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First report of *Colletotrichum siamense* causing anthracnose of dragon fruit (*Hylocereus* sp.) in Kerala and *in vitro* evaluation of fungicides and bioagents against the pathogen

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Dragon fruit (*Hylocereus*sp.) is a new and promising fruit crop that has gained popularity in Kerala in recent years. Recently, anthracnose, a damaging disease of dragon fruit, was discovered in the dragon fruit growing regions of Central Kerala, in March 2023. The highest disease incidence of 50% and severity of 29% was documented from Chirakkekode area of Thrissur. Symptoms on cladode began as orangish brown coloured, sunken lesions which later grew larger, coloured mycelia and eventually turned to an encrustation with a grey hue. A profusion of pin-head-sized, black fruiting bodies covered the encrustation. On the fruits,yellowish to brown coloured sunken patches were produced which later enlarged and subsequently the whole fruit surface was coated in a brown to blackish lesion. The pathogen up on isolation on PDA, produced grey to brown coloured mycelia and the pathogenicity on cladode and fruit were established within five and two days after inoculationrespectively.Conidia were hyaline, bullet shaped with central oil globule and measured between 12.14-14.71x 2.72-3.22 μm. Molecular characterization by amplification of ITS specific sequences followed by *in silico* analysis revealed the pathogen to be *Colletotrichum siamense.* The *in vitro* efficacy of fungicides and biocontrol agents when tested, contact fungicides like Mancozeb, Bordeaux mixture, systemic fungicides like Tebuconazole, Difenconazole and Carbendazim and the combination fungicide Carbendazim (12%) + Mancozeb (63%)were found to be superior *in vitro* exhibiting cent percentage inhibition. In case of biocontrol agents, talc based formulation of PGPM consortium showed higher per cent inhibition of the pathogen followed by *Trichoderma asperellum*.

Keywords: Anthracnose, biocontrol agents, *Colletotrichum siamense*, fungicides

INTRODUCTION

Dragon fruit (*Hylocereus* sp.)is a climbing cactus that is also known as pitaya, pitahaya, lady of the night, and queen of the night coming under the family Cactaceae, is considered to have originated in Mexico and Central and South America. This fruit crop is climate resilient and can withstand the harsh weather of India's semi-arid and arid regions. Dragon fruit is well known for its nutritional value as it contains significant amounts of minerals such as potassium, phosphorus, sodium and magnesium.

In fact, these are higher than those of mangosteen, mango and pineapple (Stintzing *et al*. 2003; Gunasena *et al*. 2007). 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).

There are 17 genera and 25 species of plant pathogens currently infecting dragon fruits including fungi, bacteria, viruses and nematodes (Balendres and Bengoa, 2019).Anthracnose is frequently reported and is relatively the most destructive fungal disease of dragon fruit. The first report of anthracnose disease of dragon fruit was from Florida by Palmateer *et al*. (2007). According to Abirami *et al.* (2019) the disease affected nearly 30 % of dragon fruit plants in Andaman and Nicobar Islandswith a consequent yield reduction.

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During March 2023, typical symptoms of anthracnose were observed on dragon fruit growing regions in Central Kerala. Subsequently, the studies were conducted to identify the pathogen, symptomatology, cultural, morphological and molecular characteristics of the pathogen and the *in vitro* efficacy of chemical fungicides and biocontrol agents against the pathogen.

MATERIALS AND METHODS

Survey and sampling was carried out on dragon fruit growing regions in Central Kerala. Disease incidence and severity was assessed for each diseased sample. Symptom development of the disease was studied under both natural and artificial conditions. Standard tissue segmentation method was used to isolate the fungus on PDA medium and further purification was 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 and fruits of dragon fruit were artificially inoculated with the mycelial bits of the pathogensafter making injury by pin prick method. Moist cotton pieces were placed over the mycelial bits to provide sufficient moisture and were maintained in humid conditions along with control at room temperature until the development of symptoms.

Based on cultural and morphological characteristics,the pathogens were identified up to genus level by comparing the description given in CMI Descriptions of Pathogenic Fungi and Bacteria. The fungal isolate obtained from the dragon fruit sample having highest disease incidence and severity were subjected to the species level identification. 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 the obtained sequence was analyzed using BLASTn program of NCBI.

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

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

Anthracnose symptom on dragon fruit cladode appeared as orange-brown coloured, round 1-2 mm sized sunken lesions which later grew larger, coalesced and eventually turned to an encrustation with a grey hue. As the disease progressed,profusion of pin-head-sized, black coloured acervuli covered the encrustation. The descriptions of the above symptoms were in line with the findings of Abirami *et al.* (2019) and Salunkhe *et al.* (2023) on dragon fruit. On the fruits, the symptoms appeared as yellowish to brown coloured, sunken patches which later enlarged and subsequently the whole fruit surface was coated in a brown to blackish lesion. Symptoms occurring in the field have been depicted in Fig. 1(A-D). Affected fruits had reduced preference in the market and were unfit for sale.

