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RESEARCH ARTICLE

Characterisation of *Phytophthora melonis* Katsura causing collar rot of pointed gourd in Eastern India and evaluation of fungicides for its management

Lellapalli Rithesh[1,2], Madhuri Pattanaik[1], Supriya Sahu[1], Dolly Pradhan[1], Muhammed Fayad A[3], Srinivas Petikam[1] & Sangeetha Ganesan[1,4*]

[1]ICAR-IIHR-Central Horticultural Experiment Station, Bhubaneswar 751 019, Odisha, India
[2]Orissa Institute of Agriculture and Technology, Bhubaneswar 751 019, Odisha, India
[3]ICAR-Indian Institute of Spices Research, Kozhikode 673 012, Kerala, India
[4]ICAR-Indian Institute of Horticultural Research, Bangalore 560 089, Karnataka, India

*Correspondence email - G.Sangeetha@icar.org.in; sangeethaau@hotmail.com

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Abstract

Pointed gourd (*Trichosanthes dioica* Roxb.) is an important perennial cucurbitaceous vegetable grown extensively in Eastern and Northern India. A disease caused by *Phytophthora melonis* infects the leaves, fruits and vines of pointed gourd, causing severe losses. Although it is known that these oomycetes cause disease in all above-ground plant parts, the mortality of plants due to extensive collar and root rot phases was observed in our study. Hence, this study aimed to characterise the pathogens associated with collar and root rot in pointed gourd, as well as its management. Two *Phytophthora* isolates were obtained from the collar and root rot phase, as well as eight isolates from symptomatic parts of leaves, vines and fruits. All 10 isolates were subjected to morphological and molecular characterisation. Basic morphological and taxonomic characteristics revealed that all 10 isolates belong to *Phytophthora* spp., which produced non-papillate sporangia with characteristic internal proliferation. The fungi were further identified by analysing genomic regions representing the inter-transcribed spacer (*ITS*) and mitochondrial cytochrome c oxidase (*COX*) regions. By comparing DNA sequences within the NCBI GenBank database and performing a multigene phylogenetic analysis involving the *ITS* and *COX* genes, the identity of all 10 isolates was confirmed as *P. melonis*. *In vitro* evaluation of fungicides using poisoned food and detached leaf assays demonstrated the efficacy of dimethomorph, tebuconazole, copper hydroxide and selected premixed fungicide combinations in controlling *P. melonis* growth. These findings highlight the potential of these fungicides for field applications in managing diseases caused by *P. melonis*.

Keywords: collar and root rot; etiology; integrated management; pointed gourd; *Phytophthora*

Introduction

Cucurbits hold immense importance in India's agricultural landscape, constituting a vital component of the country's food production system. These versatile crops play a crucial role in addressing food security and nutritional needs, serving as staple ingredients in various regional cuisines and providing essential vitamins and minerals to millions of people across the nation. From pumpkins and squash to cucumbers and gourds, cucurbits are widely cultivated throughout India, contributing significantly to both rural livelihoods and the national economy (1). Among these cucurbitaceous treasures, pointed gourd (*Trichosanthes dioica* Roxb.), a perennial cucurbitaceous vegetable crop native to South Asia, holds a significant position in agricultural landscapes, particularly in Eastern and Northern part of India, where it is extensively cultivated (2). Distinguished by its perennial growth habit, dioecism and vegetative propagation, this unique gourd species stands apart morphologically from its cucurbit counterparts. Renowned as the "King of Gourds", the pointed gourd boasts a nutritional profile superior to other gourd varieties (3). In India alone, it occupies approximately 77000 hectares of agricultural land, yielding an impressive 1008000 metric tons (First advance estimates 2024–25, Ministry of Agriculture & Farmers Welfare, Government of India), with commercial cultivation concentrated primarily in Bihar, Odisha, Jharkhand, Uttar Pradesh, Assam, Tripura and West Bengal and to a lesser extent in Madhya Pradesh, Maharashtra and Gujarat.

Despite its agricultural significance, the pointed gourd faces a plethora of challenges from various pathogens, including 11 fungal diseases, a nematode and several viruses, documented within India (4). Notable among these are downy mildew, root-knot nematode and fruit and vine rot, which inflict substantial losses upon crop yields. Leveraging *in vitro* and *in vivo* assays, this research endeavors to provide valuable insights and practical solutions to mitigate the impact of this debilitating disease on pointed gourd

cultivation. Fruit and vine rot, caused by *Phytophthora*, presents distinctive symptoms such as softening, browning and decay of the fruit and vines. Vine symptoms include wilting, browning and decay of stems, often leading to vine collapse. Affected fruits may exhibit water-soaked lesions, which eventually progressed into rotting which often led to significant yield losses for farmers (5).

However, during the period of study (2020–23), we observed rotting of the crown portion and roots, lead to sudden death of entire plant. Collar rot occurred at the lower section of the stem at the soil line, whereas root rot impacted the root system which lead to rotting and disintegration. Continuous rain during south west monsoon lead to serious collar and root rot problem mainly on the plants raised in trellis where in the plant has single tap root as against multiple root system in soil bed system of cultivation. Preliminary investigation of below ground portion revealed the rotting of entire roots and tubers and further isolation yielded *Phytophthora* species. In light of these challenges, the present study aims to expand upon previous research efforts by undertaking a comprehensive investigation into the etiology and management of collar and root rot in pointed gourds.

Materials and Methods

Sampling and isolation

Samples exhibiting rotting symptoms of pointed gourd were collected from an experimental farm at the Central Horticultural Experiment Station (ICAR-Indian Institute of Horticultural Research) in Bhubaneswar, Odisha, India (20.14° N, 85.46° E). These samples were transported to a research facility for the isolation and identification of the causative agent. Specifically, four sections measuring 5 × 5 mm^2 were excised from the symptomatic tissue and the adjacent healthy, uninfected area at the advancing edge of lesions. Following a 1-min surface sterilisation with 1 % sodium hypochlorite, the tissues were rinsed three times with sterilised distilled water. The tissue pieces were then air-dried on sterilised blotting paper and aseptically transferred to Petri plates containing fresh potato carrot infusion agar (PCA) medium, supplemented with streptomycin sulphate (100 ppm) to inhibit bacterial contamination. The plates were incubated at 25 ± 1 °C under continuous illumination, with periodic observations to monitor fungal growth. Emerging hyphal tips from the infected tissues were aseptically transferred to new PCA dishes and incubated at 25 ± 1 °C with a 12-hr photoperiod. Based on morphological characteristics, the pure cultures were initially identified to the genus level and designated as shown in Table 1. For further research, pure cultures of the isolates were maintained on PCA slants at 4 °C.

