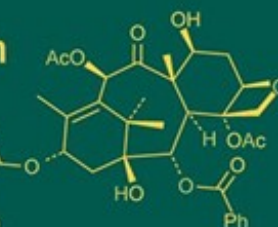


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Survey and molecular detection of viruses infecting muskmelon in Karnataka

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ABSTRACT

Muskmelon (*Cucumis melo* L.) is an economically important cucurbit crop cultivated extensively in tropical and subtropical regions for its high nutritional and commercial value. However, viral diseases have emerged as a major constraint for muskmelon production, causing significant yield losses. The increasing occurrence of mixed viral infections and the emergence of new virus strains necessitate systematic surveillance and accurate identification of viral pathogens affecting this crop. Therefore, the present investigation was undertaken to assess the viral diversity associated with muskmelon in Karnataka. An intensive roving survey was conducted in the major muskmelon-growing areas of Hiriya, Challakere and Sira taluqs to record the incidence of viral diseases. Among the surveyed locations, Sira recorded the highest disease incidence (40–46%), followed by Hiriya (20–30%), whereas the lowest incidence was observed in Challakere (15–20%). During the survey, a total of 24 fields were sampled. From each field, five symptomatic leaf samples were collected, resulting in a total of 120 samples. These samples were subjected to DAC-ELISA for the detection of major cucurbit viruses. DAC-ELISA results revealed that 40 samples tested positive for cucumber mosaic virus (CMV), 30 samples for melon yellow spot virus (MYSV), 20 samples for watermelon bud necrosis virus (WBNV), 10 samples for zucchini yellow mosaic virus (ZYMV) and 10 samples for papaya ringspot virus (PRSV-W). In addition, mixed infections of ZYMV and PRSV-W were detected in five samples, while another five samples showed mixed infection of WBNV and MYSV. Further, 12 representative samples exhibiting distinct and severe symptoms were selected for total RNA isolation. The extracted RNA was subjected to RT-PCR using coat protein (CP) or nucleocapsid (N) gene-specific primers for the respective viruses. The detection of CMV, MYSV, WBNV, ZYMV and PRSV-W was confirmed based on the presence of amplicons corresponding to the expected band sizes on agarose gel electrophoresis, indicating their occurrence in muskmelon samples collected from the surveyed regions of Karnataka.

Keywords: *Cucumis melo* L., DAC-ELISA, RT-PCR, Viral diversity, Karnataka

1. Introduction

Cucumis melo L. (muskmelon) is an annual herbaceous trailing vine belonging to the family *Cucurbitaceae* and is cultivated extensively across tropical, subtropical, and temperate regions for its high nutritional, medicinal, and commercial value. In India, muskmelon was cultivated on 80.6 thousand hectares with an estimated production of 1,753.5 thousand metric tonnes during 2025–26, with Karnataka being a major producing state contributing substantially to domestic production and supply (IndiaStat, 2026) [1]. Despite its economic importance, muskmelon production is constrained by several biotic and abiotic stresses, with viral diseases being among the most damaging constraints because of their ability to cause severe foliar symptoms, stunting, reduced fruit set, poor fruit quality, and significant yield losses.

A diverse range of viruses belonging to different genera, including *Begomovirus*, *Potyvirus*, *Cucumovirus*, *Orthotospovirus*, *Tobamovirus*, and *Polemovirus*, have been reported from muskmelon and other cucurbit crops. Important viral pathogens infecting muskmelon include cucumber mosaic virus (CMV), zucchini yellow mosaic virus (ZYMV), papaya ringspot virus (PRSV-W), melon yellow spot virus (MYSV), watermelon bud necrosis virus (WBNV), cucurbit aphid-borne yellows virus (CABYV), and tomato leaf curl New Delhi virus (ToLCNDV), inducing systemic symptoms such as mosaic, yellowing, leaf deformation, chlorosis, and plant stunting (Nagendran *et al.*, 2017; Kumari *et al.*, 2021; Radouane *et al.*, 2021) [2, 3, 4].

