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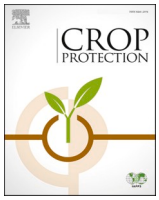

Loop-mediated isothermal amplification (LAMP) and suction spore trap-based assay for detection of mildew pathogens *Podosphaera xanthii* and *Pseudoperonospora cubensis* in cucurbits

Ananthi Palanisamy [a,1], Muthusamy Karthikeyan [b,c,**,1], Shajith Basha Jaffer [b], Shanmuga Priya Dhanabalan [b], Bavish Shanmugavel [d,e], Iruthayasamy Johnson [b], Natarajan Revathy [a], Narayanan Manikanda Boopathi [f], Ganapati Patil Santosh [g], Sambasivam Periyannan [c,h,*]

[a] Department of Plant Pathology, Agricultural College and Research Institute, Tamil Nadu Agricultural University, Madurai 625104, Tamil Nadu, India
[b] Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore 641003, Tamil Nadu, India
[c] Centre for Crop Health, University of Southern Queensland, Toowoomba 4350, Queensland, Australia
[d] Agro Climate Research Centre, Tamil Nadu Agricultural University, Coimbatore 641003, Tamil Nadu, India
[e] Centre for Applied Climate Sciences, University of Southern Queensland, Toowoomba 4350, Queensland, Australia
[f] Department of Biotechnology, Centre for Plant Molecular Biology and Biotechnology, Tamil Nadu Agricultural University, Coimbatore 641003, Tamil Nadu, India
[g] Department of Physical Science and Information Technology, Tamil Nadu Agricultural University, Coimbatore 641003, Tamil Nadu, India
[h] School of Agriculture and Environmental Science, University of Southern Queensland, Toowoomba 4350, Queensland, Australia

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ABSTRACT

The cultivation of cucurbits in India faces substantial challenges due to powdery and downy mildew diseases, caused by *Podosphaera xanthii* and *Pseudoperonospora cubensis*, respectively. Implementation of effective disease management strategies relies heavily on the rapid and early detection of the pathogen responsible for these diseases. Here, we aim to use loop-mediated isothermal amplification (LAMP) and suction spore trap-based assay for effective detection of *P. xanthii* and *P. cubensis*. The LAMP primers designed using internal transcribed spacer (ITS)1 and ITS2 and 5.8S ribosomal RNA were found suitable for detecting *P. xanthii* while the sequence of *cellulose synthase 2* (*CesA2*) gene was effective for *P. cubensis*. The specificity of the assay to their respective pathogen was further confirmed through the screening of nine different test pathogens: *Macrophomina phaseolina*, *Phytophthora capsici*, *Fusarium oxysporum*, *Sclerotium rolfsii*, *Alternaria alternata*, *Geotrichum candidum*, *Aspergillus niger*, *A. flavus* and *Colletotrichum gloeosporiodes*. Subsequently, a suction spore trap designed using a polyvinyl chloride (PVC) pipe and a cooling fan powered by a solar panel was able to capture the airborne inocula of the two mildew pathogens. In the LAMP assay, air samples from the 16 to 26th and 23rd to 29th standard weeks exhibited positive signals for *P. xanthii* and *P. cubensis*, respectively during the 2023 cropping season. Hence, the integration of LAMP assay with suction spore traps is an ideal strategy for the detection of mildew pathogens in cucurbits and will assist in implementing robust disease management practices.

1. Introduction

Cucurbits are an economically significant group of vegetables cultivated across arid to humid tropical agroclimatic conditions (Jeffrey et al., 1990). Globally, cucurbits account for 28 million tonnes of vegetables with India sharing around 5.1 million tonnes (FAO, 2021). Despite their economic importance, cucurbit cultivation faces severe challenges due to powdery and downy mildew diseases (Cohen et al.,

* Corresponding author. Centre for Crop Health, University of Southern Queensland, Toowoomba 4350, Queensland, Australia.
** Corresponding author.
E-mail addresses: ananthi261999@gmail.com (A. Palanisamy), karthikeyan.m@tnau.ac.in (M. Karthikeyan), shajithbashaagri002@gmail.com (S.B. Jaffer), dsshanmugapriya95@gmail.com (S.P. Dhanabalan), bavish.shanmugavel@unisq.edu.au (B. Shanmugavel), johnson.i@tnau.ac.in (I. Johnson), nrev@rediffmail.com (N. Revathy), nmboopathi@tnau.ac.in (N.M. Boopathi), patilsgstat@tnau.ac.in (G.P. Santosh), sambasivam.periyannan@unisq.edu.au (S. Periyannan).
[1] Contributed equally.

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2015; Lebeda and Cohen, 2011). Powdery mildew is caused predominantly by *Podosphaera xanthii* and infection is manifested by the presence of a superficial white powdery growth of conidia and mycelia on leaf surfaces, stunted growth, leaf desiccation, and poor fruit quality (Marimon et al., 2020). Downy mildew, caused by *Pseudoperonospora cubensis*, manifests water-soaked spots that progress to light green lesions, leaf death and curling. The disease culminates with the development of sporangia and sporangiophores between the veins on the lower side of the leaves (Goldenhar and Hausbeck, 2019). The rapid proliferation of these mildews, facilitated by the release of short-lived asexual spores like conidia and sporangiaspores, poses a significant threat to cucurbit crops, resulting in yield losses ranging from 30 to 80 % (Elsharkawy et al., 2014; Lebeda and Cohen, 2011). The disease development is favoured by day temperatures ranging between 24 and 30 °C and relative humidity exceeding 94% (Ghosh et al., 2015a; Gupta et al., 2001).

Recognition of these pathogens at the early stages of disease development remains crucial for effective disease management; hence, spore-trapping techniques have become a valuable tool for pathogen surveillance. Various spore traps such as Rotorod, Burkand, Hirst, and Cyclone samplers are available for the collection of airborne inocula of plant pathogens (Bello et al., 2021; Huang et al., 2016; Marimon et al., 2020). Accuracy and efficient use of spore traps for pathogen detection and identification rely heavily on the subsequent characterization of the collected spores (Jackson and Bayliss, 2011). Polymerase Chain Reaction (PCR)-based techniques have been widely employed for pathogen detection and characterization. However, PCR sensitivity can be negatively impacted by low levels of the pathogen at the initial disease development stages and is typically carried out in laboratories (Freeman et al., 1996). In contrast, loop-mediated isothermal amplification (LAMP) is a rapidly emerging pathogen detection technique known for its high specificity and sensitivity. The technique involves the use of six oligonucleotide primers designed to recognize three distinct regions of a target DNA segments and also incorporates two loop primers to expedite the amplification process. This technique relies on the strand displacement capability of *Bacillus stearothermophilus* (*Bst*) polymerase (Wastling et al., 2010) and can operate at a constant temperature between 60 and 65 °C. The amplified LAMP products generate stem-loop DNA structures, allowing the primers to bind to the target region bypassing the repeated thermal denaturation steps of the traditional PCR amplifications. Hence, loop primers significantly reduce the amplification time, making LAMP as an efficient detection technique (Nagamine et al., 2002). This assay can be monitored in real-time or visually by introducing fluorescent dyes such as SYBR green, ethidium bromide or metal ion indicators like hydroxyl naphthol blue or by assessing turbidity through $MgSO_4$ ion precipitation (Mori et al., 2001). The LAMP method can also rapidly increase the number of DNA copies without the need for additional reagents (Tomita et al., 2008).

Here we investigate and validate spore trapping and LAMP assay-based pathogen identification and quantification for early detection of pathogens to implement timely and cost-effective disease management practices.