Phenotypic characterisation

The pathogen isolates were cultured on PCA medium to assess the characteristics of the colonies. Mycelial discs, each measuring 8 mm, were excised from 7-day-old cultures of each isolate and subsequently transferred to PCA dishes. The Petri plates were incubated at a temperature of 25 ± 1 °C for one week. The mean radial growth (mm per day) and colony colour of each isolate were determined. To induce sporangial production, 5–6 mycelia plugs (8 mm diameter) were removed from the pure cultures and added with 15 mL of 2 % unsterile soil water extract (6) and incubated for 2-3 days at 25 ± 1 °C. Observations regarding conidial characteristics, including the morphology of sporangia and the presence of papillae, were conducted using an Olympus Bx53 microscope equipped with a digital camera. The conidia were mounted in 100 % lactic acid. Preliminary identification of the isolates was based on colony colour, morphology, as well as the shape, size and growth patterns of the conidia (7).

Pathogenicity tests

The pathogenicity of fungal isolates such as CHES-R2-2018 and CHES-R3-2018 was evaluated on rooted, disease-free and uniform size pointed gourd cuttings. For that healthy cuttings were selected and raised in 10 × 10 cm size plastic pots having 300 g of sterilised potting mixture consist of in red soil: coir pith and sand 2:1:1. The cuttings were allowed to establish for 30 days on green house bench and watered usually once a day. Then, both isolates were administered on total of sixty cuttings respectively by soil drenching method. The isolates CHES-R2-2018 and CHES-R3-2018 were cultured on PCA agar medium at 26 ± 1 °C for a duration of 1 week. The inoculum was prepared by sectioning the PCA culture medium into 10–15 pieces and transferring these to sterile distilled water in Petri plates.

The culture plates were incubated at 26 ± 1 °C for three days under continuous light to facilitate sporangium formation. Following the abundant formation of sporangia, the plates were maintained at 4 °C for 30 min and subsequently returned to 26 ± 1 °C for 2 hr to induce zoospore release (Fig. 1B). The zoospores were collected and quantified using a hemacytometer and the inoculum concentration was adjusted to 1000 zoospores/mL, followed by homogenisation through manual shaking. Thirty pots, each planted with pointed gourd vines, were inoculated with 10 pathogen isolates in three replicates. Each pot received an inoculation of 20 mL, resulting in a final concentration of 20000 zoospores per pot. The plants were irrigated twice daily and disease incidence was periodically assessed. The isolates obtained from the inoculated

Table 1. Isolates of *Phytophthora* used in this study

Sl. No.	Isolate name	Plant parts from where isolation is made	Location of isolation
1	CHES-R2-2018	Root	Experimental orchard, IIHR-CHES, Bhubaneswar, Odisha
2	CHES-R3-2018	Root	Experimental orchard, IIHR-CHES, Bhubaneswar, Odisha
3	CHES-F-2018	Leaf	Experimental orchard, IIHR-CHES, Bhubaneswar, Odisha
4	CHES-E-2018	Fruit	Experimental orchard, IIHR-CHES, Bhubaneswar, Odisha
5	CHES-BA-2018	Leaf	Bhadrak Dt, Odisha
6	CHES-S-2018	Fruit	Puri Dt, Odisha
7	CHES-J-2018	Fruit	Dhenkanal Dt, Odisha
8	CHES-JA-2018	Leaf	Jajpur Dt, Odisha
9	CHES-JAG-2018	Leaf	Jagatsingpur Dt, Odisha
10	CHES-H-2018	Leaf	Cuttack Dt, Odisha

plants were identified and compared to the original isolates, thereby fulfilling Koch's postulates.

Molecular characterisation

For further identification, all the isolates were molecularly characterised. The pure cultures were grown in potato carrot infusion broth amended with ampicillin (0.2 g/L) for DNA extraction and incubated at 22 ± 1 for 12 days. The mycelial mats of representative isolates were harvested, transferred through sterilised filter paper (Whatman No. 1), washed with sterile distilled water and allowed to desiccate for 20–30 min. Then, the total genomic DNA of the isolates was extracted using the CTAB method. The quality of the DNA was assessed using a 0.8 % agarose gel prior to storage at −20 ℃ for subsequent use. A segment of the internal transcribed spacer region of ribosomal DNA (*ITS*-rDNA) and the mitochondrial cytochrome c oxidase (*COX*) regions was amplified using the primer pairs ITS-4/ITS-6 (5′ -TCCTCCGCTTATTGATATGC-3′/5′-GAAGGTGAAGTCGTAACAAGG-3′) and COXF4N/COXR4N (5′-GTATTTCTTCTTTATTAGGTGC-3′/5′-CGTGAACTAATGTTACATATAC-3′) (8, 9). The polymerase chain reaction (PCR) amplification was conducted using the MasterCycler Nexus gradient thermocycler (Eppendorf). The PCR reaction mixture comprised 20 µL of emerald master mix (Takara Bio Inc.), 4 µL each of 10 pMol primers and 4 µL of genomic DNA, with the final volume adjusted to 40 µL using 8 µL of sterile nuclease-free water. The DNA amplification conditions were as follows: initial denaturation at 95 ℃ for 4 min; denaturation at 95 ℃ for 60 sec; annealing at 56 ℃ for 60 sec; extension at 72 ℃ for 1 min; and a final extension at 72 ℃ for 10 min. The PCR products, along with a DNA ladder, were subjected to electrophoresis (1 hr at 80 V) in a 2 % agarose gel in 0.5X TAE buffer and stained with ethidium bromide (10 mg mL^{-1}). The gel profile was examined using a UV transilluminator and a gel documentation system (Vilber, Marne-la-Vallée, France). The target amplicon was extracted from the gel using a gel extraction kit (QIAquick; Qiagen India, New Delhi, India) according to the manufacturer's instructions and sequenced using the Sanger sequencing method (Eurofins India Pvt Ltd, Bengaluru, India). The resulting sequences were edited and assembled using BioEdit software, V.7.0.9.0 (10). The nucleotide sequences were compiled and deposited in the GenBank database (http://www.ncbi.nlm.nih.gov).

