC (DI >50%) (Table 2). Cent per cent incidence was recorded in locations *viz.* Ayyappancheri (Alappuzha, AEU 1), Vellanikkara (Thrissur, AEU 6), Vellayani (Thiruvananthapuram, AEU 8), Pallichal (Thiruvananthapuram, AEU 8), Vaniyamkulam (Palakkad, AEU 10), Periya (Kasaragod, AEU 11), and Alakode (Idukki, AEU 12) (Table 2). Based on the type and intensity of symptoms observed in bitter gourd, score chart was prepared to assess the severity of BGMC in terms of VI. The VI of BGMC in the surveyed locations ranged between 3.08 to 80.50 where Anthikkad of Thrissur district (AEU 6) was recorded with the lowest, while the highest was recorded in Pallichal of Thiruvananthapuram district (AEU 8) (Table 2).

The intensity and incidence of bitter gourd mosaic varied across AEUs of Kerala. Highest average incidences of the disease were recorded in AEU 8 (98.71 %) followed by AEU 1 (94.57 %); whereas the lowest was recorded in AEU 10 (57.00 %). Likewise, the highest average severity of bitter gourd mosaic was also found in AEU 8 (70.21) followed by AEU 1 (59.50); but on contrary, the lowest severity was recorded in AEU 12 (36.10) (Table 2). There was no positive correlation recorded between DI and VI in some locations. In some cases, DI was high with low VI (Kodikulam and Keerikode of AEU 12); and vice versa (Thrithala of AEU 10; Cheruvathur of AEU 11) (Table 2). The probable reasons contributing this variation could be the variety cultivated, viral strains present, combination of viruses in the mosaic complex, climatic conditions of the location affecting vector population, management practices adopted etc. in different locations. In some locations, climatic conditions may not favor the rapid replication and spread of viruses or the vectors responsible for transmission, leading to lower disease severity despite of high disease incidence. Moreover, in locations where tolerant

varieties were being cultivated, even if there was widespread viral disease, the disease severity may be low due to milder effects caused by viruses on tolerant plants. Similarly, variability in viral strains can result in differences in virulence or pathogenicity. The predominant virus strains of certain locations may be less virulent and cause milder symptoms, resulting in lower disease severity. In contrast, Radhika and Umamaheswaran [1], Asna et al. [27], Resmi and Sreelathakumary [28] and Ashwini [29] observed positive correlation between DI and VI in viral diseases of bitter gourd due to changes in environmental factors and management practices adopted over locations and seasons of the crop cultivated (temporal variations). Radhika and Umamaheswaran [1] reported highest average incidences and severities of viral diseases of bitter gourd in Idukki (DI – 100 %, VI – 82), Palakkad (DI – 88 %, VI – 69) and Thiruvananthapuram (DI – 60 %, VI – 56), districts of Kerala.

Incidence and severity of BGMC also differed among varieties. The highest average DI and VI was recorded for the most preferred variety of Kerala, Preethi, (DI - 99.04 %; VI - 65.01) (Table 3). Moreover, most of the locations where Preethi variety was cultivated (77.78 %) reported 100 per cent incidence of viral diseases. The average disease incidence for hybrids (52.76 %) was comparable to that of local varieties (54.76 %). On contrary, the average disease severity was found to be low for local varieties (VI - 18.55) compared to the hybrids (37.52) (Table 3). The results suggest that Preethi variety of bitter gourd was found to be highly susceptible to BGMC in the entire state of Kerala irrespective of ecological zones or management practices, whereas the improved local varieties were less susceptible.

Nevertheless, DI and VI were not uniform among hybrids and local varieties. Some local varieties exhibited partial tolerance to viral diseases with milder symptoms compared to susceptible varieties (Table 3). Partially tolerant varieties may have high disease incidence as the virus may still infect the plant, but with low severity as the plant can partially fend off the virus or mitigate its effects [30,31,32]. These may be the probable reason for higher disease incidence and lower severity in local varieties compared to the hybrids.

3.2 Viruses Produced Varied Symptoms in Bitter Gourd

The virus complex infected bitter gourd plants displayed wide range of symptoms. The most common symptoms observed during the survey were mosaic, stunted growth, yellowing of leaves with downward curling, blistering, and puckering on leaves, upward leaf curling, chlorosis and yellow spots on leaves, and hairiness. Other symptoms include rosetting, mottling of leaves, leaf distortion, vein banding, and vein clearing (Plate 2).

