Supporting Information (SI)

A centrifugal microfluidic chip for rapid nucleic acid detection of chili pepper viruses

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Table S1. The primer sequences used in RT-LAMP assay for viral RNA detection.

virus target	Primer name	Sequence (5'-3')
ToBRFV	ToBRFV-F3	ACTAATTCACTAGGTAATCAATTCC
	ToBRFV-B3	TTTCTAGTATCGAAAGCTCCTA
	ToBRFV-FIP	GACTTGAGGGACAGGTTTCCAAACACAACAAGCTAGAACAA
	ToBRFV-BIP	GTCACTGTTAGGTTTCCTGACAGCAAAGCAGTAACTAGAGGATC T
	ToBRFV-LF	GTTCAACGGCAATTTAG
	ToBRFV-LB	TTAAGGTGTATAGGTACAATGCGG
PepMV	PepMV-F3	GGAAAAACAGCGAAAGCAC
	PepMV-B3	AGATTCTCAAGGGTGTCTG
	PepMV-FIP	ACTGTACTTGTGGGTCTCTGATCTTTACCACATTATGTCTCGTG
	PepMV-BIP	AGCATTTGCGAAGCCGCATACATTAAGCGCATAAGGACAAT
	PepMV-LF	TTTCCAAAGTATTCCT
	PepMV-LB	CAACATGTTCGACCCGTACTTAA
ToMMV	ToMMV-F3	GGAGAGTTATGGAGTTAGTGAT
	ToMMV-B3	AGTCCTTGCTTGTTGTGTT
	ToMMV-FIP	ACGGAGAAGTAATAGCGTAAGACATGATAATTTGATTGAAGATG ATACCG
	ToMMV-BIP	TCGTGTTTTTATCATCAGCATGGGTGAAACTGGTTACCTAACGAA
	ToMMV-LF	ATCCCGCGACTGACGTC
	ToMMV-LB	GTTTTTATCATCAGCA
ToRSV	ToRSV-F3	ACATTTATCAGCGCCTGG
	ToRSV-B3	ACTTCGAAAACTCTCTGCC
	ToRSV-FIP	TGCACATATGCCTGGTAATTGAGTCAGTGGCCTTGAATTGTG
	ToRSV-BIP	TACAACGATGAACATTCTATCGTGCACACACACTCCACCTACC
	ToRSV-LF	AGCCCATCAAAGCGACTATA
	ToRSV-LB	GTGGCAATCTTTTGATGG
TSWV	TSWV-F3	GGCTTGATAGCTTGATCAGG
	TSWV-B3	ATAGCCAAGACAACACTGA
	TSWV-FIP	GGTGGGAAGCAATCTTAGATTTGATGTCAGGCTTGTAGAGGAAA C
	TSWV-BIP	TTGATGATGCAAAGTCTGTGAGGCATCTCAAAGCTATCAACTGA A
	TSWV-LF	GTAATCCATAGGCTTGAATCA
	TSWV-LB	TGCCATAATGCTGGGAGGT
PMMoV	PMMoV-F3	TTTAGGTTCTGTATGGGCC
	PMMoV-B3	AGAATCTAGCACGGCATTA
	PMMoV-FIP	GTAGTTCTAGCCTGCTGTGTTTGAAATTACAAAATCTATGTACTT CGGC
	PMMoV-BIP	GGTTCAACAGCAGTTCTCTGATGGGAAAACTTTGAAACCAGTAG C
	PMMoV-LF	AACTGATTACCTAAC
	PMMoV-LB	GAAGACCATACCGACCGCT
rbcL	rbcL-F3	CCAAGGATACTGATATATTGGCA
	rbcL-B3	AGGGTAAGCTACATAAGCAATA
	rbcL-FIP	AGAAGATTCGGCAGCTACCGGCATTCCGAGTAACTCCTC
	rbcL-BIP	GACAACTGTATGGACCGATGGATTCTCCAACAACACGCTC
	rbcL-LF	TTCAGGTGGAACTCCAGGTT
	rbcL-LB	ACCAGTCTTGATCGTTACAAAGGGC