2. Materials and methods

2.1. Field survey and collection of infected leaf samples

A field survey was conducted across districts of Tamil Nadu state in India to collect cucurbits mildew infected leaf samples. Cucumber (*Cucumis sativus*), muskmelon (*C. melo*), pumpkin (*Cucurbita pepo*), bitter gourd (*Momordica charantia*), bottle gourd (*Lagenaria siceraria*) and ridge gourd (*Luffa acutangula*) were collected from Coimbatore, Madurai, Salem, Dindugul and Namakkal districts (Table 1). A total of 25 leaves were collected randomly from each field (three fields per district) and the disease severity was assessed using 0 to 9 scale (0 - No infection, 1 with 0–10%, 3 with 10–15%, 5 with 15–25%, 7 with 25–50%, 9 with more than 50% of the leaf area with infections) described in Jamadar and Desai (1997). Percentage disease index (PDI) was calculated using the following formula (Wheeler 1969).

$$\text{PDI} = \frac{\textit{Sum of all individual ratings}}{\textit{Total number of leaves observed}} \times \frac{100}{\textit{Maximum grade in the score chart}}$$

Conidia and sporangia were collected from the infected samples using a hairline brush and then suspended in sterile water and stored at −20 °C for further use.

2.2. Genomic DNA extraction

Genomic DNA from the conidia and sporangia suspension of 12 and 16 samples of downy and powdery mildew, respectively, were isolated using the CTAB extraction method described in McDermott et al. (1994). Approximately, 300 μg of conidia/sporangia were transferred to a microcentrifuge tube with 500 μl of CTAB extraction buffer (1 M Tris-base, PH 8.0; 7 M NaCl; 0.5 M EDTA, PH 8.0 and 10% CTAB (w/v) and was vortexed for few seconds before incubating at 65 °C for 10

Table 1
Survey on occurrence of cucurbit powdery and downy mildews in Tamil Nadu.

Host	Location		Global positioning system coordinates		Sample id	PM PDI (%)[a]	Sample id	DM PDI (%)[a]
	District	Village						
Bottle gourd	Coimbatore	TNAU	11.006494°N	76.931477°E	CPM1	51.68^{cd} (45.96)	CDM1	14.56^{b} (22.43)
Pumpkin		Vellimalaipattinam	10.988446°N	76.785905°E	CPM2	45.93^{fg} (42.67)	CDM2	17.82^{a} (24.97)
Ridge gourd		Thillainagar	10.976871°N	76.821681°E	CPM3	45.74^{fg} (42.55)		0^{h} (3.29)
Ridge gourd		Thondamuthur	10.99751°N	76.829686°E	CPM4	43.99^{gh} (41.54)	CDM3	18.25^{a} (25.29)
Cucumber		Poosaripalayam	11.00758°N	76.928766°E	CPM5	60.45^{a} (51.03)	CDM4	11.85^{d} (20.13)
Bitter gourd		Vellimalaipattinam	10.988372°N	76.775948°E	CPM6	58.38^{ab} (49.82)	CDM5	15.26^{b} (22.99)
Ash gourd		Narasipuram	10.994458°N	76.800727°E	CPM7	39.53^{ij} (38.96)		0^{h} (3.29)
Bitter gourd		Gengampalayam	10.597543°N	76.976979°E	CPM8	47.07^{efj} (43.31)		0^{h} (3.29)
Ridge gourd	Madurai	Narasingam	9.97035°N	78.203265°E	CPM9	50.70^{de} (45.40)	CDM6	17.46^{a} (24.70)
Ridge gourd		Ambathurai	10.254491°N	77.912284°E	CPM10	54.86^{bc} (47.79)	CDM7	13.26^{c} (21.35)
Ridge gourd		Kesampatti	10.149164°N	78.289995°E	CPM11	37.09^{j} (37.52)		0^{h} (3.29)
Bottle gourd	Salem	Chettipatti	11.731272°N	78.017735°E	CPM12	50.41^{de} (45.23)	CDM8	9.10^{f} (17.52)
Bottle gourd	Dindigul	Oddachanchatrum	10.149164°N	78.289995°E	CPM13	41.18^{hi} (39.92)	CDM9	9.14^{f} (17.60)
Cucumber	Namakkal	Tiruchengode	11.376906°N	77.812587°E	CPM14	53.45^{cd} (46.98)	CDM10	10.75^{e} (19.14)
Ridge gourd		Aalampalayam	11.367451°N	77.774352°E	CPM15	37.45^{ij} (37.73)	CDM11	8.76^{f} (17.21)
Cucumber		Thottimandhaikaadu	11.00758°N	76.928766°E	CPM16	49.68^{def} (44.80)	CDM12	4.51^{g} (12.26)
				CV(%)		1.70		1.60

[a] PM-powdery mildew, DM-downy mildew, PDI-percent disease index, numerical values are mean of three replications; 0 replace by 1/4n. Data in parenthesis indicate arcsine transformation values. In each column, figure followed by same letter do not differ significantly at 5% level by Tukey's test.

minutes (min). After incubation the mixture was centrifuged at 10,000 rpm for 10 min. The supernatant was then collected and extracted by adding phenol: chloroform: isoamyl alcohol (25:24:1). The aqueous layer was then subjected to extractions using chloroform: isoamyl alcohol (24:1) and centrifuge at 10,000 rpm for 10 min. The supernatant was collected, and DNA was precipitated by addition of a double volume of ice-cold isopropanol and incubation at −20 °C for overnight. The genomic DNA was collected by centrifugation at 13,000 rpm at 4 °C for 10 min. The pellet was washed twice with cold 70% ethanol, air-dried and resuspended in 30 μl of Tris-EDTA buffer. Extracted genomic DNA quality was assessed using 0.8% agarose gel stained with 6X loading dye.

2.3. Optimization of LAMP assay conditions for detection of mildew pathogens

The LAMP primers namely, Forward Inner Primer (FIP), Backward Inner Primer (BIP), Forward Outer Primer (F3), Backward Outer Primer (B3), Loop Forward Primer (LF), and Loop Reverse Primer (LR) were designed using Primer Explorer (v5.0) for internal transcribed spacer (ITS)I and ITS2 and 5.8 S ribosomal RNA gene of *P. xanthii* (NCBI id: OQ831627) and the *cellulose synthase 2* (*CesA2*) gene for *P. cubensis* (JF799097) (Table 2). Five different MgSO4 ion concentrations (ranging from 2-10 mM), amplification temperatures (56–68 °C) and incubation time were used to standardize the LAMP reaction. The reaction mixtures were incubated at 65 °C for 60 min, followed by termination at 80 °C for 2 min. Visual observations were made using Hydroxynaphthol Blue (HNB) dye, where a sky-blue colour indicates a positive reaction when violet colour denotes negative reaction. The results were further confirmed by running the amplified DNA product on a 2% agarose gel electrophoresis.

Next, the sensitivity of the LAMP assay was determined using various concentrations of the genomic DNA 150 ng to 1.5 fg in ten fold increments of *P. xanthii* and *P. cubensis*. The specificity of the assay was tested using nine different test pathogens: *Macrophomina phaseolina*, *Phytophthora capsici*, *Fusarium oxysporum*, *Sclerotium rolfsii*, *Alternaria alternata*, *Geotrichum candidum*, *Aspergillus niger*, *A. flavus* and *Colletotrichum gloeosporiodes*. Later, the assay was validated using 12 and 16 leaf samples infected with *P. cubensis* and *P. xanthii*, respectively collected from various locations within Tamil Nadu state in India.