The epidemiology and spread of these viral diseases are strongly influenced by insect vectors, particularly aphids, whiteflies, thrips, and leafhoppers, whose populations can increase rapidly under warm environmental conditions, facilitating primary and secondary transmission within and between fields. The situation is further complicated by the broad host range, mixed infections, and genetic variability of several cucurbit viruses, which can influence symptom expression, virus accumulation, transmission efficiency, and disease severity. In particular, the emergence and increasing incidence of whitefly-transmitted begomoviruses in cucurbits represent an important concern, with ToLCNDV being increasingly associated with muskmelon and

other cucurbit species. Recent molecular investigations have also revealed complex begomovirus infections involving ToLCNDV, tomato leaf curl Palampur virus (ToLCPaV), and papaya leaf curl virus (PaLCuV), together with evidence of recombination and host-range expansion. (Krishnan *et al.*, 2026) [5].

Accurate diagnosis is fundamental for determining the distribution, incidence, and diversity of viruses infecting muskmelon. Serological tools like Direct Antigen Coating Enzyme-Linked Immunosorbent Assay (DAC-ELISA) provide a rapid and cost-effective approach for large-scale screening of field samples and detection of important viral pathogens (Hobbs *et al.* 1987) [6]. However, because serological assays may be limited by antigenic relationships among viruses and the availability of specific antisera, molecular techniques provide complementary and more specific approaches for virus identification. In particular, RT-PCR using coat protein (CP) or nucleocapsid (N) gene-specific primers enable sensitive detection and molecular characterization of RNA viruses. The combined application of serological and molecular approaches provides reliable framework for detecting and characterizing viral pathogens associated with muskmelon.

Previous surveys in cucurbit-growing regions have reported viral disease incidence ranging from 25.5 to 60 per cent in cucurbits, demonstrating considerable variation among locations and cropping environments (Dhkal *et al.*, 2023; Anupama *et al.*, 2025) [7, 8].

Studies conducted in Karnataka have documented the occurrence of major cucurbit viruses and mixed infections across southern Karnataka (Sureshbabu, 2003; Prasad, 2023) [9, 10]. However, despite the importance of muskmelon cultivation in Karnataka, information on the current occurrence, disease incidence, virus distribution, and molecular characteristics of viruses infecting muskmelon in major production areas remains limited. Therefore, the present investigation was undertaken to survey and detection of viruses associated with muskmelon in these growing regions of Karnataka.

Therefore, the present investigation was undertaken to elucidate the current viral disease scenario in muskmelon by assessing disease incidence and distribution, detecting associated viral pathogens through DAC-ELISA and virus-specific RT-PCR in the major muskmelon-growing regions of Chitradurga and Tumakuru districts of Karnataka. The study provides up-to-date insights into the prevalence, diversity, and molecular detection of muskmelon-associated viruses, thereby establishing a foundation for improved disease surveillance, early and accurate diagnosis, and the development of effective strategies for sustainable virus disease management in muskmelon.

2. Material and methods

2.1 Survey and sample collection

An intensive roving survey was conducted 24 representative fields in major muskmelon- production areas of Hiriya and Challakere taluks of Chitradurga district and Sira taluk of Tumakuru district, Karnataka, to assess the occurrence and incidence of viral diseases. A total of 120 symptomatic leaf samples exhibiting characteristic symptoms-such as mosaic, mottling, yellow spots, necrotic spots, vein clearing, leaf curling, stunting, leaf blistering, leaf elongation, and fruit necrosis-were collected in sterile polythene bags. Relevant field

information, including geographical location, crop age (days after planting), disease incidence (%), and predominant symptoms, was recorded for each field using a standardized survey proforma. The disease incidence was calculated using the following formula:

$$DI (\%) = \frac{\text{Number of diseased plants}}{\text{Total number of plants observed}} \times 100$$

The collected samples were transported to the laboratory under cooled conditions and processed immediately or stored at -80°C until further serological and molecular analyses

2.2 Serological assay (DAC-ELISA)

The symptomatic leaf samples collected during the roving survey were initially screened for the presence of major cucurbit viruses using DAC-ELISA according to the protocol described by Hobbs *et al.* (1987) [6]. Virus-specific polyclonal antibodies used against major cucurbit viruses known to infect cucurbit crops were used for serological detection. Briefly, leaf tissues from each field sample were processed and subjected to antigen coating, followed by incubation with the respective virus-specific primary antibodies and enzyme-conjugated secondary antibodies. The enzymatic reaction was developed using the appropriate substrate, and absorbance was measured at 405 nm using an ELISA microplate reader. Samples exhibiting absorbance values twice that of the healthy control were considered as positive for corresponding viral infection. The DAC-ELISA-positive samples were subsequently selected for molecular detection by RT-PCR to confirm the presence of the associated viral pathogens.