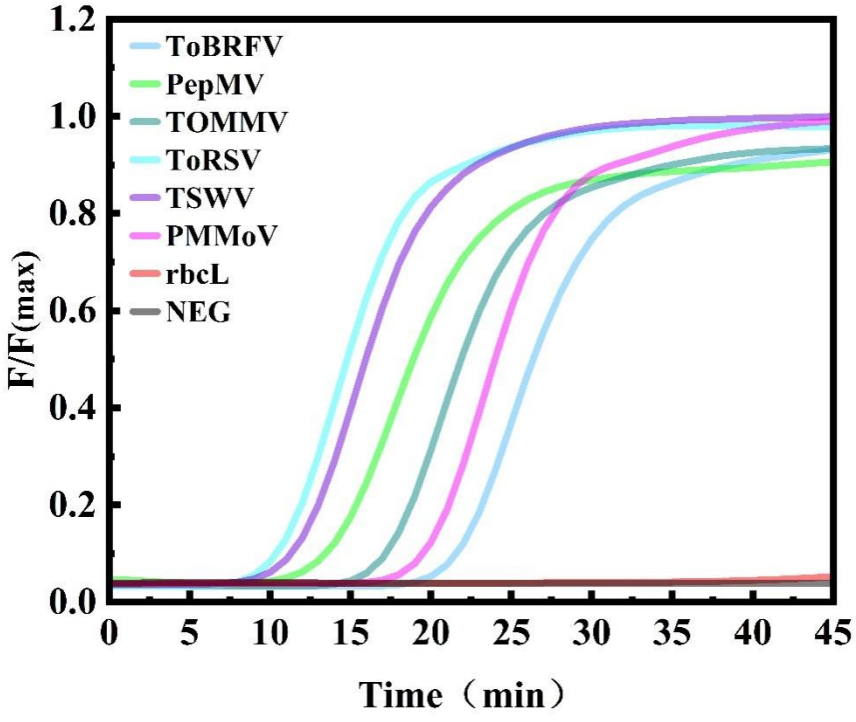

Figure S1. The amplification results of the microfluidic chip RT-LAMP assay utilizing a mixture of six types of standard plasmids with highly conserved region from the viruses of ToBRFV, PepMV, ToMMV, ToRSV, TSWV and PMMoV respectively.

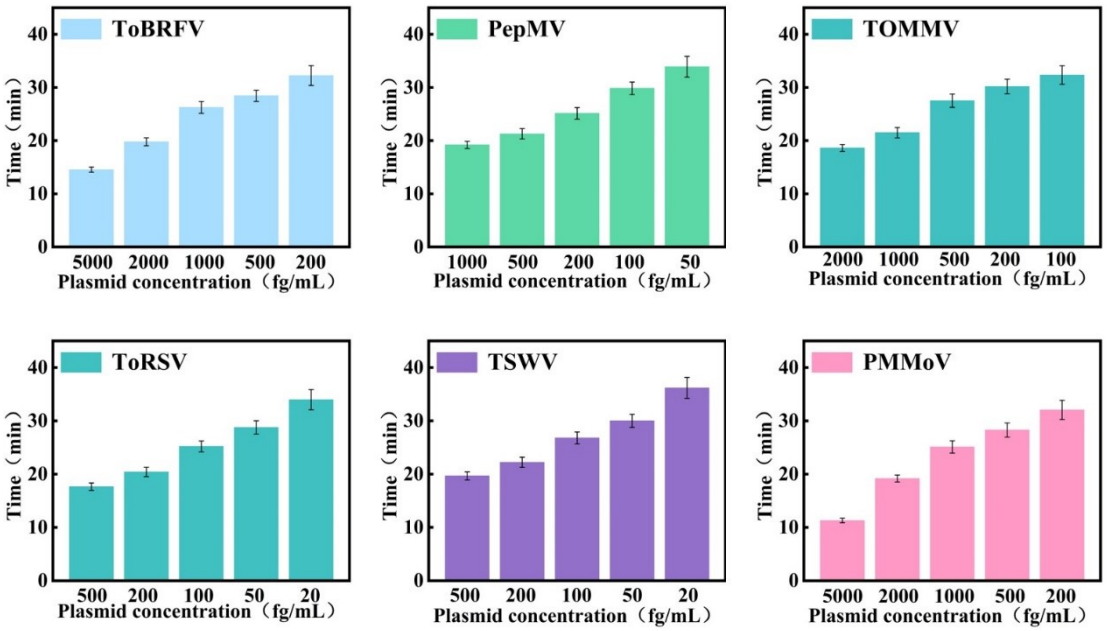

Figure S2. The time-to-positivity (Tp) values of the microfluidic chip RT-LAMP assay utilizing different concentrations of standard plasmids with highly conserved region from the viruses of (a) ToBRFV; (b) PepMV; (c) ToMMV(d) ToRSV; (e) TSWV and (f) PMMoV. The error bars are standard deviations across six repetitive trials.