2.4. Design of traps for collecting airborne spores of cucurbit mildew pathogens

Suction spore trap was constructed using a polyvinyl chloride (PVC) pipe with in and out-let caps, suction DC cooling fan motor, rechargeable lithium battery rated at 6 volts (V) and 4.5 ampere-hours (Ah), sampling plates, a solar panel module SEPL010P, electronic control board with a 2 A switch, and a sturdy steel stand (Basha et al., 2021) (Fig. 1). Sampling plates were soaked in hexane for 24 h and subsequently rinsed two to three times with water. Plates were autoclaved for 30 min and air-dried under a laminar airflow chamber in an aseptic environment. A fine layer of silicone vacuum grease (Dow Corning) was applied using gloved hands and stored carefully in sterilized containers. Three greased suction plates were placed in the suction spore trap to

Table 2
LAMP primers for *Podosphaera xanthii* and *Pseudoperonospora cubensis*.

Primers for *P. xanthi*	Sequence
FIP Px	CACCGCCACTGCGTTTAGGGAGAACACCCCTCAAGCCT
BIP Px	CTGGTGTGCTCTCCGCGTAGCCAAAGACTGGGGTTCTGG
F3 Px	CATGCCTGTTCGAGCGTC
B3 Px	CCTACCTGATCCGAGGTCAT
LF Px	GAGCCCCAAGACCAAGCT
LB Px	TCATGTATCTCGCGACAGAGT
Primers for *P. cubensis*	**Sequence**
FIP Pc	AGTTCGTGCAGCACATGTCGATCCAGGTGAATGGCACCATG
BIP Pc	GTGACACGAGGCTTGGCAAGAGACATATCCCCGCGAACAC
F3 Pc	GTGCAGAGTGCGGTACAC
B3 Pc	CGCACATCGTCTGAGTAGG
LF Pc	TTGCTGACACGCAGCTCGC
LB Pc	GCGGTGTGTGTCGCAAATGC

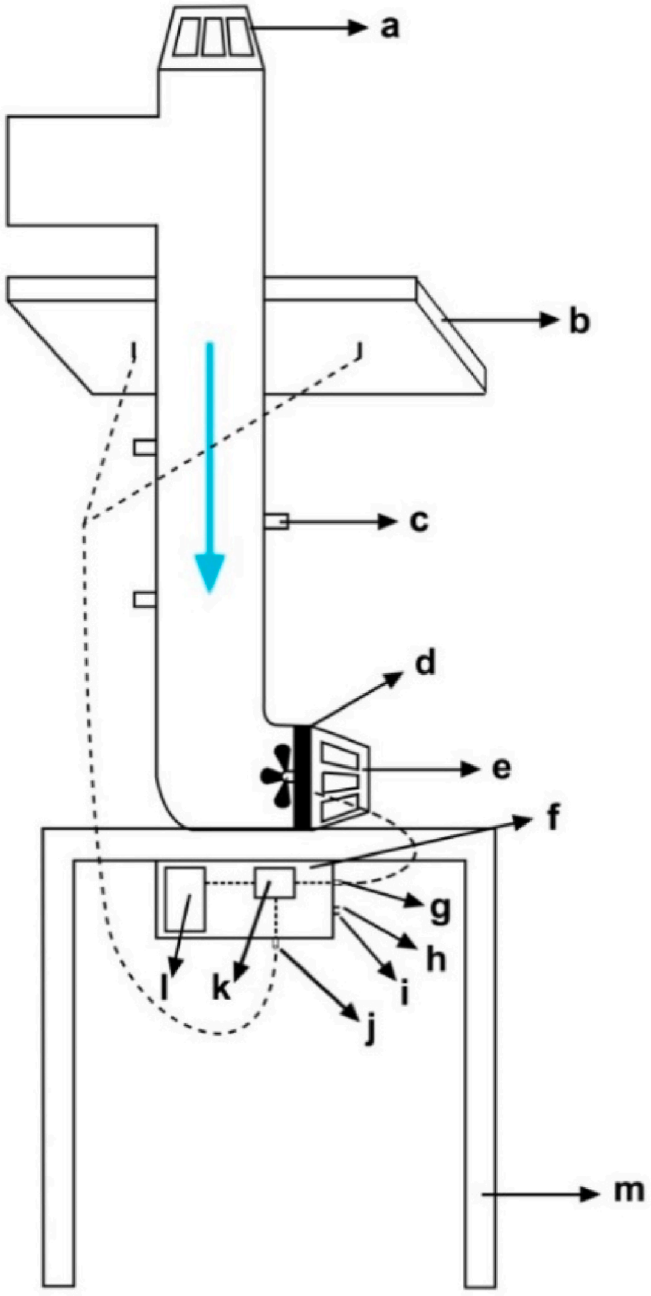


Fig. 1. Solar-operated suction spore trap design for the detection of airborne inocula of cucurbits mildews. a) Air Inlet; b) Solar panel 300 × 350 × 21 mm, module type; SEPL010P Peak power voltage; 18 V, Peak power current; 0.56 A, Nominal Operating Cell Temperature; 43 °C, Weight approximately: 1.3 kg, Application class: A; c) stainless steel sampling plate (65 × 50 × 2 mm) placed through slit opening; d) 12 V-2.4 W cooling fan DC motor; e) Air Outlet; f) 170 × 70 × 110 mm dimension of junction box g) Motor wire socket; h) DPDT On-Off switch rocker switch for solar panel i) DPDT On-Off switch rocker switch for motor; j) Solar panel power socket k) Voltage regulator circuit board; l) 6-V, 4.5 Ah rechargeable battery. Blue colour arrow indicates the direction of air flow. A dotted line indicates Atekt DC power cable 12 V - 5 A and has 18 AWG (American wire gauge); m) Stainless steel stand.

capture airborne conidia and sporangia. The suction spore trap was positioned within the cucumber experimental plot located at Tamil Nadu Agricultural University (Latitude: 11.0086° N, Longitude: 76.9284° E), Coimbatore district, Tamil Nadu, India. The spore traps were positioned at the height of 1 m above ground level and installed at the rate of 2–3 traps per hectare. The suction spore traps were operated continuously throughout the cropping season in 2023. Sampling plates were collected weekly and stored at −20 °C for future studies.

2.5. Genomic DNA extraction from air samples

The genomic DNA extraction from the sampling plate's airborne inocula was based on Thiessen et al.'s method (2016) with slight modifications. Using sterilized toothpick, the inocula sticking on silicone vacuum grease of the sampling plate was transferred to 14 mL falcon snap-cap tubes containing 300 µl of CTAB extraction buffer (comprising 50 mM Tris-HCl, PH 8.0; 0.7 M NaCl; and 1% CTAB w/v). The tubes were vortexed at high speed for 1 min and the contents were transferred to microcentrifuge tubes and incubated at 60 °C for 20 min. Following this incubation, a volume of phenol: chloroform: isoamyl alcohol (25:24:1) equivalent to the sample volume was added, and the mixture was centrifuged at 10,000 rpm for 10 min. The resulting aqueous phase was carefully transferred to 1.5 mL microcentrifuge tubes, and DNA was precipitated by adding an equal volume of ice-cold isopropanol, followed by incubation at −20 °C overnight. The DNA was then collected by centrifugation at 13,000 rpm at 4 °C for 10 min. The resulting pellet was washed twice with 70% ethanol, air-dried, and finally resuspended in 30 µl of nuclease-free water. The DNA was stored at −20 °C for further studies.