Table 2. Disease incidence and severity of Bitter gourd mosaic complex (BGMC) in different agro-ecological units of Kerala

Agro-ecological units (AEUs)		Districts	Locations	Variety	Management practices	DI* (%)	VI*
AEU 1	Southern coastal plain	Alappuzha	Ayyappancheri	Preethi	Traditional	100.00	78.00
			Kuthiathode	Hybrid	Integrated	88.47	57.50
			Kanjikkuzhy	Preethi	Integrated	95.25	43.00
AEU 6	Kole land	Thrissur	Vellanikkara	Preethi	Integrated	100.00	68.82
			Cherpu	Hybrid	Integrated	82.05	39.14
			Anthikkad	Hybrid	Integrated	7.50	3.08
AEU 8	Southern laterite	Thiruvananthapuram	Vellayani	Preethi	Integrated	100.00	60.83
			Pallichal	Preethi	Traditional	100.00	80.50
			Kalliyoor	Preethi	Integrated	96.15	69.30
AEU 10	North Central laterite	Palakkad	Vaniyamkulam	Preethi	Traditional	100.00	56.80
			Pattambi	Hybrid	Integrated	60.00	43.00
			Thrithala	Hybrid	Integrated	11.00	20.80
AEU 11	Northern laterite	Kasaragod	Kanhangad	Hybrid	Integrated	74.35	35.66
			Periya	Preethi	Traditional	100.00	56.67
			Cheruvathur	Hybrid	Integrated	45.96	63.50
AEU 12	South and Central foot hills	Idukki	Alakode	Preethi	Traditional	100.00	71.20
			Kodikulam	Local	Organic	46.00	19.60
			Keerikode	Local	Organic	62.50	17.50

** Number of plants observed in each location: 50*

Table 3. Average disease incidence and severity of bitter gourd mosaic complex (BGMC) in different varieties of bitter gourd cultivated across six AEUs of Kerala

Variety	Average DI (%)*	Average VI*
Preethi	99.04	65.01
Hybrid varieties	52.76	37.52
Local varieties	54.25	18.55

