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First report of *Neoscytalidium dimidiatum* causing stem canker of dragon fruit *Hyalocereus* sp.) in Kerala

P. SUHAIRA[1*], C. R. RASHMI[1], SIBLE GEORGE VARGHESE[1], C. ANJU[1] AND A. ASWINI [2]
[1]Department. of Plant Pathology, College of Agriculture, Vellanikkara, Thrissur - 680656, Kerala
[2]Department of Fruit Science, College of Agriculture, Kerala Agricultural University, Vellanikkara, Thrissur - 680656, Kerala

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Dragon fruit (*Hylocereus sp*.) is a new and promising fruit crop that has gained popularity in Kerala. Recently, stem canker, a new dragon fruit disease, was discovered in the dragon fruit growing regions of Thrissur and Wayanad districts of Kerala during March and June 2023, with a highest disease incidence and severity of 55.00% and 23.45% from Chirakkekode region. Symptoms began as deep chlorotic patches with a little orange core that gradually grew in size. Finally, several coalescing lesions produced a stem canker symptom, with numerous pycnidia developing on the cankerous growth. The pathogen up on isolation on PDA, produced white mycelia which later turned to olive green to greyish black with black pigmentation and the pathogenicity was established within five days after inoculation. Arthroconidia were dark brown in colour, ellipsoid to ovoid in shape and ranged in size from 7.21-9.16 3.45-5.40μm. Molecular characterization by amplification of ITS specific sequences followed by *in silico* analysis revealed the pathogen to be *Neoscytalidium dimidiatum.* Preliminary studies on the efficacy of fungicides and biocontrol agents were attempted *in vitro* using poisoned food technique. The contact fungicides like Propineb, Mancozeb and the systemic fungicide Tebuconazole, Difenconazole were found to be superior *in vitro* exhibiting cent percentage inhibition at all the three tested concentrations. Among the combination fungicides evaluated, Tebuconazole (50%) + Trifloxystrobin (25%) and Carbendazim (12%) + Mancozeb (63%)showed complete inhibition at all the three doses tested. Among the biocontrol agents, PGPM showed higher inhibition of the pathogen followed by *Trichoderma asperellum.*

Keywords: Dragon fruit, *Neoscytalidium dimidiatum*, Stem canker disease, symptomatology

INTRODUCTION

Pitaya (Hylocereus spp.) originated from the tropical and subtropical forest regions of Latin Americas, including North, Central and South America (Crane and Balerdi, 2005), is known as dragon fruit in Asia as its skin is covered with bracts like dragon (Mizrahi *et al*. 2002).

India has 3000 hectares of area under dragon fruit cultivation with a productivity of 8.0 to 10.5 MT ha^{-1}. Total production is estimated to be 4200 MT per year which accounts for a share of only 0.2 per cent in global dragon fruit production (Wakchaure *et al*. 2020).

Recently a canker disease infecting both fruits and cladodes of dragon fruit was observed across various dragon fruit growing regions of Thrissur and Wayand district. The disease initially appeared as deep chlorotic patches with little orange centre on the cladodes of dragon fruit.In India first report of stem canker of dragon fruit was given by Salunkhe *et al*. (2023) from Maharashtra. According to the literature stem canker has not been reported to cause infection on dragon fruit in Kerala. Recently another pathogen causing anthracnose disease of dragon fruit was reported from Kerala (Suhaira *et al*. 2024). The objectives of the study were to reveal the occurrence of stem canker on dragon fruit, symptomatology, identify the pathogen based on cultural, morphological and molecular characteristics and assess the *in vitro* efficacy of chemical fungicides and biocontrol agents against the pathogen.

MATERIALS AND METHODS

The characteristic stem canker symptoms were observed from the dragon fruit growing fields of

*Correspondence : suhairap92@gmail.com

Thrissur and Wayanad districts and disease incidence and severity was assessed. Symptom development of the pathogen was studied under both natural and artificial conditions. The fungus was isolated by standard tissue segmentation technique on PDA medium and further purification done by hyphal tip method.