2.6. LAMP assay for detection of cucurbit mildew from the airborne inocula

DNA of the airborne inocula of *P. xanthii* and *P. cubensis* were used as templates to standardize the LAMP assay for pathogen detection and quantification. A reaction volume of 25 µl containing specific concentrations of 1.6 µM FIP and BIP, 0.2 µM F3 and B3, 0.4 µM LF and LB for both *P. xanthii* and *P. cubensis* primers, 8U of *Bst* DNA polymerase (New England Biolabs), 2.5 µl of a 10X Thermopol buffer, 1.6 mM of each dNTP, 0.8 M betaine, 4 and 6 mM $MgSO_4$, 120 µM hydroxyl naphthol blue and 2.5 µl of template DNA of *P. xanthii / P. cubensis*. The LAMP reaction mixture was then subjected to incubation at 65 °C for 60 min, followed by termination at 80 °C for 2 min. Positive reactions showed the development of a sky blue colour, while negative reactions retained a violet colour. To validate the results, the LAMP-amplified products were reconfirmed through electrophoresis using a 2% agarose gel.

2.7. Assessment of cucurbit mildew disease severity

Powdery and downy mildew severity in the cucumber field were assessed using a cluster sampling method (Choudhury et al., 2016). The field was divided into two sections and denoted as an 'X' pattern to separate individual sampling points situated at the arm end of 'X'. The disease severity of cucurbits mildew was rated at each sampling point (presence or absence on individual leaves) by estimating the proportion of infected leaves. Data was collected weekly, from 14 to 30th standard weeks, during 3rd April to 30th July in 2023. The severity of mildew diseases was determined using a scoring system ranging from 0 to 9, as specified in section 2.1. The results were then converted into PDI using the formula of Wheeler (1969).

2.8. Statistical analysis

The PDI data were statistically analysed and the means of all pairs of groups in a dataset were compared using Tukey's Honestly Significant Difference (HSD) test (Tukey, 1949).

3. Results

3.1. Disease severity

Disease survey was conducted in the major cucurbits cultivating area of Tamil Nadu state of India. Powdery mildew severity ranged from 37.09 to 60.45 % while downy mildew severity varied between 4.51 and 18.25 % (Table 1).

3.2. Standardization of LAMP reaction condition

Six LAMP primers designed using ITS1, ITS2 and 5.8S ribosomal RNA gene sequence of *P. xanthii* and *CesA2* gene of *P. cubensis* detected the respective pathogens specifically. Among the various $MgSO_4$ concentrations examined, a strong sky-blue coloration was observed at 6 mM for *P. xanthii* (Fig. 2a) and 4 mM for *P. cubensis* (Fig. 2b). Lower (2 mM) and higher (10 mM) concentrations of $MgSO_4$ failed to facilitate template DNA amplification, indicated by the absence of the sky-blue colour development. Subsequently, under various incubation temperatures, colour development observed at 59, 62 and 65 °C for *P. xanthii* (Fig. 2c) and 62, 65 and 68 °C for *P. cubensis* (Fig. 2d). Robust sky-blue colour development was explicitly observed at 65 °C, while other incubation temperatures resulted in less intense colour. Subsequently, the incubation times, 60 and 75 min for *P. xanthii* (Fig. 2e) and 45, 60 and 75 min for *P. cubensis* (Fig. 2f) showed positive responses and an intense sky-blue coloration was observed at the 60-min. The above three parameters were subsequently validated through agarose gel electrophoresis, where a distinct and intense ladder-like pattern was observed with $MgSO_4$ concentrations of 6 mM for *P. xanthii* and 4 mM for *P. cubensis* and 65 °C incubation temperature and 60 min incubation time.

3.3. Sensitivity and specificity of the LAMP assay

An intense sky-blue colour was developed in all the tested template DNA concentrations except at 1.5 fg for both *P. xanthii* (Fig. 3a) and *P. cubensis* (Fig. 3b). These findings were further validated using agarose gel electrophoresis. In the LAMP assay specificity test, a distinct sky-blue colour was observed for *P. xanthii* (Fig. 3c) and *P. cubensis* (Fig. 3d), while a violet colour was noticed for other pathogen samples, indicating the absence of the target DNA molecule. The results were further validated in the agarose gel electrophoresis.

3.4. Validation of LAMP assay for detection of P. xanthii and P. cubensis

In the validation test, the LAMP assay displayed sky blue coloration in all 16 samples of *P. xanthii*, (Fig. 3e) and 12 samples of *P. cubensis* (Fig. 3f) collected from various locations in Tamil Nadu. As a negative control, the tube containing nuclease-free water remained violet colour even after a 60-min incubation at 65 °C. To confirm these results, 2% agarose gel electrophoresis showed a distinct ladder-like pattern in all the Lamp-positive samples, while the tube containing nuclease-free water failed to exhibit such a pattern.

3.5. Detecting airborne inocula of cucurbit mildew using suction spore trap and LAMP assay

The suction spore trap designed using PVC pipe and a cooling fan powered by a solar panel was able to capture 1953.86 L of air per minute. In the LAMP assay of pathogen inocula collected from traps during the 14, 15, 27, 28, 29 and 30th standard weeks, the reaction tube remained violet in colour. However, a distinct sky-blue colour development was noticed in samples from the 16 to 26th standard weeks for *P. xanthii* (Fig. 4a). In case of *P. cubensis*, samples from the 14th to 22nd standard weeks showed negative results, while a positive reaction was detected in samples from 23rd to 29th standard weeks (Fig. 4b). These results were further verified through 2% agarose gel electrophoresis,