** Number of plants observed: Preethi – 450; Hybrids – 350; Local varieties – 100*

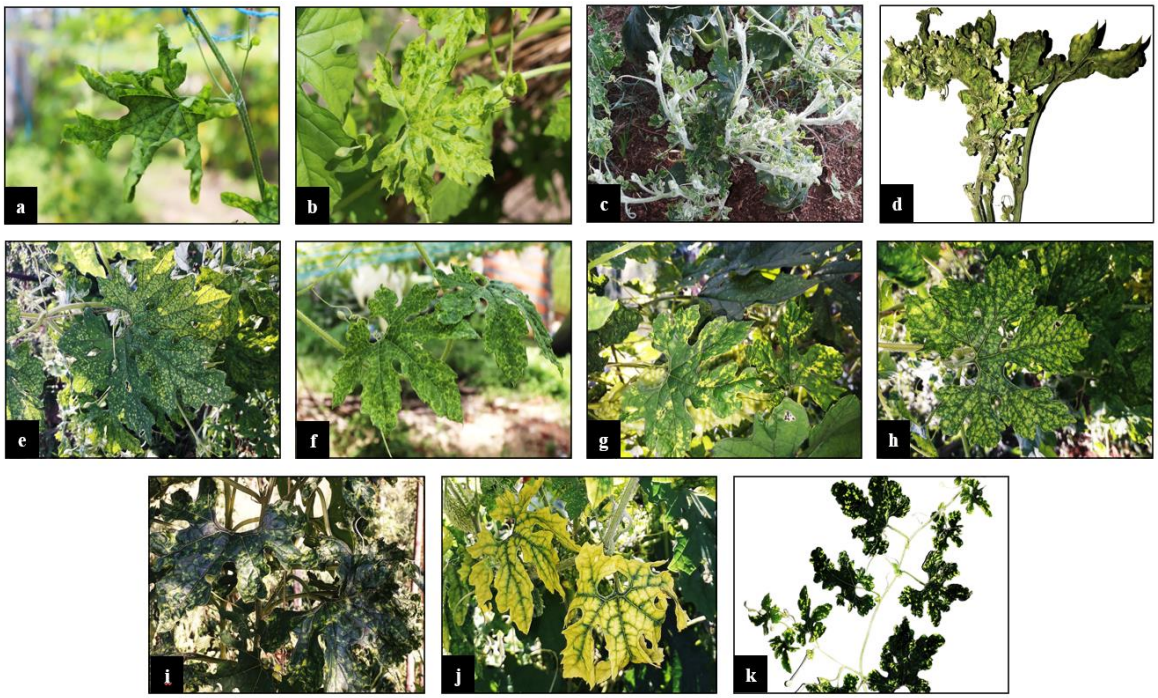

Plate 2. Symptoms of bitter gourd mosaic complex (BGMC) observed in fields in six agroecological units (a. upward leaf curling, b. mosaic, c. stunting and hairiness, d. rosetting, e. yellow spots on leaves, f. vein clearing, g. leaf mottling, h. vein banding, i. blistering of leaves, j. yellowing of leaves with downward curling, k. leaf distortion)

Mosaic and stunted growth were prevalent (100 %) throughout the locations of all AEUs surveyed. Symptoms such as yellowing of leaves with downward curling, blistering and puckering on leaves were observed in 77.78% of locations surveyed (Ayyappancheri and Kuthiathode of AEU 1, Cherpu and Anthikkad of AEU 6, Vaniyamkulam and Thrithala of AEU 10, Kanhangad and Cheruvathur of AEU 11, AEU 8 and AEU 12; Table 4). Symptoms like upward leaf curling and hairiness (Ayyappancheri and Kanjikkuzhy of AEU 1, Vellanikkara of AEU 6, Pattambi of AEU 10, Periya of AEU 11, Keerikode of AEU 12 and AEU 8,), as well as chlorosis and yellow spots on leaves (Kuthiathode of AEU 1, Cherpu and Anthikkad of AEU 6, Vaniyamkulam and Thrithala of AEU 10, Kanhangad and Cheruvathur of AEU 11, Alakode and Kodikulam of AEU 12) were recorded in 50 per cent of the locations (Table 4). Symptoms like rosetting was prevalent in 44.44 percent of the areas surveyed (Ayyappancheri and Kuthiathode of AEU 1, Cherpu of AEU 6, Pattambi of AEU 10, Keerikode of AEU 12 and AEU 8). Mottling of leaves, and leaf distortion had prevalence of 16.67 per cent each (Ayyappancheri of AEU 1, Pallichal of AEU 8, Vaniyamkulam of AEU 10), whereas, vein banding (Kanhangad of AEU 11, Kodikulam of AEU 12) and vein clearing (Ayyappancheri of AEU 1, Vellayani of AEU 8) accounted for only 11.11 per cent (Table 4). Yellow mottling, mosaic, leaf curl, reduction in leaf size, yellow mosaic, blistering, stunting, reduced flowering and fruiting and hairiness on the stem associated with viral diseases in bitter gourd were reported in Southern Kerala by Radhika and Umamaheswaran [1] and also in Southern Karnataka by Naik et al. [20].

Table 4. Prevalence of different symptoms of bitter gourd mosaic complex (BGMC) in different agro-ecological units (AEUs) of Kerala

Symptoms	Surveyed locations																	
	AEU 1			AEU 6			AEU 8			AEU 10			AEU 11			AEU 12		
	Ayyappancheri	Kuthiathode	Kanjikkuzhy	Vellanikkara	Cherpu	Anthikkad	Vellayani	Pallichal	Kalliyoor	Vaniyamkulam	Pattambi	Thrithala	Kanhangad	Periya	Cheruvathur	Alakode	Kodikulam	Keerikode
Upward leaf curling	+	-	+	+	-	-	+	+	+	-	+	-	-	+	-	-	-	+
Mosaic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Chlorosis and yellow spots in leaves	-	+	-	-	+	+	-	-	-	+	-	+	+	-	+	+	+	-
Mottling of leaves	+	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-
Yellowing of leaves with downward curling	+	+	-	-	+	+	+	+	+	+	-	+	+	-	+	+	+	+
Blisters and puckering on leaves	+	+	-	-	+	+	x	+	+	+	-	+	+	-	+	+	+	+
Leaf distortion	+	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-
Stunted growth	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hairiness	+	-	+	+	-	-	+	+	+	-	+	-	-	+	-	-	-	+
Rosetting	+	+	-	-	+	-	+	+	+	-	+	-	-	-	-	-	-	+
Vein banding	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-
Vein clearing	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-