Phylogenetic analysis

The sequences of the reference strain and *Phytophthora* spp. that are similar to the isolates in this study were obtained from the NCBI GenBank database using the Basic Local Alignment Search Tool (BLAST) (11). The sequences of two genes (*ITS* and *COX*) from the current isolates, together with the chosen reference sequences of *Phytophthora* spp. (Table 2), were aligned using the ClustalW software. The nucleotide sequence identity matrices for *Phytophthora* spp. were created using BioEdit. The evolutionary history was inferred using the Maximum Likelihood approach and the Tamura-Nei model with the completed dataset consisting of a total of 27 nucleotide sequences and 2388 locations (12). The evolutionary studies were performed using MEGA11 software (13), with a bootstrap analysis conducted using 1000 bootstrap replications (14). The initial trees utilised in the heuristic search were generated automatically through the application of Neighbor-Join and

Table 2. Information on the *Phytophthora* spp., utilised in this study, along with their respective NCBI GenBank accession numbers

Species	Isolate	GenBank accession numbers		Host/Source	Country
		ITS	*COX*		
P. melonis	CHES-R2-2018*	OK606118	OP382209	*T. dioica*	India
P. melonis	CHES-R3-2018*	OK606119	OP382208	*T. dioica*	India
P. melonis	CHES-F-2018*	OK606126	OP382203	*T. dioica*	India
P. melonis	CHES-E-2018*	OK606125	OP382202	*T. dioica*	India
P. melonis	CHES-BA-2018*	OK606120	OP292975	*T. dioica*	India
P. melonis	CHES-S-2018*	OK606127	OP382210		India
P. melonis	CHES-J-2018*	OK606123	OP382205	*T. dioica*	India
P. melonis	CHES-JA-2018*	OK606121	OP382206	*T. dioica*	India
P. melonis	CHES-JAG-2018*	OK606122	OP382207	*T. dioica*	India
P. melonis	CHES-H-2018*	OK606124	OP382204	*T. dioica*	India
P. melonis	PMFJHL1	EF213746	EF372621	*Lagenaria siceraria*	China
P. melonis	PMNJDG1	EF208118	EF372620	*Benincasa hispida*	China
P. boehmeriae	CBS 291.29	KT183036	AY564165	Irrigation water	USA
P. infestans	CPHST BL 142	MG865512	MH136906	*Solanum tuberosum*	The Netherlands
P. nicotianae	FD00203	LC783859	LC783861	*Echeveria* spp.	South Korea
P. cactorum	1384	MH397244	MK105843	*Pyrus communis*	Argentina
P. palmivora	CBS 236.30	KY475624	MH760220	*Cocos nucifera*	India
P. heveae	IMI180616	AF266770	AY564182	*Hevea brasiliensis*	Malaysia
P. katsurae	P45	GU993899	GU945485	*Castanea sativa*	Japan
P. citricola	1E1	FJ392326	GU071239	Irrigation water	USA
P. citrophthora	M189	GU993889	GU945471	*Citrus* spp.	USA
P. colocasiae	IMI368918	AF266786	AY564173	*Colocasia esculenta*	Malaysia
P. megasperma	CBS 402.72	MF615274	LC595926	*Alcea rosea*	USA
P. humicola	IMI302303	AF266792	AY564184	Citrus orchard soil	Taiwan
P. inundata	SCRP644	EF210200	EF210206	*Salix* sp.	UK
P. inundata	NRCPh-196	KT633842	KU186666	*Citrus* sp.	India
P. fragariae	IMI330736	AF266762	AY564177	*Fragaria* x *ananassa*	Scotland
P. sojae	Pm-1	AB688353	KX371904	*Glycine max*	Japan
P. cryptogea	CPHST BL 16	MG865483	MH136878	*solanum lycopersicum*	Ireland
P. drechsleri	20-043	MW471127	MW480541	*Dendranthema grandiflora*	USA
P. syringae	MAFF645010	AB688409	AB688299	*Malus pumila*	Japan
P. lateralis	IMI040503	AF266804	AY564191	*Chamaecyparis lawsoniana*	USA
P. ramorum	P2058	MW300319	MW323671	*Rhododendron* sp.	USA
Pythium phragmiticola	P56	KC145165	KC145166	Reed rhizosphere soil	Germany

BioNJ algorithms to a matrix of pairwise distances estimated via the Tamura-Nei model. The topology with the highest log likelihood value was subsequently chosen as the final tree. The phylogenetic tree was established by using *Pythium phragmiticola* isolate p56 as the root.

In vitro management of *Phytophthora melonis*

Poisoned food technique

The effectiveness of the latest fungicides at concentrations of 500 ppm and 1000 ppm was assessed using the poisoned food technique against *Phytophthora* isolates. The requisite amount of each fungicide was individually incorporated into sterilised molten PCA media to achieve the desired concentration. Subsequently, 20 mL of the poisoned media was dispensed into sterilised Petri plates. Mycelium discs, each with a diameter of 8 mm, were excised from a 7-day-old culture of the fungus using a sterile cork borer and four such discs were positioned at the four corners of each plate. PCA medium devoid of any fungicide served as the control. The fungicides utilised in this study are enumerated in Table 3. Owing to its properties, potassium phosphite was tested *in vitro* at varying concentrations, specifically 500 ppm, 1000 ppm, 1500 ppm, 2000 ppm and 3000 ppm, against *P. melonis*. The plates were incubated at room temperature for 7 days, with radial growth measured daily until maximum growth was observed in the control plates.

Detached leaf method

The fungicides identified as most effective, based on their minimum inhibitory concentration determined through poisoned food assays, were utilised in this study. For the detached leaf assay, mature leaves of the pointed gourd were detached and surface sterilised by immersion in 1 % (w:v) sodium hypochlorite for 1 min. Subsequently, the leaves were rinsed three times with sterile double-distilled water, blotted dry on sterile Whatman filter paper and placed with the abaxial surface facing upward in moist chambers. These chambers were constructed from transparent plastic boxes containing two layers of water-soaked blotting papers. Fungicide concentrations were applied according to the effectiveness observed in *in vitro* screening. Initially, the leaves were sprayed with the fungicide and allowed to air dry. Mycelial plugs from a 7-day-old culture were then used to inoculate the centre of the leaves and disease development was monitored. The experiment was replicated three times using this method. Leaves inoculated with the pathogen served as controls. Measurements of lesion length and breadth were recorded.