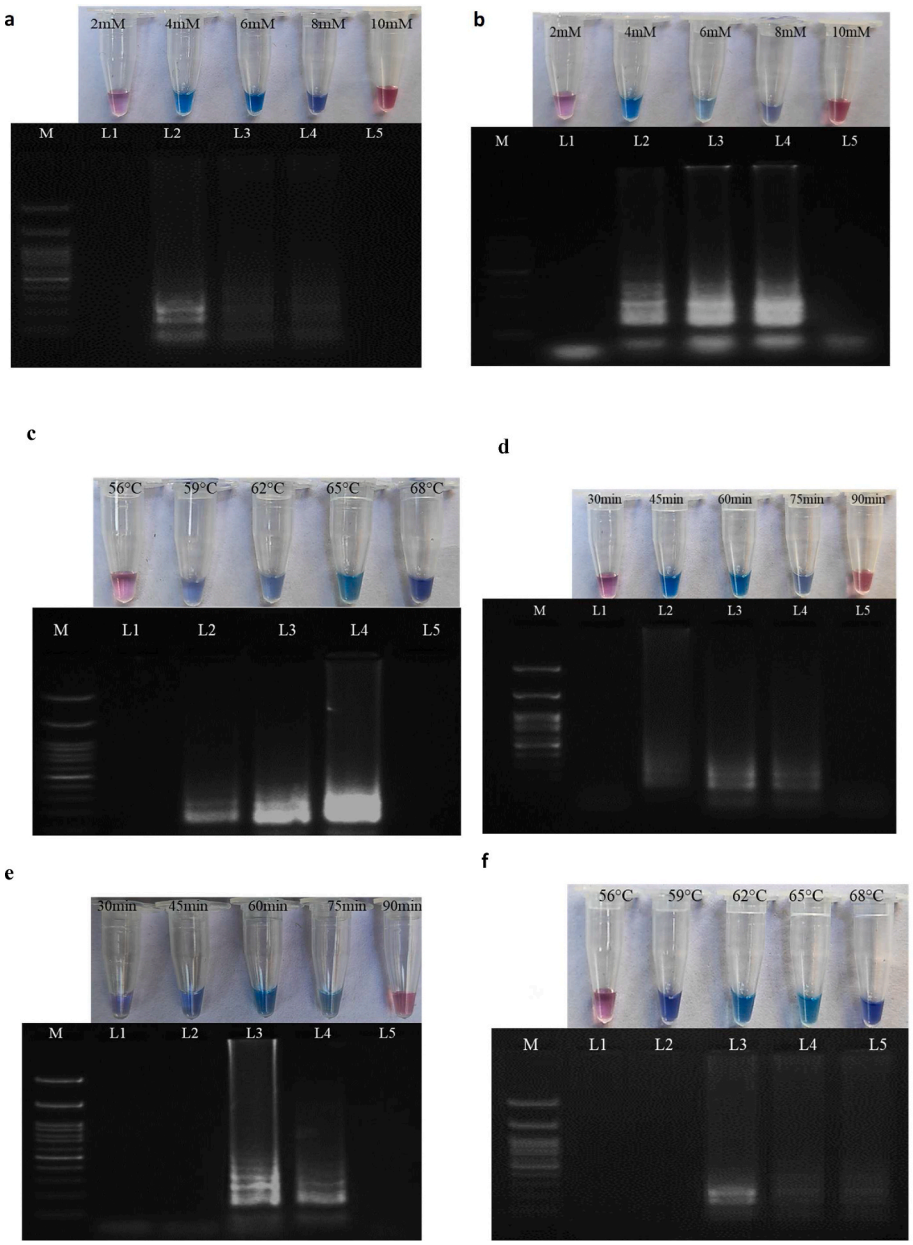


Fig. 2. Optimization of LAMP assay for *Podosphaera xanthii* and *Pseudoperonospora cubensis*. M: 100 bp ladder as a molecular marker. For $MgSO_4$ concentration, **a)** *P. xanthii,* **b)** *P. cubensis,* L1: 2 mM, L2: 4 mM, L3: 6 mM, L4: 8 mM and L5: 10 mM. Incubation temperature **c)** *P. xanthii* **d)** *P. cubensis*, Lane L 1: 56 °C, L2: 59 °C, L3: 62 °C, L4: 65 °C and L5: 68 °C. Incubation time, **e)** *P. xanthii*, **f)** *P. cubensis*, L1: 30 min, L2: 45 min, L3: 60 min, L4: 75 min and L5: 90 min.

which produced a characteristic ladder-like banding pattern in a positively reacted sample.

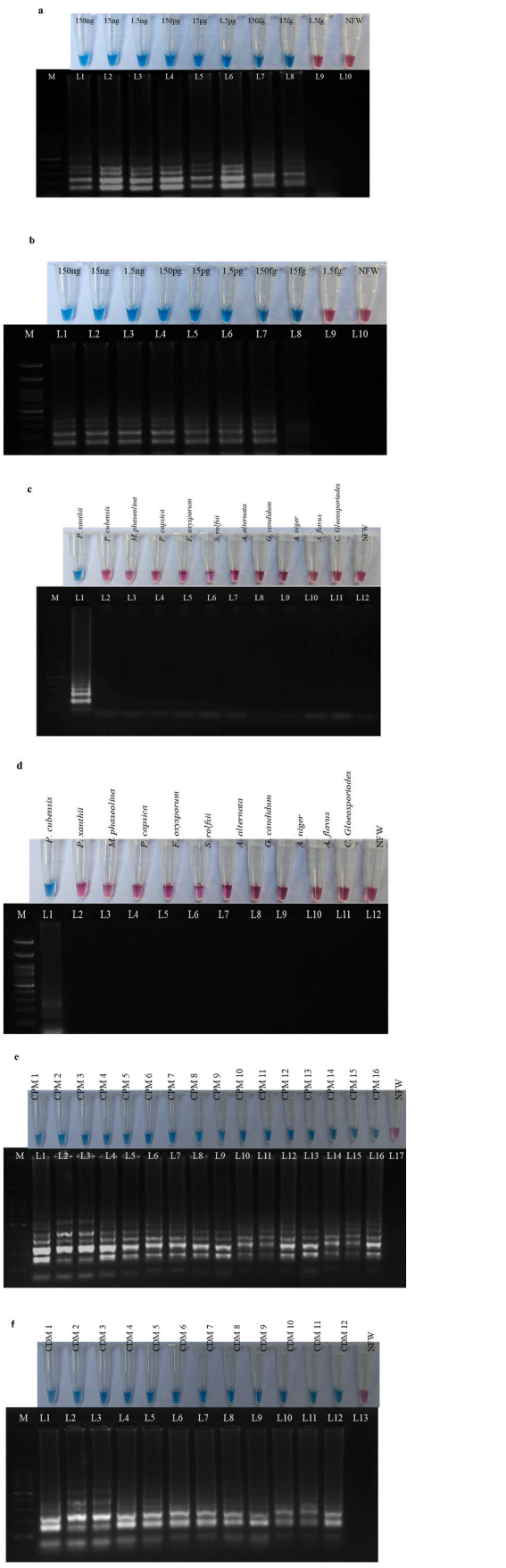
3.6. Assessment of cucurbit mildew severity in the field

Powdery mildew symptoms appeared during the 17th standard week, approximately 30 days after sowing, with a disease index of 2.8%. However, during the 22nd standard week, there was a slight decline in the severity. The highest occurrence of powdery mildew, reaching a 10.1% index, was recorded during the 24th standard week (Fig. 5). For downy mildew, the disease was first observed in the 23rd standard week with a disease index of 5.4%. Subsequently, the severity rose to 11.2% during the 27th Standard week and then decreased gradually. From 14th to 22nd standard weeks, there is no disease occurrence of downy mildew (Fig. 5).

4. Discussion

4.1. Conserved DNA segments for LAMP primer design

The effectiveness of the LAMP assay depends on the specificity of the selected DNA segments to the targeted plant pathogen. In this present study, ITS1, ITS2 and 5.8S ribosomal RNA sequence for *P. xanthii* and *CesA2* gene for *P. cubensis* were found ideal for designing pathogen-specific LAMP primers. Similarly, Thiessen et al. (2016) designed LAMP primers targeting the sequences of the ITS1, ITS2, and 5S ribosomal RNA encoding regions of *Erysiphe necator* when ITS2 region was used to develop LAMP primers for the detection of *Phytophthora infestans,* causing late blight in potato (Ristaino et al., 2020). Alternatively, *β-tubulin* gene were also targeted for designing LAMP primers as demonstrated for Spanish cucurbit powdery mildew, *P. xanthii* (Vielba-Fernández et al., 2019). Gene *TEF-1α* is the other targeted region



(caption on next column)

Fig. 3. Sensitivity, specificity, and validation of LAMP assay for the detection of *Podosphaera xanthii* and *Pseudoperonospora cubensis.* M for 100 bp ladder as a molecular marker and control is nuclease-free water (NFW). Sensitivity of LAMP assay; **a**) *P. xanthii* and **b**) *P. cubensis*. Specificity of LAMP assay; **c**) *P. xanthii* and **d**) *P. cubensis*. For *P. xanthii* (**c**) L1: *P. xanthii* and L2: *P. cubensis* while for *P. cubensis* (**d**) L1: *P. cubensis* and L2: *P. xanthii*. For both **c** and **d** L3: *Macrophomina phaseolina,* L4: *Phytophthora capsici,* L5: *Fusarium oxysporum,* L6: *Sclerotium rolfsii,* L7: *Alternaria alternata,* L8: *Geotrichum candidum*, L9: *Aspergillus niger,* L10: *A. flavus,* L11: *Colletotrichum gloeosporiodes*. Validation of LAMP assay; **e**) *P. xanthii*, L1: Positive control, L2 to L16: CPM 1 – CPM 16 powdery mildew infected samples, **f**) *P. cubensis* L1: Positive control, L2 to L13: CDM 1 – CDM 12 downy mildew infected samples.

that is used for the detection of *F. acuminatum* and *F. solani* pathogens (Wang et al., 2022).