The Mycelial Bit Inoculation method (Rocha *et al.* 1998) was used to prove the pathogenicity of the fungus. Healthy cladodes of dragon fruit were artificially inoculated with the mycelial bits of the pathogen after making injury by pin prick method. Moist cotton pieces were placed over the mycelial bit to provide sufficient moisture and were maintained in humid conditions along with control at room temperature until the development of symptoms.

The pathogen was identified up to genus level based on cultural and morphological characteristics by comparing the description given in CMI Descriptions of Pathogenic Fungi and Bacteria. The molecular characterization was done by isolating the genomic DNA, followed by amplification of specific ITS regions using universal primers of ITS (ITS – IF (TCCGTAGGTGAACCTGCGG) and ITS-4R(TCCTCCGCTTATTGATATGC)) by PCR and obtained sequence was analyzed in BLASTn program of NCBI.

Efficacy of five contact fungicides like Chlorothalonil (75 WP), Copper hydroxide (50% WP), Propineb (70% WP), Mancozeb (80% WP), Bordeaux mixture (35% WG), three systemic fungicides *viz.*, Tebuconazole (250 EC), Difenoconazole (25% EC), Carbendazim (50% WP) and three combination fungicides like Tebuconazole (50% WG) + Trifloxystrobin (25% WG), Carbendazim (12% WP) + Mancozeb (63% WP), Azoxystrobin (18.2% SC) + Difenoconazole (11.4% SC) were tested against *Neoscytalidium dimidiatum* at three different dosages (lower, recommended, higher) by poisoned food technique. The biocontrol agents like *Trichoderma asperellum*, *Pseudomonas fluorescens*, and microbial consortia *viz.*, PGPM were assessed for their antagonistic activity against the pathogen by dual culture assay and poisoned food technique. The per cent inhibition of the fungal pathogens were calculated using the formula given by Vincent *et al*. (1927).

$$\text{Per cent inhibition} = \frac{C-T}{C} X\ 100$$

Where, C- growth of the pathogen in control (cm); T- growth of the pathogen in treatment (cm)

RESULTS AND DISCUSSION

Symptomatology and pathogenicity

The stem canker symptom on cladode initiated as deep chlorotic patches that occasionally had a little orange centre. The patches get larger over time and frequently merge to form massive brown lesions which later turned to grey coloured patches. Grey cankerous growths are frequently encircled by yellow halos or water-soaked tissue. In advanced stages numerous pycnidia developed on the cankerous growth. In later stages, the lesion often getting detached from the cladode and often producing shot hole symptom (Fig.1). The descriptions of the above symptom were in line with the findings of Mohd *et al*. (2013), Dy *et al*. (2022) and Sanahuja *et al*. (2016) on dragon fruit.

On fruits the symptom initiated as minute brown patches on the entire fruit surface that eventually coalesced. The samples when subjected to isolation on PDA medium, the fungus produced white mycelia on PDA which later turned to olive green to greyish black with black pigmentation. In order to establish pathogenicity, mycelial bits taken from four days old culture of the pathogen was inoculated on healthy cladode and was kept in humid conditions for disease development. The symptoms started to appear on the inoculated cladodes after five days of inoculation (Fig.2). The symptom initiated as orange to brown coloured spots. The dots expanded and joined over time to form a huge encrustation on the cladode that later turned to black colour. The pathogen was re-isolated from inoculated plants and the re-isolated fungal isolate was found to be morphologically similarto the initially isolated culture of pathogen.