Analysis of percent inhibition (I)

Percent inhibition (I) in the growth of the organism in different fungicide treatments over the control was calculated in both the poison food technique and detached leaf assay with the following formula (15).

$$I = \frac{C-T}{C} X\ 100$$

Where I is percent inhibition, C is the growth of the test pathogen in the absence of treatment (cm) and T is the growth of the test pathogen in the presence of treatment (cm).

Analysis of lesion area (A)

In the detached leaf assay, lesions were measured two times, usually on the fourth and seventh days after inoculation. The largest length and width (perpendicular to the length) of each lesion were measured and the area was calculated

$$A = ¼ \times \pi \times \text{length} \times \text{width}$$

Statistical analysis

The experimental design employed a completely randomised design (CRD) for all experiments. Statistical analysis of the data was conducted using analysis of variance at a significance level of ($p \leq 0.05$) with Grapes 1.1.0 software (16). Each experiment was performed in triplicate and the data were analysed using standard statistical procedures (17).

Results

Symptomatology

The pointed gourd collar and root rot symptoms were observed in ICAR-IIHR-CHES experimental orchard at Bhubaneswar with different degrees of severity, spanning from partial to complete rotting of the stem. Observations were made in three randomly selected distinct plots. The symptoms of collar and root rot typically manifest as wilting and decline of the plant, often accompanied by yellowing or browning of leaves. As the disease progresses, the stem near the soil line may develop water-soaked lesions that darken over time. These lesions can girdle the stem, leading to the rapid collapse of foliage above the affected area (Fig. 1A–C). In saturated soil conditions, the roots may rot, further exacerbating the

Table 3. List of fungicides used in this study

Sl. No.	Active ingredient & formulation	Trade name	Manufacturing company
1	Tebuconazole 25 % EC	Tebu	Heranba industries
2	Fosetyl – Al 80 % WP	Aliette	Bayer
3	Propineb 70 % WP	Antracol	Bayer
4	Copper Hydroxide 53.8 % DF	Kocide	Dupont
5	Propiconazole 25 % EC	Tilt	Syngenta
6	Azoxystrobin 23 % WP	Mirador	Adama
7	Potassium Phosphite 98 % WP		Skyliteagrochem
8	Dimethomorph 50 % WP	Acrobat	BASF
9	Pyraclostrobin 10 % WG	Headline	BASF
10	Copperoxy chloride 50 % WP	Blitox50	Tata Rallis
11	Hexaconazole 5 % EC	Contaf	Tata Rallis
12	Fluopicolide 62.5 % + Propamocarb hydrochloride 62.5 SC	Infinito	Bayer
13	Pyraclostrobin 5 % + Metiram 55 % WG	Clutch	Pi industries
14	Tebuconazole 50 % + Trifloxystrobin 25 % WG	Nativo	Bayer
15	Cymoxanil 8 % + Mancozeb 64 % WP	Cyman	Heranba industries

Fig. 1. (A–C) Symptoms of collar and root rot in main field condition as well as on young cuttings (D–F) in pointed gourd.

symptoms. Ultimately, severe infections can result in plant death. Additionally, in some cases, white mycelial growth may be visible on infected plant parts, particularly under wet conditions. Under nursery conditions, cuttings exhibited collar and root rot symptoms (Fig. 1D–F).

Isolation and pathogenicity

Isolation was carried out from the infected symptomatic stem and the isolates were further selected for pathogenicity evaluation and characterisation. The pathogen was artificially inoculated into the pointed gourd seedlings for the pathogenicity experiments (Fig. 2). Each isolate exhibited disease signs similar to those seen in the field, such as plant wilting and decline, sometimes along with yellowing or browning of the leaves. The stem close to the soil level may get water-soaked lesions that intensify with time as the disease advances. Koch's hypotheses were fulfilled by the pathogens found on artificially infected symptomatic stems, which had the exact same symptoms as the field.

The organism causing collar and root rot (root phase) as well as fruit and vine rot (foliar phase) were found to be same i.e., *P. melonis*. So far the collar and root rot phase of the disease caused by *P. melonis* was not studied by earlier workers. The major diagnostic symptom of collar and root rot includes shredding of vascular bundles both in young cuttings as well as in matured plants in addition to wilting and drying of plants. From the diseased plant paths both *Fusarium* species and *Phytophthora* species were isolated. Upon artificial inoculation, *Phytophthora* species produced diagnostic symptoms of collar and root rot however artificial inoculation *Fusarium* not yielded any symptoms

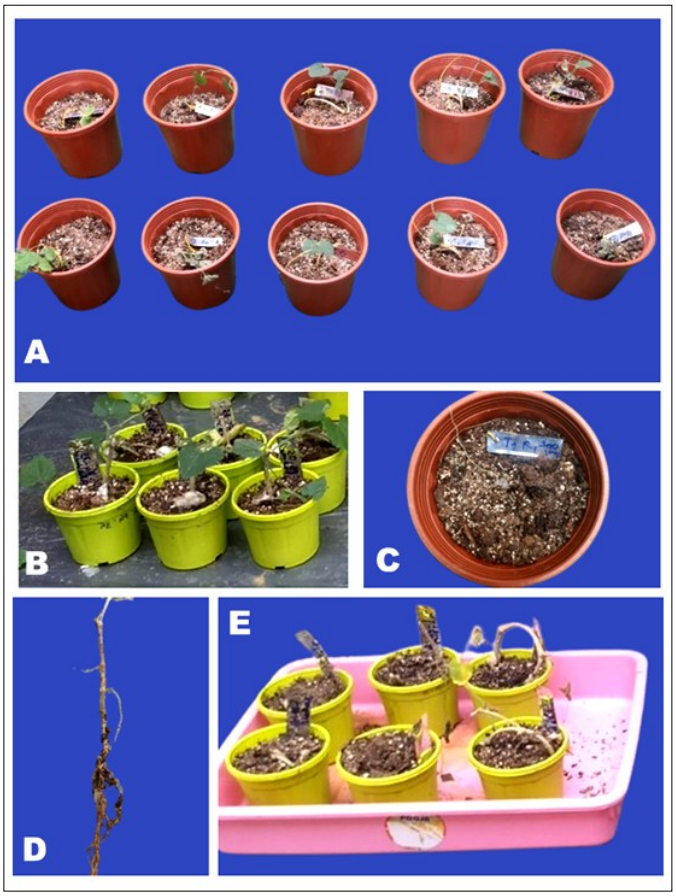

Fig. 2. (A) Artificial inoculation on seedlings. (B) Artificial inoculation on rooted cuttings. (C, D) Seedlings inoculated with *P. melonis*. (E) Rooted vine cuttings inoculated with *P. melonis*.

even after repeated attempts with different inoculation methodology. Further during pathogenicity study, it was observed and confirmed that certain *Fusarium* species were having endophytic relationship with the host plant without causing any disease symptoms. Koch's postulates successfully proved for collar and root rot and the associated pathogen was confirmed as *P. melonis*.