4.2. Standardization of the LAMP assay protocol

Optimization of $MgSO_4$ concentration, incubation time, and temperature in LAMP reaction plays a crucial role for DNA polymerization and to generate pyrophosphate ions whose interaction with magnesium results in observable turbidity in positive samples (Mori et al., 2001). In our study $MgSO_4$ concentration of 4 mM, resulted in a strong sky blue colour for *P. cubensis*, as similarly noticed for the LAMP-based detection of *Plasmopara viticola*, responsible for downy mildew disease in grapes (Marimuthu et al., 2020). For *P. xanthii* a 6 mM concentration was ideal as observed for *Bipolaris oryzae* detection in rice (Lakshmi et al., 2022).

The LAMP technique utilizes *Bst* polymerase, a thermophilic enzyme to enhance DNA strand displacement under isothermal conditions, typically operate within the temperature range of 60–65 °C (Chander et al., 2014). In our present study, optimal conditions of 62 °C temperature and 60 min of incubation time produced an intense sky-blue colour for *P. cubensis* similar to *Fusarium fujikuroi* detection in rice (Sunani et al., 2019). In the optimization of LAMP assay for *F. oxysporum* f. sp. *ciceris*, causing wilt in chickpea, an incubation condition of 63 °C for 60 min resulted in an intense sky blue colour as noticed also for the recognition of *Ustilago tritici,* causing loose smut of wheat (Ghosh et al., 2015b; Yan et al., 2019).

As noticed earlier for *Phytophthora sojae* in soybean (Zhao et al., 2015), an incubation condition of 65 °C and 60 min was ideal for *P. xanthii* detection. However, Yang et al. (2022) found a similar temperature condition but with a reduced incubation period of 35 min as ideal for screening *Plasmodiophora brassicae*, which causes club-root disease in *Brassica* species.

4.3. Sensitivity of LAMP assay to detect pathogen at low concentrations

In our study, the LAMP assays were able to detect these pathogens at a very low genomic DNA concentration of 15 fg as compared to 33 fg required for *P. viticola* (Kong et al., 2016). Whereas a slightly higher DNA concentration of 100 fg was required for *Puccinia triticina* and *F. graminearum,* causing leaf rust and head blight disease, respectively in wheat (Gupta et al., 2020; Manjunatha et al., 2018). Recently, for the detection of *F. oxysporum* f. sp. *ciceris*, causing *Fusarium* wilt in chickpea, a DNA concentration of at least 0. 9 pg/μl was found essential (Achari et al., 2023). DNA concentrations of 5 and 100 pg remained vital for *M. oryzae* and *Colletotrichum falcatum* detections (Chandra et al., 2015). Interestingly, a higher dose of 1 ng/μl was required for the detection of Spanish cucurbit powdery mildew pathogen *P. xanthii* (Vielba-Fernández et al., 2019).

4.4. Specificity of LAMP assay for the detection of P. xanthii and P. cubensis

LAMP assay is one of the highly effective tools for detecting specific pathogens. Here we demonstrate the specificity of the assay for cucurbit

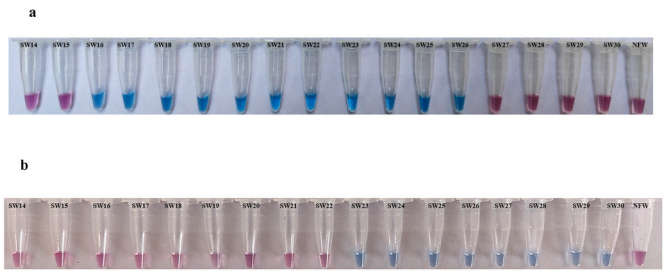


Fig. 4. LAMP assay to detect airborne inocula of **a**) *Podosphaera xanthii* and **b**) *Pseudoperonospora cubensis* collected from suction spore trap at Tamil Nadu Agricultural University orchard. DNA extracted from the sampling plates of suction spore trap used as a template. M - 100 bp ladder as a molecular marker, SW 14 to SW30: DNA samples analysed are from air samples collected from 14 to 30th standard weeks of 2023 and Nuclease free water (NFW).

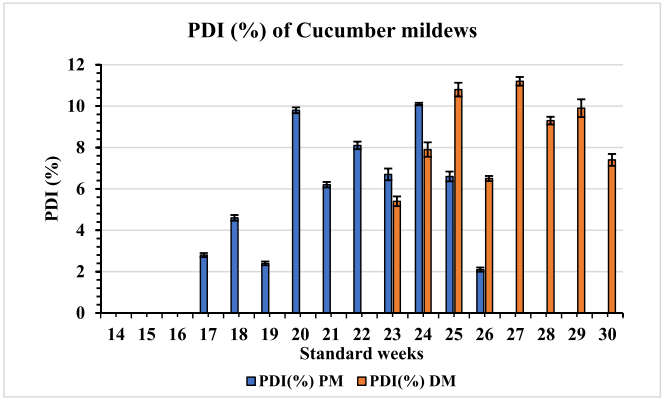


Fig. 5. Percent disease index (PDI) of cucurbit mildew diseases under field conditions in Tamil Nadu Agricultural University orchard. Blue bars represent PDI for powdery mildew (PM), while the orange bar is for downy mildew (DM) at different weeks during the cropping season. Error bar in each standard week represent the standard deviation of PDI (n = 3).

mildew pathogens by testing with 9 diverse plant pathogens namely *M. phaseolina*, *P. capsici*, *F. oxysporum*, *S. rolfsii*, *A. alternata*, *G. candidum*, *A. niger*, *A. flavus* and *C. gloeosporiodes*. Earlier studies have demonstrated the specificity of LAMP assay with collections more extensive than the current study as the assay developed for *P. viticola* was tested with 38 plant pathogens. Further, the LAMP assay can also distinguish pathogens at the species level, as evident from the studies on *C. falcatum* (Kong et al., 2016; Chandra et al., 2015).

4.5. Validation of LAMP assay for the detection of P. xanthii and P. cubensis

Using the LAMP assay, we effectively confirmed the identification of *P. xanthii* and *P. cubensis* in the infected samples collected from various cucurbit-growing areas in Tamil Nadu. Similar to cucurbit mildew, LAMP assays were validated for *Plasmopara viticola,* causing downy mildew disease in grapes (Marimuthu et al., 2020) and for *Venturia inaequalis,* causing apple scab in apples (Xu et al., 2021).

4.6. Design of suction spore trap

In addition to the LAMP assay, here we also designed a suction spore trap to detect mildew pathogen from airborne inocula. Further, the provincially made device is operated through solar panels, thereby readily applicable to Indian farming settings where access to uninterrupted electricity is rare. The trap was designed based on the kit used for capturing grapevine mildew inocula (Basha et al., 2021). A battery-operated trap can also be an alternative in the absence of solar and electric power supplies (Rahman et al., 2021; Saha et al., 2020).

4.7. Detection of airborne inocula of cucurbit mildew using LAMP assay

LAMP assays are highly recommended for the detection and quantification of airborne inocula of crop pathogens. Here, a LAMP assay successfully detected the presence of *P. xanthii* and *P. cubensis* in the inoculum collected via suction spore traps during the primary crop season. Similarly, a LAMP assay enabled the detection of grapevine powdery mildew pathogen *Erysiphe necator* from the air samples (Basha et al., 2021).