Characterization and identification of the pathogen

The pathogen cultured on PDA media, produced white mycelia on PDA which later turned to olive

Table 1: Disease incidence and severity

Location		Geographical coordinates	Per cent disease incidence (%)	Disease severity (%)
Chirakkekode	Field 1		23.45	55.00
	Field 2	10.561672/76.294555	17.77	25.00
	Field 3		19.67	35.00
Vellanikkara		10.552624/76.278157	13.45	44.00
Klapetta		11.3810/76.445	6.16	10.00

Fig 1: Development of symptoms of stem canker under field conditions

Fig 2 : Development of symptoms on artificially inoculated plant

Table 2 : *In vitro* evaluation of fungicides against *Neoscytalidium dimidiatim*

Fungicides	Concentration (%)	Inhibition (%)
Chlorothalonil (75% WP)	0.1	73.44 (58.98)[f]
	0.20	78.70 (62.51)[e]
	0.30	81.30 (64.43)[d]
Copper hydroxide (77% WP)	0.15	0.00 (9.59)[l]
	0.20	0.00 (9.59)[l]
	0.30	0.00 (9.59)[l]
Propineb (70% WP)	0.10	100.00 (80.40)[a]
	0.20	100.00 (80.40)[a]
	0.30	100.00 (80.40)[a]
Mancozeb (75% WP)	0.10	100.00 (80.40)[a]
	0.20	100.00 (80.40)[a]
	0.30	100.00 (80.40)[a]
Bordeaux Mixture	0.50	0.00 (9.59)[l]
	1.00	20.09 (29.63)[k]
	1.50	58.52 (49.90)[j]
Tebuconazole (25.9% EC)	0.10	100.00 (80.40)[a]
	0.15	100.00 (80.40)[a]
	0.20	100.00 (80.40)[a]
Difenoconazole (25% EC)	0.05	100.00 (80.40)[a]
	0.10	100.00 (80.40)[a]
	0.15	100.00 (80.40)[a]
Carbendazim (50% WP)	0.05	65.00 (53.73)[i]
	0.10	66.87 (54.86)[h]
	0.15	71.80 (57.93)[g]
Trifloxystrobin 25%WP +Tebuconazole 55% WG	0.03	100.00 (80.40)[a]
	0.04	100.00 (80.40)[a]
	0.05	100.00 (80.40)[a]
Carbendazim 12%+ Mancozeb 63%	0.15	100.00 (80.40)[a]
	0.20	100.00 (80.40)[a]
	0.25	100.00 (80.40)[a]
Azoxystrobin 18.2% + Difenoconazole 11.4% SC	0.05	86.70 (68.61)[c]
	0.10	89.64 (71.23)[b]
	0.15	89.84 (71.43)[b]
CD(0.05)	0.885	
CV	0.841	

* Data in parenthesis are angular transformed values

Table 3: *In vitro* evaluation of biocontrol formulations against *Neoscytalidium dimidiatum*

Biocontrol agent	Concentration (%)	Inhibition (%)
T. asperellum	1	40.83 (39.72)[f]
	2	61.70 (51.77)[c]
	3	68.92 (56.12)[b]
P. fluorescens	1	39.21 (38.76)[f]
	2	45.21 (42.25)[e]
	3	49.96 (44.97)[d]
PGPM	1	48.32 (44.04)[d]
	2	67.33 (55.14)[b]
	3	73.39 (58.44)[a]
CD(0.05)	1.60	
CV	1.94	