Phenotypic characterisation

The isolates were subjected to morphological characterisation under a light microscope according to the method described in previous study (18). The colonies of all the isolates on the PCA medium had a white aerial and coenocytic mycelium (Fig. 3A). The average growth rate of the mycelia of *P. melonis* isolates varied between 3.61 and 5.95 mm/day after incubation at 25 ± 1 °C and a 12:12 hr (light:dark) photoperiod. Sporangia are oval to obclavate and non-papillate (Fig. 3B) with characteristic internal proliferation (Fig. 3C). All the isolates caused spreading lesions in the detached twigs. The isolates were identified as *Phytophthora* spp., based on morphological and conidial characteristics (7).

Molecular characterisation

For molecular characterisation, nucleotide sequences of the *ITS* and *COX* genes were generated to identify *Phytophthora* isolates at the species level. The amplified PCR products of the *ITS* (~900 bp) and *COX* (~450 bp) genes were sequenced using custom Sanger sequencing services and subjected to NCBI BLASTn analysis. Both forward and reverse sequences were compiled and submitted to NCBI GenBank, where accession numbers were obtained. BLAST searches of isolates exhibiting collar rot symptoms from the NCBI GenBank database revealed that the *ITS* sequences exhibited 99.19–100.00 % similarity with *P. melonis* (AF228094, OK606126), 99.88 % with *Phytophthora drechsleri* (AY590268, KU211341) and 98.23 % similarity with *Phytophthora sojae* (KU211341, KU211340). The *COX* gene showed 99.89–100 % similarity with *P. melonis* (KF317110, EF372620), 96–98 % similarity with *P. pistaciae* isolate (KF358237) and 95–96.54 % similarity with *Phytophthora niederhauserii* isolates (AM943026, GU477617). Due to conflicts observed in single-gene phylogenies for all isolates (data not shown), the two genes were concatenated. A multigene phylogenetic analysis was conducted using the combined *ITS* and *COX* gene dataset of *Phytophthora* spp., including 23 isolates from GenBank corresponding to a wide range of known *Phytophthora* spp., one outgroup taxon (*Pythium phragmiticola* isolate p56) and 8 isolates (CHES-BA-2018 to CHES-E-2018) from this study (Table 2). The maximum likelihood tree is presented in Fig. 4, with bootstrap support values indicated above the nodal branches. All *Phytophthora* isolates from the pointed gourd formed a common clade with reference isolates of *P. melonis* (PMNJDG1, PMFJHL1) obtained from GenBank reference strains. In contrast, *P. phragmiticola* isolate p56 formed a separate subgroup. Consequently, the pathogenic isolates (CHES-BA-2018 to CHES-E-2018) were identified as *P. melonis*.

In vitro management of *Phytophthora melonis* isolates

Evaluation of fungicides by poison food technique

A series of the latest fungicide molecules were evaluated by poison food technique *in vitro* against *P. melonis*, at two different concentrations (500 ppm and 1000 ppm) (Fig. 5). Among the fungicide molecules evaluated, tebuconazole, copper hydroxide, showed cent percent inhibition at 500 ppm, while propineb and fosetyl – Al showed cent percent inhibition at 1000 ppm. Among the combination fungicide molecules, fluopicolide + propamocarb hydrochloride and cymoxanil + mancozeb exhibited cent percent inhibition at both 500 and 1000 ppm concentrations compared to the control (without fungicide amendment). Potassium phosphite at 3000 ppm exhibited cent percent growth inhibition of the test pathogen as compared to the control (Fig. 6).

In the detached leaf assay, fungicides such as dimethomorph and copper hydroxide, both at 2000 ppm conc. exhibited 100 % and 92 % growth inhibition over control, respectively. Potassium phosphite at 4000 ppm conc. exhibited 89 % growth inhibition over control significantly. Fenamidone 10 % + Mancozeb 50 % WG at 0.2 % concentration exhibited 82. 5 % control (Table 4).

Discussion

The pointed gourd (*Trichosanthes dioica* Roxb.), a member of the Cucurbitaceae family, is notably vulnerable to various diseases that adversely affect its yield and quality. Among these, collar and root rot, induced by *P. melonis*, is particularly detrimental, especially in regions such as West Bengal and Odisha. The symptoms of this disease have been documented in the ICAR-IIHR-CHES experimental orchard in Bhubaneswar, where they present with varying severity, ranging from partial stem rotting to the complete collapse of the plant. These symptoms were