5. Conclusion

Using ITS1 and ITS2 and 5.8S ribosomal RNA and *CesA2* sequences, we successfully developed a LAMP and suction spore trap-based assay for the detection of *P. xanthii* and *P. cubensis*, causing mildew disease in cucurbits. While the LAMP technique can only detect individual pathogens, the next step is to develop reverse transcription LAMP (RT-LAMP) and multiplex-based assays (Venbrux et al., 2023) for real-time and simultaneous detection of multiple pathogens that will eventually reduce the time and cost in addition to early and accurate detection of diseases to minimise crop yield losses.

Ethics approval and consent to participate

Experiments were conducted in accordance with the relevant institutional guidelines. Doesn't involve any experiments with animals.

Consent for publication

Not applicable.

Availability of data and materials

Seeds of plant materials used in this study are available from the corresponding author by request.

Funding

Not applicable.

CRediT authorship contribution statement

Ananthi Palanisamy: Writing – original draft, Validation, Investigation, Formal analysis, Data curation. **Muthusamy Karthikeyan:** Writing – original draft, Supervision, Methodology, Investigation, Data curation, Conceptualization. **Shajith Basha Jaffer:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Shanmuga Priya Dhanabalan:** Writing – review & editing, Investigation, Formal analysis. **Bavish Shanmugavel:** Formal analysis, Data curation. **Iruthayasamy Johnson:** Writing – review & editing, Supervision, Data curation. **Natarajan Revathy:** Writing – review & editing, Supervision, Data curation. **Narayanan Manikanda Boopathi:** Writing – review & editing, Supervision, Data curation. **Ganapati Patil Santosh:** Writing – review & editing, Supervision, Formal analysis. **Sambasivam Periyannan:** Writing – review & editing, Supervision, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Sambasivam Periyannan reports administrative support and article publishing charges were provided by University of Southern

Queensland. Sambasivam Periyannan reports a relationship with University of Southern Queensland that includes: employment. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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References

Achari, S.R., Mann, R.C., Sharma, M., Edwards, J., 2023. Diagnosis of *Fusarium oxysporum* f. sp. *ciceris* causing Fusarium wilt of chickpea using loop-mediated isothermal amplification (LAMP) and conventional end-point PCR. Sci. Rep. 13 (1), 2640. https://doi:10.1038/s41598-023-29730-6.

Basha, J.S., Kamalakannan, A., Saraswathy, S., Johnson, I., Ganapati, P.S., Lakshmi, K.R. S., 2021. Rapid detection of airborne inocula of grapevine mildews using PCR and LAMP assay. Int. J. Soil Sci. 33, 12–21. https://doi:10.9734/ijpss/2021/v33i2230677.

Bello, J.C., Sakalidis, M.L., Perla, D.E., Hausbeck, M.K., 2021. Detection of airborne sporangia of *Pseudoperonospora cubensis* and *P. humuli* in Michigan using Burkard spore traps coupled to quantitative PCR. Plant Dis. 105 (5), 1373–1381. https://doi:10.1094/PDIS-07-20-1534-RE.

Chander, Y., Koelbl, J., Puckett, J., Moser, M.J., Klingele, A.J., Liles, M.R., 2014. A novel thermostable polymerase for RNA and DNA loop-mediated isothermal amplification (LAMP). Front. Microbiol. 5, 395. https://doi:10.3389/fmicb.2014.00395.

Chandra, A., Keizerweerd, A.T., Que, Y., Grisham, M.P., 2015. Loop-mediated isothermal amplification (LAMP) based detection of *Colletotrichum falcatum* causing red rot in sugarcane. Mol. Biol. Rep. 42, 1309–1316. https://doi:10.1007/s11033-015-3875-9.

Choudhury, R.A., Koike, S.T., Fox, A.D., Anchieta, A., Subbarao, K.V., Klosterman, S.J., 2016. Season-long dynamics of spinach downy mildew determined by spore trapping and disease incidence. Phytopathology 106 (11), 1311–1318. https://doi:10.1094/PHYTO-12-15-0333-R.

Cohen, Y., Van den Langenberg, K.M., Wehner, T.C., Ojiambo, P.S., Hausbeck, M., Quesada-Ocampo, L.M., 2015. Resurgence of *Pseudoperonospora cubensis*: the causal agent of cucurbit downy mildew. Phytopathology 105 (7), 998–1012. https://doi:10.1094/PHYTO-11-14-0334-FI.

Elsharkawy, M.M., Kamel, S.M., El-Khateeb, N.M., 2014. Biological control of powdery and downy mildews of cucumber under greenhouse conditions. Egypt. J. Biol. Pest Control 30, 1–7. https://doi.org/10.1186/s41938-020-00267-4.

FAO, 2021. https://www.fao.org/state-of-food-security-nutrition/2021.

Freeman, W.M., Vrana, S.L., Vrana, K.E., 1996. Use of elevated reverse transcription reaction temperatures in RT-PCR. Biotechniques 20 (5), 782–783. https://doi:10.2144/96205bm09.

Ghosh, D., Bhattacharya, I., Dutta, S., Saha, A., Mazumdar, D., 2015a. Dependence of the weather on outbreak of cucumber downy mildew (*Pseudoperonospora cubensis*) in eastern India. J. Agric. Meteorol. 17 (1), 43–50.

Ghosh, R., Nagavardhini, A., Sengupta, A., Sharma, M., 2015b. Development of Loop-Mediated Isothermal amplification (LAMP) assay for rapid detection of *Fusarium oxysporum* f. sp. *ciceris*-wilt pathogen of chickpea. BMC Res. Notes 8, 1–10. https://doi:10.1186/s13104-015-0997-z.

Goldenhar, K.E., Hausbeck, M.K., 2019. Fungicides for control of downy mildew on pickling cucumber in Michigan. Plant Health Prog. 20 (3), 165–169. https://doi.org/10.1094/PHP-04-19-0025-RS.

Gupta, S.K., Gupta, A., Shyam, K.R., Bhardwaj, R., 2001. Morphological characterization and effect of meteorological factors on development of cucumber powdery mildew. Indian Phytopathol. 54 (3), 311–315.

Gupta, S., Saharan, M.S., Gurjar, M.S., Singh, J., Bashyal, B.M., Aggarwal, R., 2020. Molecular detection of *Fusarium graminearum* causing head blight of wheat by loop mediated isothermal amplification (LAMP) assay. Indian Phytopathol. 73, 667–672. https://doi.org/10.1007/s42360-020-00261-8.

Huang, C.M., Liao, D.J., Wu, H.S., Shen, W.C., Chung, C.L., 2016. Cyclone-based spore trapping, quantitative real-time polymerase chain reaction and high resolution melting analysis for monitoring airborne inoculum of *Magnaporthe oryzae*. Ann. Appl. Biol. 169 (1), 75–90. https://doi:10.1111/aab.12282.

Jackson, S.L., Bayliss, K.L., 2011. Spore traps need improvement to fulfil Plant Biosecurity requirements. Plant Pathol. 60 (5), 801–810. https://doi:10.1111/j.1365-3059.2011.02445.x.

Jamadar, M.M., Desai, S.A., 1997. Bioefficacy of dimethomorph against downy mildew of grapevine. Adv. Agric. Res. India 7, 81–85.

Jeffrey, C., Bates, D.M., Robinson, R.W., Jeffrey, C., 1990. Systematic of the cucurbitaceae. In: Biology and Utilization of the Cucurbitaceae. Cornel University Press, Ithaca, pp. 449–463.