2.3 RNA isolation and RT-PCR amplification

Total RNA was extracted from the viral disease symptomatic leaf samples of muskmelon using RNeasy Plant Mini Kit (Qiagen), following the manufacturer's protocol. The integrity and quality of the total RNA was checked on 1.2% agarose gel and quantified by using Nanodrop (Thermo Fisher Scientific, USA).

First-strand viral cDNA synthesis was carried out using 8 μL of total RNA and 1.0 μL of the virus-specific B3 reverse primer. The RNA-primer mixture was incubated at 70°C for 5 min and immediately chilled on ice for 5 min. Subsequently, 4 μL of $5\times$ RT buffer, 2.5 μL of 10 mM dNTP mixture, 4 μL of RNase-free water and 0.5 μL of PrimeScriptTM Reverse Transcriptase were added to obtain a final reaction volume of 20 μL . Reverse transcription was performed at 42°C for 60 min, followed by enzyme inactivation at 70°C for 10 min. The synthesized cDNA was stored at -20°C and subsequently used as template for PCR amplification.

Then PCR amplification was performed using specific forward and reverse primers of coat protein or nucleocapsid (N) coding region described in table. Amplification was performed in an Eppendorf thermal cycler as per the protocol described by Nireesh Kumar *et al.*, (2025) [11]. The amplified products were analyzed by electrophoresis on a 1.2% agarose gel stained with ethidium bromide and visualized under UV illumination.

Primers used for molecular detection of viruses associated with muskmelon, target genomic regions and expected amplicon sizes.

Virus	Primer	Primer sequence (5'–3')	Target region	Expected size (bp)	Reference
CMV	F	TATGATAAGAAGCTTGTTCGCGCA	CP gene	500	Bariana <i>et al.</i> (1994) [12]
	R	TTTATGCCGTAAGCTGGATGGACAACCC			
MYSV	F	GCCATGGCATGCATGTCTACCGTTACTAAGCTGACA	N gene	850	Peng <i>et al.</i> (2011) [13]
	R	GTCTAGAGGTACCAACTTCAATGGACTTAGCTCTGGA			
WBNV	F	CAAAGACTTGTGGCTGGTG	N gene	554	Basavaraj <i>et al.</i> (2018) [14]
	R	GRTTGAAMATWCCTGCTYYGTTTG			

(continued on next page)

ZYMV	F	GGGGATCCAGCGTCTCATCAGCAATTCG	CP gene	498	Kaldis <i>et al.</i> (2018) [15]
	R	GGGGATCCGTGTGCCGTTCACTGTCTTC			
PRSV-W	F	TCACAGCGGCAATGATAGAG	Nib-CP region	650	Mohammed <i>et al.</i> (2012) [16]
	R	ATTGTGAATGAGTGGCACGA			

IUPAC nucleotide codes: R = A/G, M = A/C, W = A/T and Y = C/T.

3. Results

3.1 Survey and disease incidence across locations

An intensive roving survey across 24 representative fields in Challakere, Hiriyur and Sira districts recorded disease incidence ranging from 15.0 to 46.0 per cent. Sira taluk recorded the highest disease incidence (40–46%) across 10 villages, where crops were at an advanced growth stage of 54–65 days. Hiriyur taluk recorded 20–30% disease incidence across 5 villages with crop age ranging from 38 to 50 days. Challakere taluk recorded the lowest disease incidence (15–20%) across 9 villages, corresponding to younger crops aged 22–32 days (Table 1). The symptomatic muskmelon plants exhibited diverse foliar symptoms, including leaf puckering, crinkling, mosaic and chlorotic mottling, leaf deformation, rugosity, and distortion (Fig. 1A–D). Other prominent symptoms included yellow spotting, leaf cupping, puckering and blistering, and interveinal chlorosis, indicating considerable variation in symptom expression among the surveyed plants (Fig. 1E–H).