+ denotes presence of symptom in plants; - denotes absence of symptom in plants

Table 5. Viruses detected in bitter gourd mosaic complex (BGMC) samples from different agro-ecological units (AEUs) of Kerala

Virus	Surveyed locations in AEUs																		Prevalence (%)
	AEU 1			AEU 6			AEU 8			AEU 10			AEU 11			AEU 12			
	Ayyappancheri	Kuthiathode	Kanjikkuzhy	Vellanikkara	Cherpu	Anthikkad	Vellayani	Pallichal	Kalliyoor	Vaniyamkulam	Pattambi	Thrithala	Kanhangad	Periya	Cheruvathur	Alakode	Kodikulam	Keerikode	
ToLCV	+	-	+	+	-	-	+	+	+	-	+	-	-	+	-	-	-	+	50.00
PRSV	+	+	-	-	+	+	+	+	+	+	-	+	+	-	+	+	+	+	77.78
CMV	-	+	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	16.67

+ denotes samples tested positive to virus and – denotes samples tested negative to virus in serological and molecular detection

Multiple viruses produced complex symptoms in bitter gourd. After the survey, it was found that mosaic and stunted growth were observed to be the general symptoms of viral disease in bitter gourd plants irrespective of the viruses associated (Table 4.). Nevertheless, after detection and screening of viruses (Table 5.), some specific symptoms were found to be associated with specific viruses (ToLCV or PRSV or CMV). It was found that upward leaf curling and hairiness were the common symptoms in ToLCV infected bitter gourd plants, whereas yellowing of leaves with downward curling, and blistering and puckering on leaves were found to be associated with PRSV infection. Mottling and distortion of leaves were observed specifically in plants tested positive for CMV (Tables 4 & 5).

When ToLCV and CMV was found together (Pattambi of AEU 10), prominent symptoms including upward leaf curling along with mottling and leaf distortion were observed; whereas upward leaf curling with blistering and puckering was observed in case of mixed infection of ToLCV and PRSV (Ayyappancheri of AEU 1, Vellayani and Kalliyoor of AEU 8, Keerikode of AEU 12) (Tables 4 & 5). Combination of CMV and PRSV produced downward leaf curling and yellowing and puckering of leaves with or without distortion (Kuthiathode of AEU 1). Triple infection of CMV, ToLCV and PRSV produced complex symptoms including prominent upward leaf curling, blistering and puckering, leaf distortion, hairiness as well as stunted growth (Pallichal of AEU 8; Table 4 & 5). Symptoms such as vein clearing, thin leaves, reduced leaf size, and yellowing were reported as typical symptoms of *Potyvirus* in bitter gourd [29]. Mohanan and Sharma [33] reported variable mosaic and leaf curl symptoms associated with whitefly transmitted *Begomovirus.* Similarly, Kumari et al. [34] reported symptoms such as leaf curl, mosaic, puckering, reduced growth, malformation of shoots and fruits, mottling, etc. in cucurbits infected with *Begomovirus.*

3.3 PRSV Dominated in Bitter Gourd Mosaic Complex Across Kerala over ToLCV and CMV

The presence of ToLCV, PRSV and CMV was tested using ELISA and DIBA, and further confirmed with PCR amplification of the specific regions of viruses in symptomatic bitter gourd samples collected from six AEUs surveyed (Table 5). All three viruses were detected in bitter gourd plants in the different AEUs of the state (Table 5). PRSV and ToLCV were present in all AEUs surveyed. PRSV was detected in two locations each in AEU 1 (Ayyappancheri, Kuthiathode), AEU 6 (Cherpu, Anthikkad), AEU 10 (Vaniyamkulam, Thrithala), AEU 11 (Kanhangad, Cheruvathur), and three each in AEU 8, and AEU 12. Samples collected from fields of two locations in AEU 1 (Ayyappancheri, Kanjikkuzhy), one each in AEU 6 (Vellanikkara), AEU 10 (Pattambi), AEU11 (Periya), and AEU 12 (Keerikode); and three in AEU 8 were tested positive to ToLCV. On contrary, CMV was present only in one location each in AEU 1, 8 and 10 (Kuthiathode AEU 1, Pallichal AEU 8 and Pattambi AEU 10). When we compared the prevalence of each virus in 18 locations of six AEUs, PRSV was found to be the dominant (77.78 %) followed by ToLCV (50 %), whereas, CMV was reported to be the least prevalent (16.67 %) (Table 5).