Kong, X., Qin, W., Huang, X., Kong, F., Schoen, C.D., Feng, J., 2016. Development and application of loop-mediated isothermal amplification (LAMP) for detection of *Plasmopara viticola*. Sci. Rep. 6 (1), 28935. https://doi:10.1038/srep28935.

Lakshmi, K.R.S., Kamalakannan, A., Gopalakrishnan, C., Rajesh, S., Panneerselvam, S., Ganapati, P.S., 2022. Loop-mediated isothermal amplification assay: a specific and sensitive tool for the detection of *Bipolaris oryzae* causing brown spot disease in rice. Phytoparasitica 50 (3), 543–553. https://doi:10.1007/s12600-022-00979-3.

Lebeda, A., Cohen, Y., 2011. Cucurbit downy mildew (*Pseudoperonospora cubensis*) -biology, ecology, epidemiology. Eur. J. Plant Pathol. 129, 157–192. https://doi:10.1007/s10658-010-9658-1.

Manjunatha, C., Sharma, S., Kulshreshtha, D., Gupta, S., Singh, K., Bhardwaj, S.C., 2018. Rapid detection of *Puccinia triticina* causing leaf rust of wheat by PCR and loop mediated isothermal amplification. PLoS One 13 (4), e0196409. https://doi:10.1371/journal.pone.0196409.

Marimon, N., Eduardo, I., León, M., Berbegal, M., Armengol, J., Luque, J., 2020. A qPCR-based method for the detection and quantification of the peach powdery mildew (*Podosphaera pannosa*) in epidemiological studies. Eur. J. Plant Pathol. 158 (4), 1005–1016. https://doi:10.1007/s10658-020-02136-0.

Marimuthu, K., Ayyanar, K., Ganesan, M.V., Vaikuntavasan, P., Uthandi, S., Mathiyazhagan, K., 2020. Loop-mediated isothermal amplification assay for the detection of *Plasmopara viticola* infecting grapes. J. Phytopathol. 168 (3), 144–155. https://doi:10.1111/jph.12866.

McDermott, J.M., Brandle, U., Dutly, F., Haemmerli, U.A., Keller, S., Muller, K.E., 1994. Genetic variation in powdery mildew of barley: development of RAPD, SCAR, and VNTR markers. Phytopathology 84 (11), 1316–1321. https://doi.org/10.1094/phyto-84-1316.

Mori, Y., Nagamine, K., Tomita, N., Notomi, T., 2001. Detection of loop-mediated isothermal amplification reaction by turbidity derived from magnesium pyrophosphate formation. Biochem. Biophys. Res. Commun. 289 (1), 150–154. https://doi:10.1006/bbrc.2001.5921.

Nagamine, K., Hase, T., Notomi, T., 2002. Accelerated reaction by loop-mediated isothermal amplification using loop primers. Mol. Cell. Probes 16 (3), 223–229. https://doi:10.1006/mcpr.2002.0415.

Rahman, A., Standish, J.R., Darcangelo, K.N., Quesada-Ocampo, L.M., 2021. Clade-specific biosurveillance of *Pseudoperonospora cubensis* using spore traps for precision disease management of cucurbit downy mildew. Phytopathology 111 (2), 312–320. https://doi:10.1094/PHYTO-06-20-0231-R.

Ristaino, J.B., Saville, A.C., Paul, R., Cooper, D.C., Wei, Q., 2020. Detection of *Phytophthora infestans* by loop-mediated isothermal amplification, real-time LAMP, and droplet digital PCR. Plant Dis. 104 (3), 708–716. https://doi:10.1094/PDIS-06-19-1186-RE.

Saha, M., Chakraborty, A., Bhattacharya, K., 2020. Aerobiology, epidemiology and disease forecasting of false smut disease of rice in West Bengal, India. Aerobiologia 36, 299–304. https://doi:10.1007/s10453-020-09631-1.

Sunani, S.K., Bashyal, B.M., Rawat, K., Manjunatha, C., Sharma, S., Prakash, G., 2019. Development of PCR and loop mediated isothermal amplification assay for the detection of bakanae pathogen Fusarium fujikuroi. Eur. J. Plant Pathol. 154, 715–725. https://doi:10.1007/s10658-019-01694-2.

Thiessen, L.D., Keune, J.A., Neill, T.M., Turechek, W.W., Grove, G.G., Mahaffee, W.F., 2016. Development of a grower-conducted inoculum detection assay for management of grape powdery mildew. Plant Pathol. 65 (2), 238–249. https://doi:10.1111/ppa.12421.

Tomita, N., Mori, Y., Kanda, H., Notomi, T., 2008. Loop-mediated isothermal amplification (LAMP) of gene sequences and simple visual detection of products. Nat. Protoc. 3 (5), 877–882. https://doi:10.1038/nprot.2008.57.

Tukey, J.W., 1949. Comparing individual means in the analysis of variance. Biometrics 5 (2), 99–114. https://doi.org/10.2307/3001913.

Venbrux, M., Crauwels, S., Rediers, H., 2023. Current and emerging trends in techniques for plant pathogen detection. Front. Plant Sci. 14, 1120968. https://doi.org/10.3389/fpls.2023.1120968.

Vielba-Fernández, A., de Vicente, A., Pérez-García, A., Fernández-Ortuño, D., 2019. Monitoring methyl benzimidazole carbamate-resistant isolates of the cucurbit powdery mildew pathogen, *Podosphaera xanthii*, using loop-mediated isothermal amplification. Plant Dis. 103 (7), 1515–1524. https://doi:10.1094/PDIS-12-18-2256-RE.

Wang, Y., Wang, C., Ma, Y., Zhang, X., Yang, H., Li, G., 2022. Rapid and specific detection of *Fusarium acuminatum* and *Fusarium solani* associated with root rot on *Astragalus membranaceus* using loop-mediated isothermal amplification (LAMP). Eur. J. Plant Pathol. 163 (2), 305–320. https://doi:10.1007/s10658-022-02478-x.

Wastling, S.L., Picozzi, K., Kakembo, A.S., Welburn, S.C., 2010. LAMP for human African trypanosomiasis: a comparative study of detection formats. PLoS Neglected Trop. Dis. 4 (11), e865. https://doi:10.1371/journal.pntd.0000865.

Wheeler, B.E.J., 1969. An Introduction to Plant Disease. John Wiley and Sons Ltd, London, UK, p. p254.

Xu, L., Wang, Y., Zhu, S., Li, J., Chang, Y., Huang, L., 2021. Development and application of a LAMP assay for the detection of the latent apple tree pathogen Valsamali. Plant Dis. 105 (4), 1065–1071. https://doi:10.1094/PDIS-07-20-1449-RE.

Yan, H., Zhang, J., Ma, D., Yin, J., 2019. qPCR and loop mediated isothermal amplification for rapid detection of *Ustilago tritici*. PeerJ 7, e7766. https://doi:10.7717/peerj.7766.

Yang, X., Sun, L., Sun, H., Hong, Y., Xia, Z., Pang, W., 2022. A loop-mediated isothermal DNA amplification (LAMP) assay for detection of the club root pathogen *Plasmodiophora brassicae*. Plant Dis. 106 (6), 1730–1735. https://doi:10.1094/PDIS-11-21-2430-RE.

Zhao, W., Wang, T., Qi, R., 2015. *Ypt1* gene-based detection of *Phytophthora sojae* in a loop-mediated isothermal amplification assay. J. Plant Dis. Prot. 122, 66–73. https://doi:10.1007/BF03356533.