* Data in parenthesis are angular transformed values

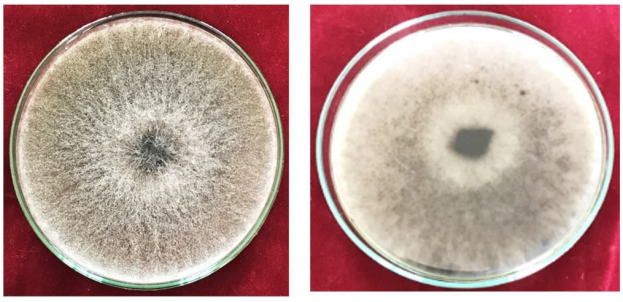

Fig 3: Growth of N.dimidiatum on PDA

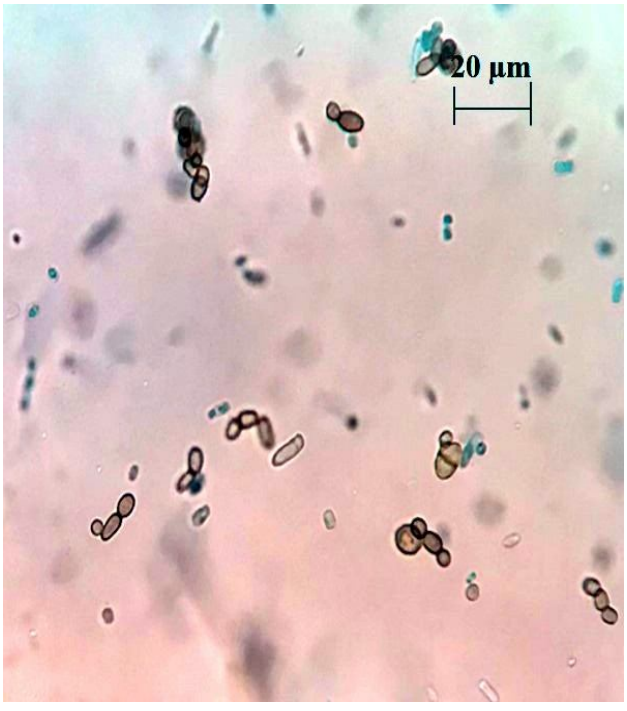

Fig 4: Arthroconidia of *N.dimidiatum*

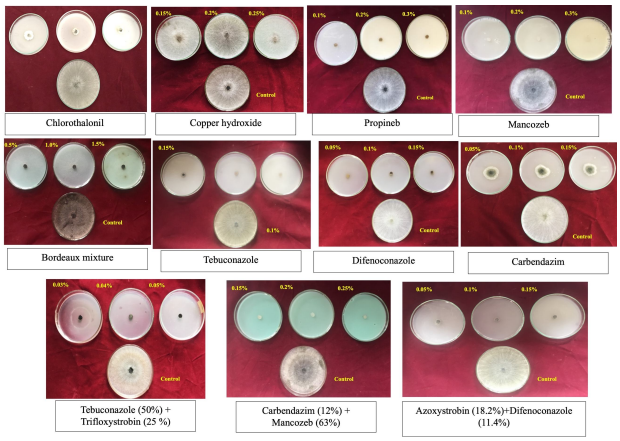

Fig. 5: *In vitro* efficacy of fungicides against *N.dimidiatum*

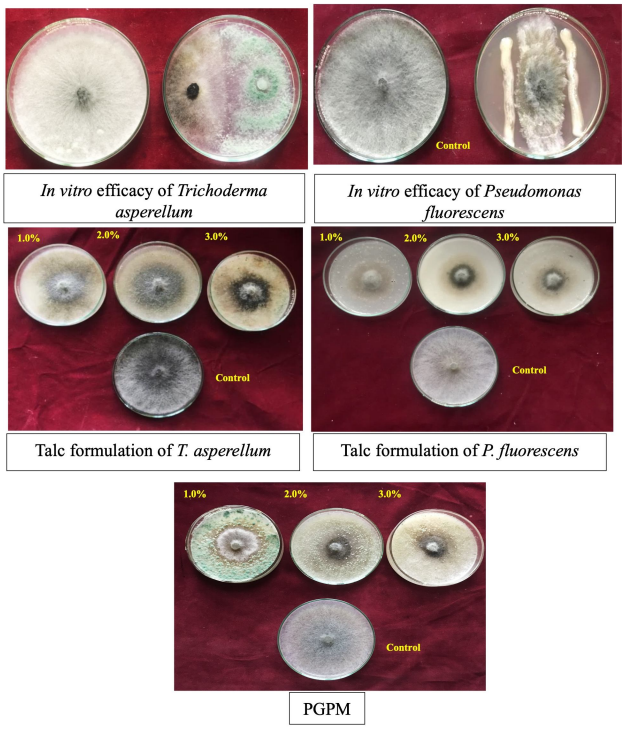

Fig. 6: *In vitro* efficacy of bioagents and their formulations against *N.dimidiatum*

green to greyish black with black pigmentation (Fig.3). The culture attained full growth in the Petri plate (9 cm) after 3 days of incubation at room temperature and the growth rate was 3 cm per day. The mycelium was branched, septate and brown. The hyphae were fragmented to produce numerous arthroconidia. The arthroconidia produced in chains with ellipsoid to ovoid in shape and measured between 7.21-9.16 $\times$ 3.45-5.40μm. Based on cultural and morphological characters, the pathogen was identified at the genus level as *Neoscytalidium* sp.