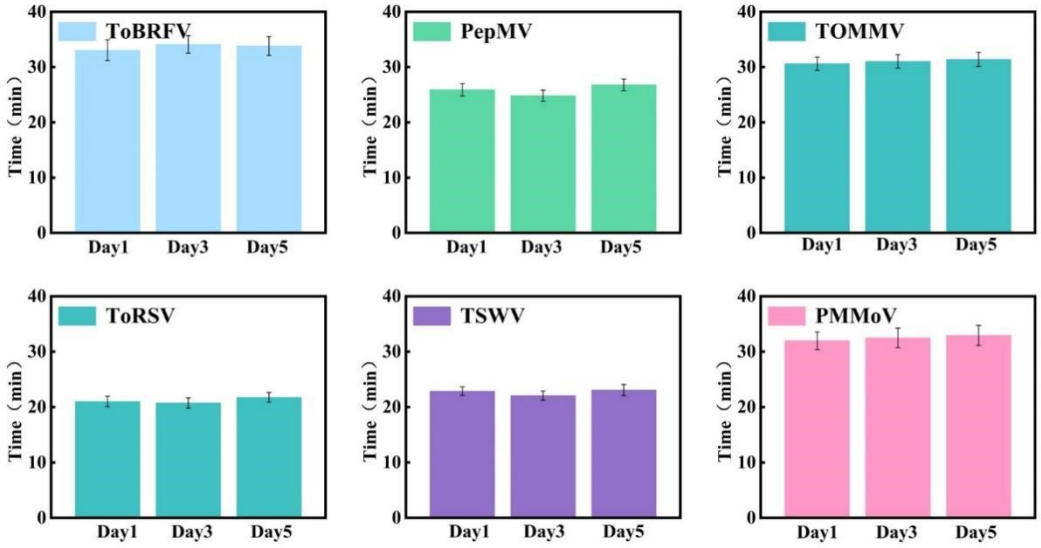

Figure S3. The reproducibility (inter-assay precision) investigation via testing samples containing all plasmids at same concentration of 200 fg/mL on different dates. The error bars are standard deviations across six repetitive trials.

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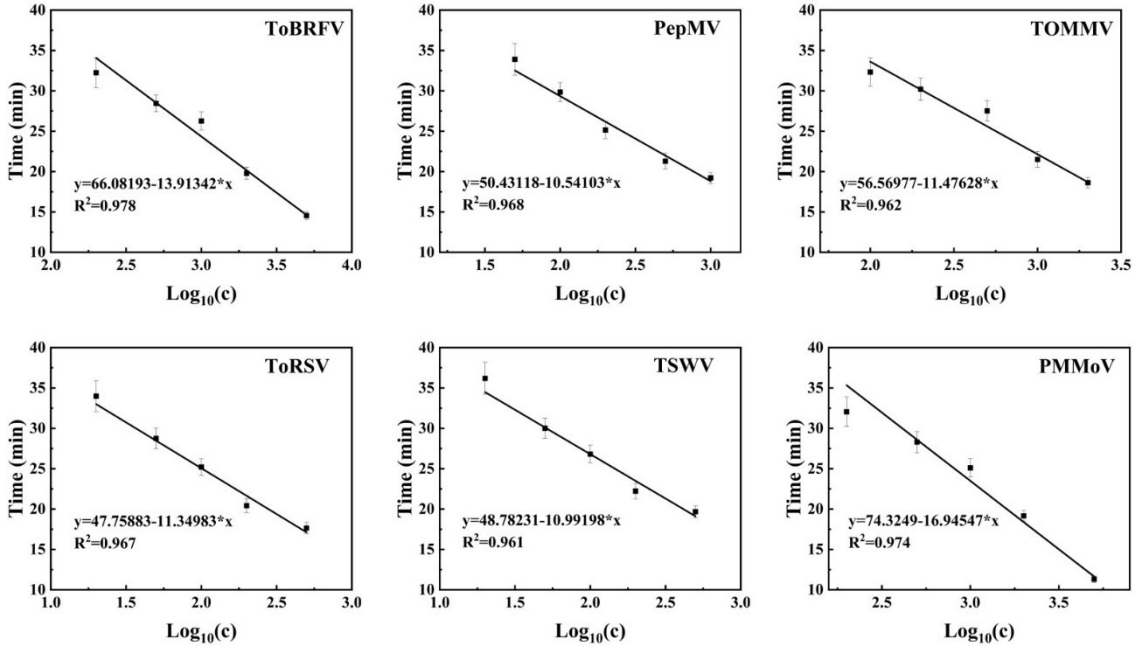

Figure S4. The semi-logarithmic regression between the Tp values and standards concentration of different plasmids(fg/mL). The error bars are standard deviations across six repetitive trials.

Table S2. The LOD of microfluidic chip RT-LAMP assay for standard plasmids with highly conserved region detection of different types of viruses.