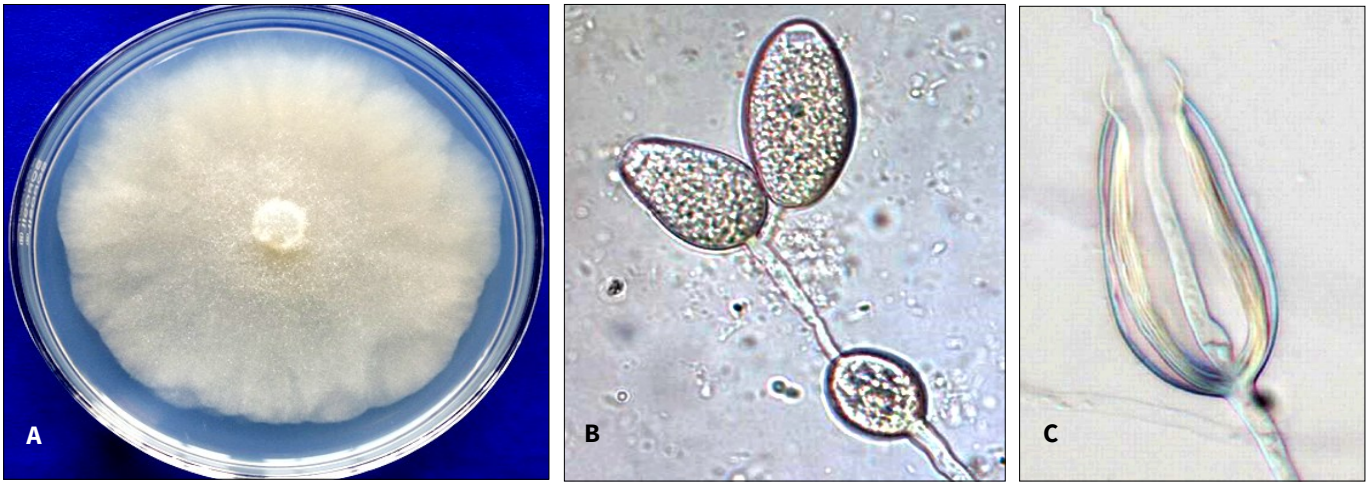

Fig. 3. *P. melonis*. (A) Culture on PCA. (B) Sporangia. (C) Characteristic intercalary growth of mycelium.

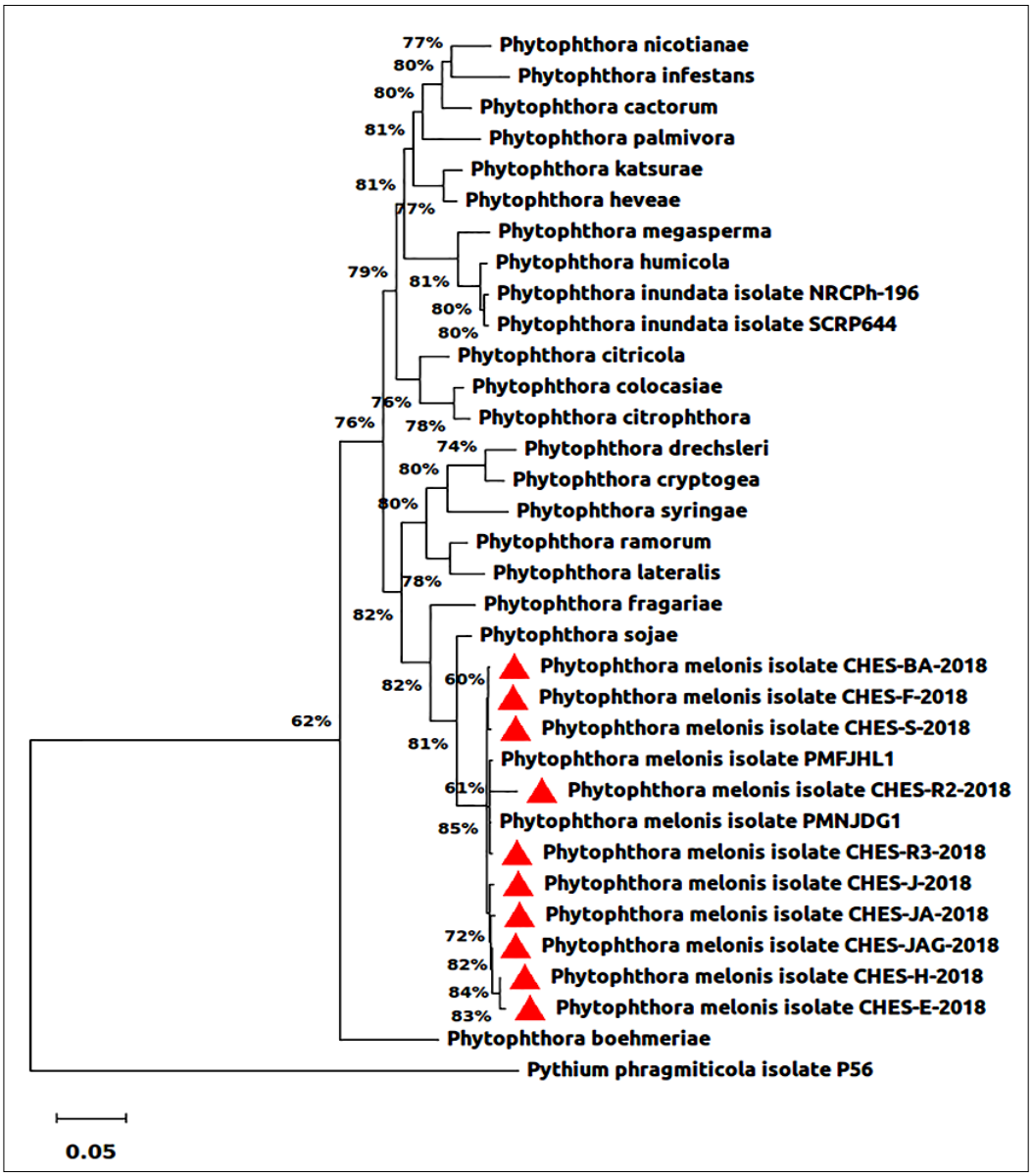

Fig. 4. Multigene phylogenetic tree of *ITS* and *COX* regions of *P. melonis* isolates (marked as red triangle bullets) obtained by maximum likelihood method.