The forward and reverse nucleotide sequence by ITS sequencing was compared with known sequences of nucleotides available in NCBI, and it revealed the sequence similarity of 99.81 % with 87% query coverage and maximum score of 961 with accession number KF612309.1 of *Neoscytalidium dimidiatum.*

In vitro evaluation of chemical fungicides and biocontrol agents against Myrothecium inundatum

The *in vitro* analysis was conducted in Completely Randomized Design (CRD) with three replications each. Among the contact fungicides tested, Propineb (70 % WP) and Mancozeb (80 % WP) were found highly effective against *N. dimidiatum* exhibiting cent percentage inhibition at all three concentrations (Fig.5). The least effective among them was Copper hydroxide (77 % WP) with zero percent inhibition. In case of systemic fungicides, the Tebuconazole (250 EC) and Difenoconazole (25% EC) showed higher inhibition (100 %) at all the three doses tested. The least effective fungicide was Carbendazim (50 % WP), which showed 65.00 %, 66.87 % and 71.80 % inhibition at 0.05%, 0.1 %, 0.15% doses. Among the combination fungicides evaluated, Tebuconazole (50% WG)+ Trifloxystrobin (25% WG) and Carbendazim (12% WP) + Mancozeb (63% WP)showed complete inhibition at all three concentration. Azoxystrobin (18.2% SC) + Difenoconazole (11.4 % SC)were found to be comparatively less effective exhibiting inhibition at a range of 86.70 – 89.84 % (Table 1).Sur *et al.* (2021) conducted studies on *in vitro* assessment of fungicides against *Neoscytalidium* sp. isolated from Apricot, where cent percentage inhibition was recorded with Tebuconazole which is comparable to the present study.

The response of *Trichoderma asperellum* against *N. dimidiatum* showed 51.32 % inhibition of the pathogen through intermingling of hyphae mechanism and 54.89 % inhibition was found in dual culture assay with *P. fluorescens* antagonist (Fig 6). When tested with *T. asperellum* talc formulation (Table 2), the antagonist showed 40.83 %, 61.70 % and 68.92 % inhibition at 1 %, 2 5 and 3 % respectively. However, the talc formulation of *P. fluorescens* showed 49.96 %, 45.21 % and 39.21 % inhibition at 3%, 2% and 1% concentrations of talc formulations. Studies conducted by Intana *et al*. (2023) revealed that the *T. asperelllum* K1- 02 train recorded an inhibition of 84.45 % in dual culture assay against *Neoscytalidium dimidiatum*. Talc based PGPM formulation was observed to show per cent inhibition of 73.39 %, 67.33 %, and 48.32% at 3%, 2 and 1 % concentrations. According to a review of the literature, *Neoscytalidium* has not yet been recorded from dragon fruit in Kerala. As a result, this appears to be the first record of *Neoscytalidium* stem canker on dragon fruit in Kerala. The nucleotide sequence of the fungus was deposited in gene bank of NCBI with accession number OR975327.

CONCLUSION

The paper describes the occurrence and symptoms of stem canker associated with dragon fruit in Kerala. The pathogen was identified using cultural and morphological characteristics, and its identity was validated by molecular characterisation as *Neoscytalidium dimidiatum*. According to the findings, contact fungicides such as Propineb (70% WP), Mancozeb (80% WP), systemic chemicals such as Tebuconazole (250 EC), Difenoconazole (25% EC), and the combination fungicide Tebuconazole (50% WG) + Trifloxystrobin (25% WG), Carbendazim (12% WP) + Mancozeb (63% WP) were found effective in *in vitro* against *Neoscytalidium*. PGPM was noticed to be extremely effective against *Neoscytalium dimidiatum* among the biocontrol agents tested.

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