Type of virus	LOD (fg/mL)
ToBRFV	200
PepMV	50
ToMMV	100
ToRSV	20
TSWV	20
PMMoV	200

Table S3. The primer sequences used in RT-PCR assay for viral RNA detection.

virus target	Primer name	Sequence (5'-3')
ToBRFV	ToBRFV-F	ACTAATTCACTAGGTAATCAATTCC
	ToBRFV-B	TTTCTAGTATCGAAAGCTCCTA
PepMV	PepMV-F	GCACTTTACCRCATTATGTCT
	PepMV-B	CTTTATGHACAGCAGCTCTTC
ToMMV	ToMMV-F	GGTGTATAGGTATAATGCGGTT
	ToMMV-B	TTCGAAAGTACTCTGGTTGT
ToRSV	ToRSV-F	GACGAAGTTATCAATGGCAGC
	ToRSV-B	TCCGTCCAATCACGCGAATA
TSWV	TSWV-F	AGGATTGGAGCCACTGAC
	TSWV-B	GCTGGAGCTGAGTATAGCAG
PMMoV	PMMoV-F	AGAACTCGGAGTCATCGGAC
	PMMoV-B	GAGTTATCGTACTCGCCACG
rbcL	rbcL-F	CCTCATGGGATCCAAGTT
	rbcL-B	CGATCTCTCCAACGCATAA

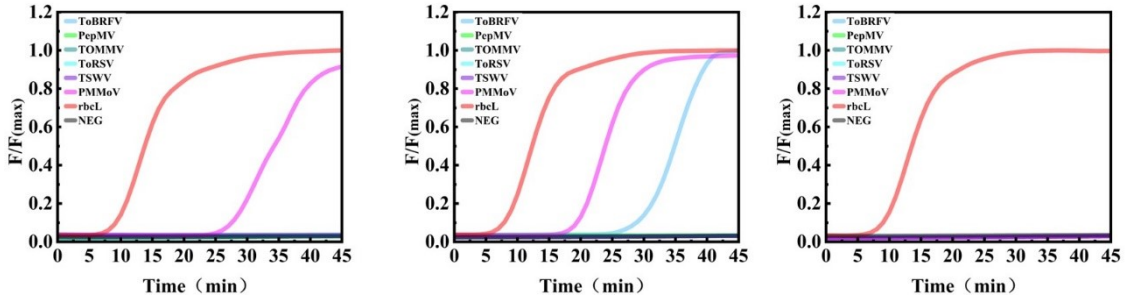

Figure S5. Microfluidic chip RT-LAMP results for RNA viruses in three representative leaf tissue samples collected from seedlings germinated from pepper seeds. (A) Co-infection with PMMoV and ToBRFV; (B) PMMoV infection only; (C) no detectable amplification for any of the six viruses tested.

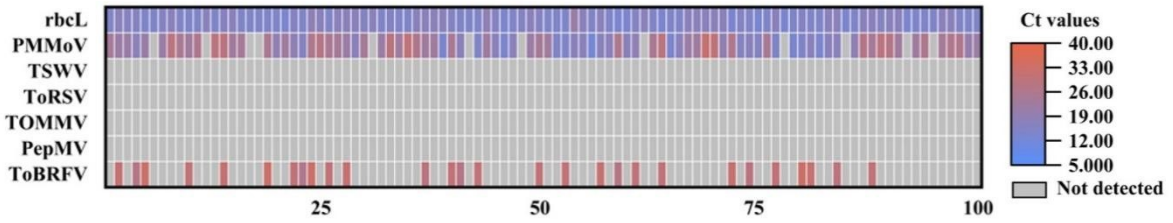

Figure S6. Results of viral RNA detection in leaf tissues from 100 chili pepper seed samples using the RT-PCR assay. The x-axis shows the sample (infected plant) number, and the y-axis lists the six target viruses and the rbcL internal control. The color scale represents the Ct values, whereas gray indicates no detectable amplification.

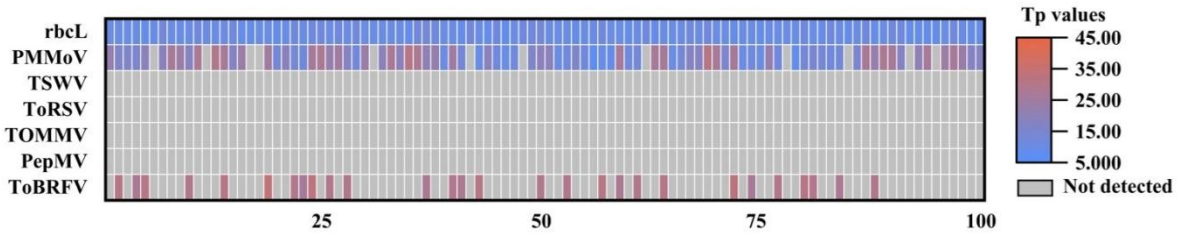

Figure S7. Results of viral RNA detection in leaf tissues from 100 chili pepper seed samples with trisodium phosphate treatment using the developed microfluidic chip RT-LAMP assay. The x-axis shows the sample (infected plant) number, and the y-axis lists the six target viruses and the rbcL internal control. The color scale represents the time-to-positivity (Tp) values, whereas gray indicates no detectable amplification.