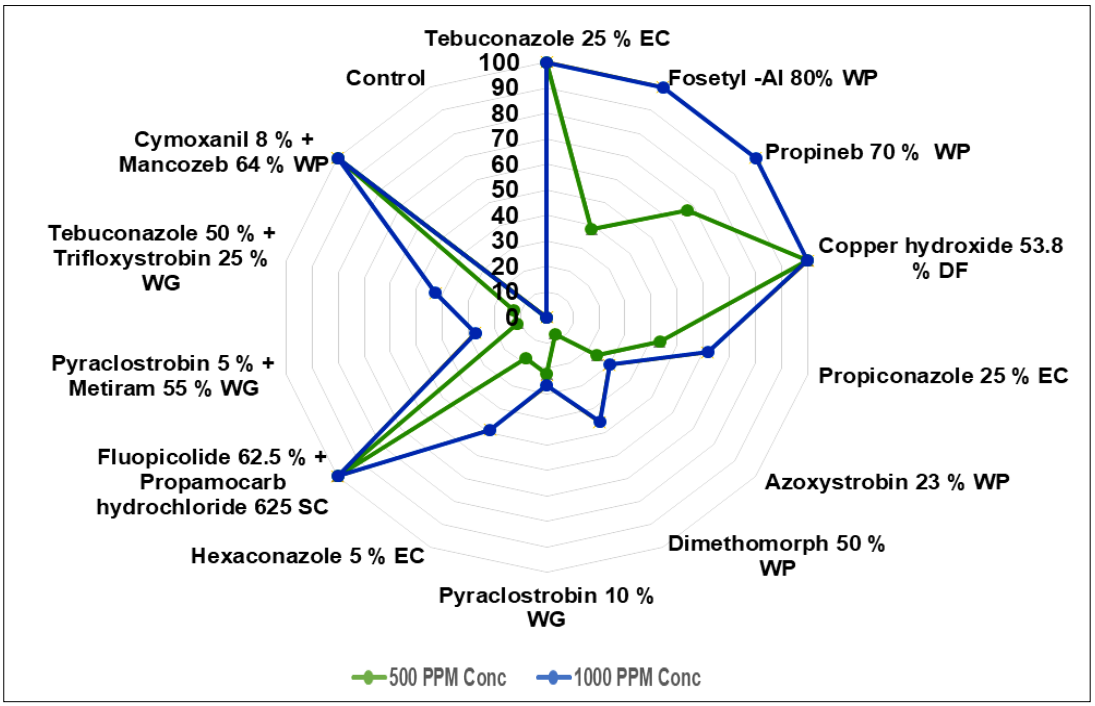

Fig. 5. Efficacy of various fungicides against *P. melonis*.

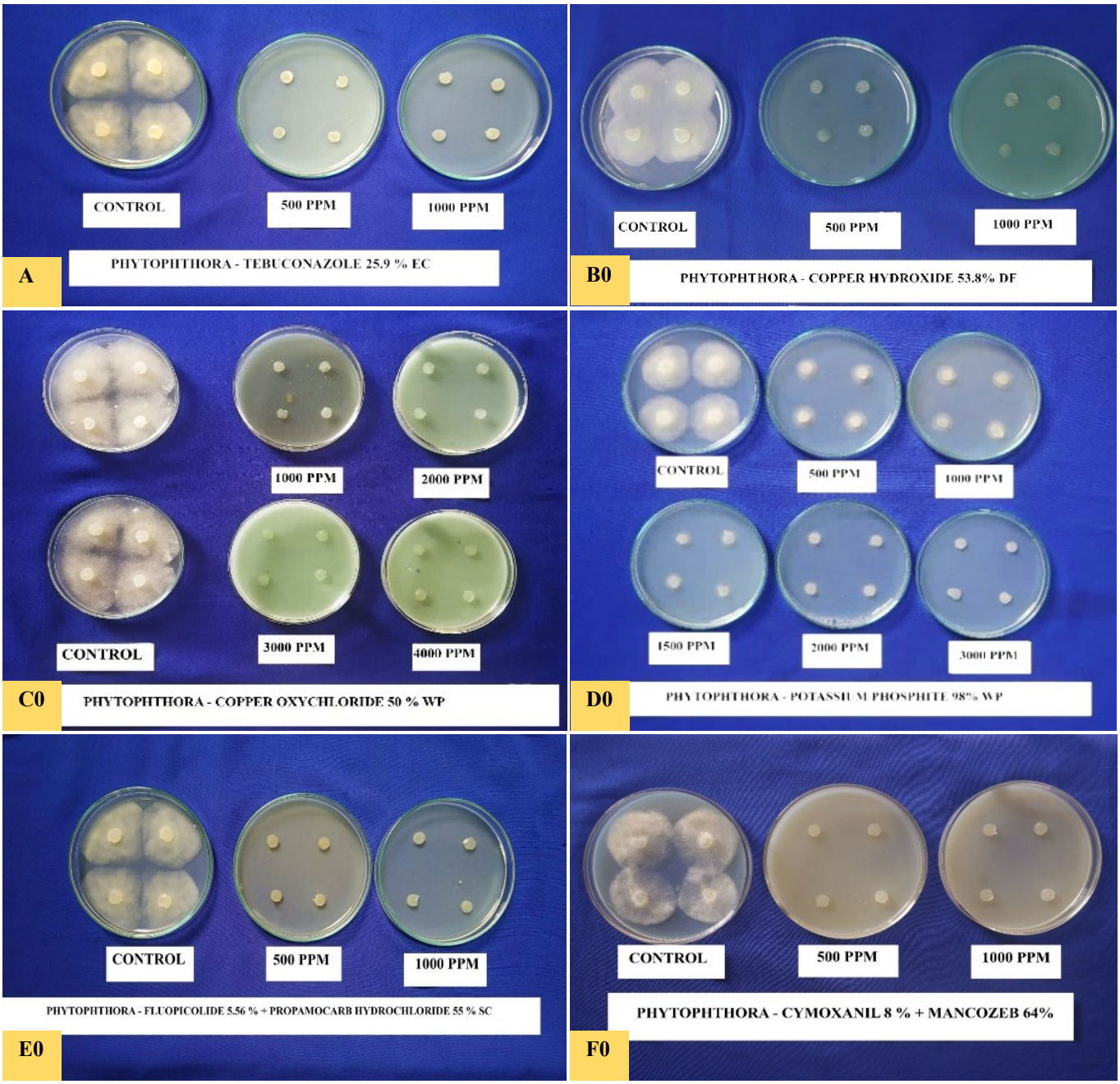

Fig. 6. Efficacy of fungicide molecules and commercial pre-packed fungicide mixtures against *Phytophthora melonis* in by poisoned food assay. (A) Tebuconazole, (B) copper hydroxide, (C) copper oxychloride, (D) potassium phosphite, (E) Fluopicolide 62.5 % + propamocarb hydrochloride 625 SC, (F) cymoxanil 8 % + mancozeb 64 %.

consistently observed across three randomly selected plots, underscoring the disease's prevalence. Typical manifestations include wilting and decline of the plant, accompanied by yellowing or browning of leaves. As the disease advances, water-soaked lesions emerge on the stem near the soil line, which darken over time. These lesions can encircle the stem, leading to the collapse of the foliage above. In saturated soil conditions, root rot exacerbates the disease, often resulting in plant death. Additionally, white mycelial growth is frequently visible on the affected plant parts, particularly under wet conditions. In nursery settings, similar symptoms were observed in cuttings, further corroborating the pathogenicity of *P. melonis* in both mature plants and young seedlings.