3.2 Serological detection using DAC-ELISA

Serological screening of 120 symptomatic samples by DAC-ELISA confirmed the presence of five major viruses: cucumber mosaic virus (CMV) in 40 samples (33.3%), melon yellow spot virus (MYSV) in 30 samples (25.0%), watermelon bud necrosis virus (WBNV) in 20 samples (16.7%), zucchini yellow mosaic virus (ZYMV) in 10 samples (8.3%), and papaya ringspot virus-W (PRSV-W) in 10 samples (8.3%). Among the virus-positive samples, mixed viral infections were detected in 10 samples (8.4% of the total samples), comprising PRSV-W + ZYMV co-infection in five samples (4.2%) and MYSV + WBNV co-infection in five samples (4.2%) (Fig. 2).

3.3 RT-PCR validation

RT-PCR amplification using virus-specific primer pairs targeting the coat protein (CP) or nucleocapsid (N) gene was performed to molecularly confirm the viruses detected by DAC-ELISA. The amplified products were separated by 1.2% agarose gel electrophoresis and visualized under UV illumination. Virus-specific amplification products corresponding to the expected sizes were obtained for cucumber mosaic virus (CMV), melon yellow spot virus (MYSV), watermelon bud necrosis virus (WBNV), zucchini yellow mosaic virus (ZYMV), and papaya ringspot virus-W (PRSV-W). The expected amplicon sizes were [CMV: 500 bp], [MYSV: 850 bp], [WBNV: 554 bp], [ZYMV: 498 bp], and [PRSV-W: 650 bp], respectively (Fig. 3). The presence of virus-specific bands at the expected molecular sizes confirmed the occurrence of these viruses in the symptomatic muskmelon samples.

4. Discussion

The present survey revealed a substantial incidence of virus-like diseases in muskmelon across the major production areas of Chitradurga and Tumakuru districts of Karnataka, with disease incidence ranging from 15.0 to 46.0 per cent. The highest incidence was recorded in Sira (40–46%), followed by Hiriyur (20–30%) and Challakere (15–20%). This spatial variation indicates considerable differences in the epidemiology and distribution of viral diseases among the surveyed locations. Similar variability in viral disease incidence has been reported in cucurbit-growing regions of India. Dhkal and Sharma (2023) [7] recorded viral disease incidence of up to 60% in

muskmelon in Punjab, while surveys in Uttar Pradesh documented substantial variation in viral incidence among cucurbit crops and locations.

DAC-ELISA screening revealed diverse virus infecting muskmelon, with CMV showing the highest detection frequency (33.3%), followed by MYSV (25.0%), WBNV (16.7%), ZYMV (8.3%), and PRSV-W (8.3%). The predominance of CMV is consistent with its widespread occurrence in cucurbit crops in Karnataka and other parts of India, where both single and mixed infections have been reported (Ashwathappa *et al.*, 2021) [17]. The detection of MYSV and WBNV at relatively high frequencies further highlights the importance of orthotospoviruses in muskmelon, as both viruses are known to cause diverse foliar and necrotic symptoms in cucurbits (Somnath *et al.*, 2019) [18]. The occurrence of ZYMV (8.3%) agrees with earlier reports of ZYMV infecting muskmelon in India, confirming its continued distribution in cucurbit-growing regions (Anthony Johnson *et al.*, 2013) [19]. Similarly, detection of PRSV-W (8.3%) is consistent with previous reports of PRSV-W in Indian cucurbits (Kumar *et al.*, 2021) [20]. The occurrence of mixed infections (8.4%) involving PRSV-W + ZYMV and MYSV + WBNV further indicates the complexity of the muskmelon virus disease complex. Such mixed infections may modify symptom expression and complicate field diagnosis, emphasizing the need for combined serological and molecular approaches for accurate virus detection and characterization.

5. Conclusion

The present study revealed a substantial and diverse viruses occur in muskmelon cultivated in the major production areas of Chitradurga and Tumakuru districts of Karnataka, with disease incidence ranging from 15.0 to 46.0%. DAC-ELISA and RT-PCR confirmed the occurrence of CMV, MYSV, WBNV, ZYMV, and PRSV-W, with CMV being the most frequently detected virus. The detection of mixed infections involving PRSV-W + ZYMV and MYSV + WBNV further highlights the complexity of viral diseases affecting muskmelon. The concordance between serological and molecular detection demonstrated the effectiveness of combining DAC-ELISA with virus-specific RT-PCR for reliable diagnosis. These findings provide updated information on the occurrence and distribution of major viruses infecting muskmelon in Karnataka and emphasize the need for regular surveillance, early diagnosis, and integrated management strategies to minimize virus-associated yield losses and sustain muskmelon production.