3.4 Viruses Were Present Single and in Combinations

In all the locations surveyed, the viruses were present either singly or in combinations of two or three (Table 5). Bitter gourd mosaic with ToLCV infection alone was found in AEU 1 (Kanjikkuzhy), AEU 6 (Cherpu) and AEU 11 (Periya), and that of PRSV infection in AEU 6 (Cherpu, Anthikkad), AEU 10 (Vaniyamkulam, Thrithala), AEU 11 (Kanhangad, Cheruvathur) and AEU 12 (Alakode, Kodikulam). CMV was always found in combinations, either with ToLCV or with PRSV or with both (Table 5). Combined infection of ToLCV and PRSV were found in AEU 1 (Ayyappancheri), AEU 8 (Vellayani, Kalliyoor) and AEU 12 (Keerikode); whereas ToLCV and CMV combination was found only in AEU 10 (Pattambi). Likewise, combined infection of PRSV and CMV was recorded in AEU 1 (Kuthiathode). All the three viruses were present together only in one location surveyed, i.e., in Pallichal location of Thiruvananthapuram district coming under AEU 8 (Table 5).

While comparing the infection types of ToLCV, PRSV and CMV in all the six AEUs, highest percentage was found out for single infections (61.11 %) followed by double infections of either ToLCV and PRSV, or PRSV and CMV, or ToLCV and CMV (33.33 %); and the lowest was for triple infections of ToLCV, PRSV and CMV (5.56 %)(Table 6). This pattern varied with AEUs. Single infections were more in AEU 6 and 11 (100 %) whereas, multiple infections were prominent in AEU 8 (100 %) (Table 6).

Table 6. Viruses associated with the bitter gourd mosaic complex (BGMC) disease in different agro-ecological units (AEUs) of Kerala

AEU	Single virus infection			Double virus infection			Triple virus infection
	ToLCV	PRSV	CMV	ToLCV + PRSV	PRSV + CMV	ToLCV + CMV	ToLCV + PRSV + CMV
AEU 1	1/3	0/3	0/3	1/3	1/3	0/3	0/3
AEU 6	1/3	2/3	0/3	0/3	0/3	0/3	0/3
AEU 8	0/3	0/3	0/3	2/3	0/3	0/3	1/3
AEU 10	0/3	2/3	0/3	0/3	0/3	1/3	0/3
AEU 11	1/3	2/3	0/3	0/3	0/3	0/3	0/3
AEU 12	0/3	2/3	0/3	1/3	0/3	0/3	0/3
Prevalence (%)	16.67	44.44	0	22.22	5.55	5.56	5.56
	61.11			33.33			

The study to determine the viruses infecting cucurbitaceous crops in different agro-climatic zones of Uttar Pradesh, India, Kumari et al. [35] observed the dominance of *Begomovirus* (93%) followed by *Potyvirus* (46%), *Cucumber green mottle mosaic virus* (CGMMV-39%), *Polerovirus* (9%), *Cucumber mosaic virus* (CMV-2%) and *Orthotospovirus* (2%) in cucurbits. Moreover, nearly 65% of samples were co-infected with more than one virus. Study conducted by Nagendran et al. [16] in Tamil Nadu, India also reported similar findings. Hence, it may be inferred that the dominance of viruses can vary depending upon locations, environmental factors, varieties cultivated, vector populations, seasons of the crop growth as well as management practices.

4. CONCLUSION

The study was conducted to examine the prevalence of the bitter gourd mosaic complex in bitter gourd cultivating AEUs of Kerala and to detect the prevalent viruses in these AEUs. Bitter gourd mosaic complex was found to be widespread throughout AEUs of Kerala. PRSV, ToLCV and CMV were the major viruses associated with BGMC in the different AEUs. PRSV was found to be the most prevalent virus among the three where viruses were present singly as well as in combinations. This indicates an alarming situation for the bitter gourd cultivation in the future. Hence, there is a need to monitor the viruses associated with the disease complex and vector populations periodically and check the varieties being cultivated, so that the knowledge of these complex interactions can help in developing targeted, sustainable and integrated management strategies to safeguard bitter gourd cultivation.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Authors hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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