The pathogenic role of *P. melonis* in causing collar and root rot in pointed gourd was further validated through isolation and pathogenicity testing. Adhering to Koch's postulates, this study unequivocally demonstrated the association between *P. melonis* and disease development, reinforcing the role of this pathogen as the causal agent. This association was further corroborated by the symptomatology observed, which aligned with previously documented manifestations (5, 18). The destructive potential of this pathogen is particularly pronounced in environments conducive to its growth, such as regions with high humidity and adequate moisture levels, which favor the germination and proliferation of *P. melonis* spores. This observation further substantiates the conclusion that *P. melonis* is the primary pathogen responsible for collar and root rot.

Phenotypic characterisation of the pathogen isolates revealed distinct morphological traits characteristic of *P. melonis*. All isolates grown on PCA displayed a white, aerial, coenocytic mycelium, as well as the presence of oval to obclavate, non-papillate, single and terminal sporangia. The sporangia were non-caducous and exhibited a sympodial branching pattern with dominant internal proliferation. These

findings are consistent with previous reports that described *P. melonis* sporangia as ovoid to ellipsoid with rounded bases and produced through internal proliferation (7, 18). Additionally, sporangiophores were found to be indeterminate and simple, presenting sympodially. Colony morphology was slightly rosaceous in some isolates, which aligns with previous observations. The mycelial growth rate averaged between 3.61 mm/day and 5.95 mm/day under standard incubation conditions (25 ± 1 °C with a 12:12 light/dark photoperiod), which is consistent with findings of previous researchers who reported growth rates exceeding 3.5 mm/day (19). This growth rate indicates the aggressive nature of the pathogen, which can cause rapid disease progression under favorable conditions. The molecular identification of *P. melonis* provides deeper insights into the diversity and geographic distribution of this pathogen, aiding in the refinement of disease management strategies tailored to specific regions.

Molecular characterisation using *ITS* and *COX* gene sequences further confirmed the identity of this pathogen. The PCR amplification of these genes yielded products of approximately 900 bp and 450 bp, respectively, which were subsequently sequenced and compared to sequences in the NCBI GenBank database. The *ITS* gene sequences demonstrated 99.19–00.00 % similarity with *P. melonis* isolates (AF228094, OK606126), while the *COX* gene sequences exhibited 99.89–100 % similarity with *P. melonis* (KF317110, EF372620). Phylogenetic analysis of the concatenated *ITS* and *COX* gene sequences positioned the isolates within a clade containing reference strains of *P. melonis*, thereby confirming their identity as *P. melonis*. The high genetic similarity between the isolates and *P. melonis* strengthens the evidence for its role as the causative agent of collar and root rot in pointed gourd. This molecular confirmation aligns with previous studies that have identified *P. melonis* as the key pathogen of pointed gourd in other areas, further validating the results of this study (18, 20).

The investigation of potential fungicides for the control of *P. melonis* has identified several promising compounds. Among the fungicides evaluated, tebuconazole and copper hydroxide completely inhibited the pathogen at a concentration of 500 ppm, whereas copper oxychloride, propineb and fosetyl - Al were effective at 1000 ppm. Furthermore, combination fungicides, such as fluopicolide + propamocarb hydrochloride and cymoxanil + mancozeb, demonstrated high efficacy, achieving complete inhibition at both 500 and 1000 ppm concentrations (21-23). Notably, potassium phosphite achieved complete inhibition at 3000 ppm, highlighting its potential as an effective fungicide. Unlike traditional fungicides, potassium phosphite is characterised by its unique properties, as it readily translocate to metabolically active tissues, providing both direct and indirect defense responses to pathogens. This mode of action is complex and involves the activation of plant defense mechanisms, rendering potassium phosphite a promising candidate for inclusion in integrated disease management (IDM) strategies. These findings align with previous studies demonstrating the efficacy of potassium phosphite in managing *Phytophthora* infections in other crops (24, 25). The results suggest that the aforementioned fungicides, particularly when used in combination, could serve as effective tools for the management of *P. melonis* in pointed gourd cultivation, thereby enhancing both disease control and yield.

Subsequent evaluations using detached leaf assays corroborated the effectiveness of various fungicides. Specifically, dimethomorph and copper hydroxide achieved growth inhibition rates of 100 % and 92 % at a concentration of 2000 ppm, respectively. In contrast, potassium phosphite at 4000 ppm resulted in an 89 % inhibition rate. Additionally, a formulation of fenamidone 10 % combined with mancozeb 50 % WG at a 2000 ppm concentration demonstrated an 82.5 % control rate. These findings further substantiate the potential of these fungicides in managing *P. melonis* in pointed gourd cultivation.

Conclusion

This study provides comprehensive evidence identifying *P. melonis* as the causative agent of collar and root rot in pointed (*T. dioica*). Extensive phenotypic, molecular and pathogenicity analyses have confirmed the pathogen's role in disease development. The findings underscore the significance of *P. melonis* in both the root and foliar phases of the disease, a context not previously explored in pointed gourd. The morphological and molecular characteristics of the pathogen will contribute to a broader understanding of its biology and subsequently aid in formulating accurate disease management strategies. The results further highlight the potential efficacy of various fungicides, including tebuconazole, copper hydroxide and potassium phosphite, in managing *P. melonis*. These compounds, particularly when used in combination, provide a robust foundation for developing integrated disease management strategies. Incorporating these fungicides into integrated disease management (IDM) programs, alongside biological control measures, will not only mitigate the impact of *P. melonis* but also enhance the yield of pointed gourd cultivation in affected regions. This research emphasises the necessity for sustainable and eco-friendly disease management practices to ensure the long-term productivity of pointed gourd farming and to strengthen food security in areas dependent on this crop.

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Authors' contributions

SG and LR conceived the study and participated in its design and conceptualisation and in writing the original draft. MP, MFA and SS participated in sequence alignment and phylogenetic analysis. DP participated in the study design and performed the statistical analysis. SP participated in the conceptualisation of the experiment. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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