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Conflict of interest

The author declares that there is no conflict of interest.

Table 1. Survey of viral disease incidence in muskmelon-growing regions during 2025–26

Sl. No.	Location (Taluk)	Villages Covered	Crop Age (Days)	Disease Incidence (%)	Predominant Symptoms Observed
1	Challakere	9	22–32	15–20	Mosaic, mottling, leaf deformation
2	Hiriyur	5	38–50	20–30	Mosaic, yellowing, vein clearing
3	Sira	10	54–65	40–46	Severe mosaic, yellowing, and necrosis

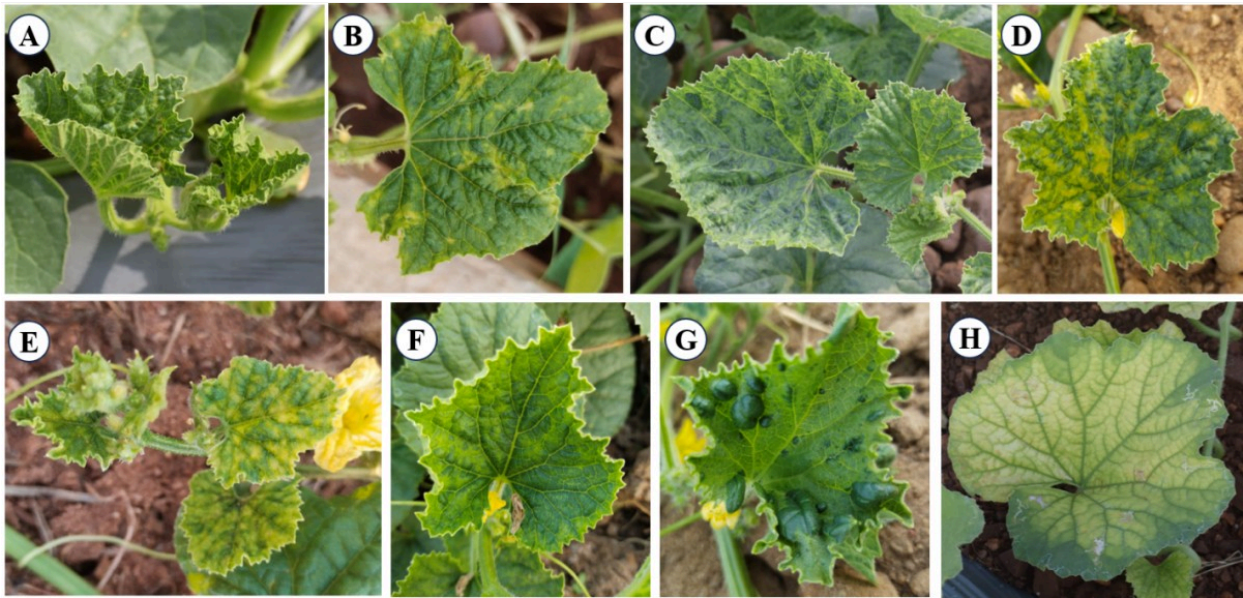


Figure 1. Representative virus disease symptoms observed in muskmelon plants. (A) Leaf puckering, crinkling and mosaic mottling; (B) Chlorotic mottling and leaf deformation; (C) Mosaic mottling with rugosity and puckering; (D) Severe mosaic and leaf distortion; (E) Severe yellow spot; (F) Leaf cupping; (G) Puckering and blistering; and (H) Interveinal chlorosis.

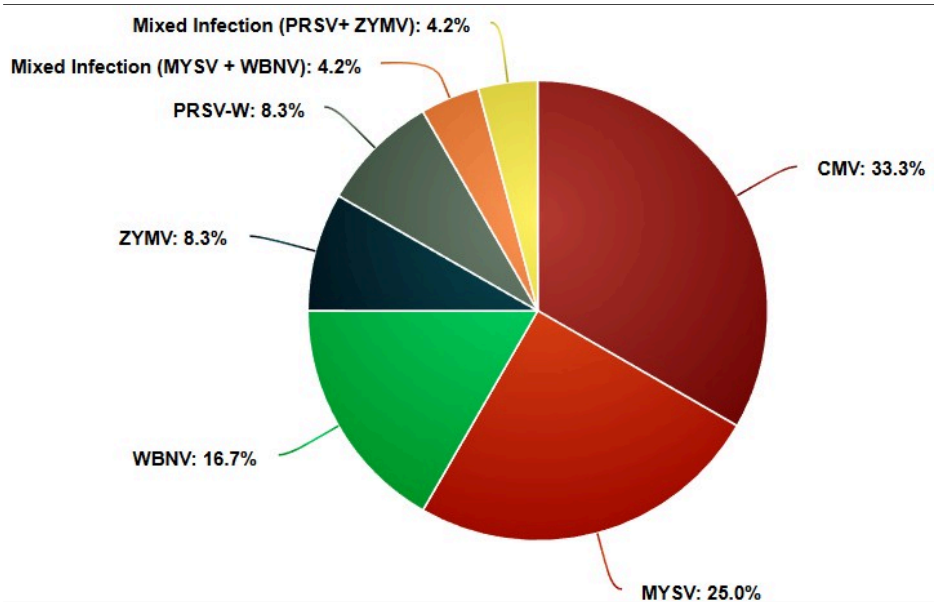


Figure 2. Distribution of viruses detected in muskmelon samples based on DAC-ELISA, showing single and mixed infections.

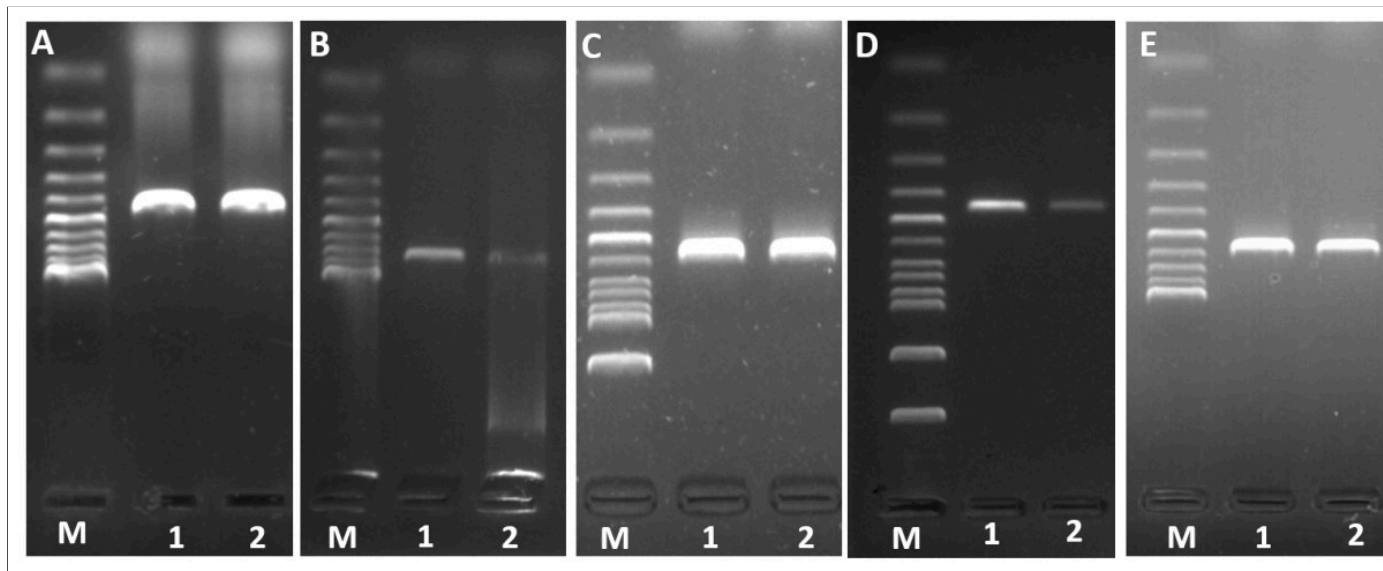


Figure 3. RT-PCR-based molecular detection of viruses associated with muskmelon. Agarose gel (1.2%) electrophoresis of virus-specific RT-PCR products: (A) CMV (500 bp), (B) MYSV (850 bp), (C) WBNV (554 bp), (D) ZYMV (498 bp), and (E) PRSV-W (650 bp). M: 100-bp DNA molecular marker; lanes 1 and 2: representative positive muskmelon samples showing virus-specific amplification products of the expected sizes.

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