The occurrence of anthracnose disease was recorded from various dragon fruit orchards in Central Kerala. The highest disease incidence (50.00 %) and severity (28.95 %) was recorded

Table. 1: Disease incidence and severity of anthracnose of dragon fruit

Location		Geographical coordinates	Per cent disease incidence (%)	Disease severity (%)
Chirakkekode	Field 1	10.561672/76.294555	28.95	50.00
	Field 2		19.95	20.00
Vellanikkara		10.552624/76.278157	8.43	9.00
Vaniyampara		10.5788/76.4129	16.38	20.00

Table 2: *In vitro* evaluation of fungicides against *Colletotrichum siamense*on PDA

Fungicides	Concentration (%)	Inhibition (%)
Chlorothalonil (75% WP)	0.1	100.00 (80.40)^{a}
	0.20	100.00 (80.40)^{a}
	0.30	100.00 (80.40)^{a}
Copper hydroxide (77% WP)	0.15	64.50 (56.48)^{h}
	0.20	72.23 (58.19)^{h}
	0.30	77.22 (61.49)^{g}
Propineb (70% WP)	0.10	47.55 (43.59)^{j}
	0.20	56.52 (48.74)^{l}
	0.30	77.30 (61.54)^{g}
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	86.93 (9.34)^{c}
	1.00	100.00 (80.40)^{a}
	1.50	100.00 (80.40)^{a}
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	100.00 (80.40)^{a}
	0.10	100.00 (80.40)^{a}
	0.15	100.00 (80.40)^{a}
Trifloxystrobin 25%WP +Tebuconazole 55% WG	0.03	80.31 (68.81)^{f}
	0.04	83.37 (65.93)^{de}
	0.05	91.48 (73.03)^{b}
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	81.58 (64.43)^{ef}
	0.10	83.76 (66.24)^{de}
	0.15	85.06 (67.39)^{cd}
CD (0.05)		1.836
CV		1.543

Data in parenthesis are angular transformed values

Table 3. *In vitro* evaluation of biocontrol formulations against *Colletotrichum siamense*

Biocontrol agent	Concentration (%)	Inhibition (%)
T. asperellum	1	80.06 (63.48)[f]
	2	81.55 (64.56)[e]
	3	88.29 (69.99)[b]
P. fluorescens	1	65.11 (53.79)[h]
	2	75.17 (60.11)[g]
	3	82.58 (65.33)[d]
PGPM	1	87.74 (69.50)[c]
	2	100.00 (80.40)[a]
	3	100.00 (80.40)[a]
CD (0.05)	0.304	
CV	0.352	

Data in parenthesis are angular transformed values

from Chirakkekode, whereas the lowest disease incidence of 8.43 % and severity of 9.00 % was recorded from Vellanikkara area of Thrissur district.

Isolation and pathogenicity

The samples when subjected to isolation on PDA medium, the fungus produced white to brown coloured woolly mycelia on PDA. In order to establish pathogenicity, mycelial bits taken from the margins of actively growing colonies (six days old culture) of the pathogen were placed on healthy cladode and fruits. The cladodes and fruits were wounded with sterile dissection needle before inoculation for the better establishment of the pathogen and were kept in humid conditions for disease development. The symptoms started to appear on the inoculated cladodes after five days of inoculation and on the fruits within 2 days of inoculation (Fig. 1 E-H). On the cladode, symptoms initiated as orange to brown spots of one to two mm size. The dots expanded and coalesced over time to form a huge encrustation of 1.5 to 2 cm. On the fruits, brown coloured water soaked lesions appeared which later expanded and covered the entire fruit surface. Thesame fungus was re-isolated from inoculated plants fulfilling the Koch's postulates. The re-isolated fungal isolate was found to be morphologically similar to the initially isolated culture ofthe pathogen.

Characterization and identification of the pathogen

The pathogen cultured on PDA medium produced grey to brown coloured mycelium (Fig.2).The culture attained full growth in the Petri plate (9 cm) after 6 days of incubation at room temperature and the growth rate was 1.5 cm per day. The mycelium was branched, septate andhyaline.The pathogen produced conidia having bullet shape with central oil globule and measured between 12.14- 14.71 x 2.72- 3.22 μm. Based on the cultural and morphological characters, the pathogen was identified at the genus level as *Colletotrichm* sp.

The forward and reverse nucleotide sequence obtained by ITS sequencing was compared with known sequences of nucleotides available in NCBI, and revealed a sequence similarity of 99.47 % with 99 % query coverage and maximum score

Fig. 1: (A-D) Development of symptoms of *Colletotrichum* anthracnose under field condition and **(E-H)**on artificially inoculated plant

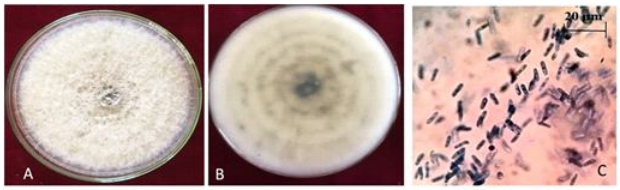

Fig. 2: Growth of mycelium of pathogen on PDA medium (A,B) and microscopic observation of conidia (C)

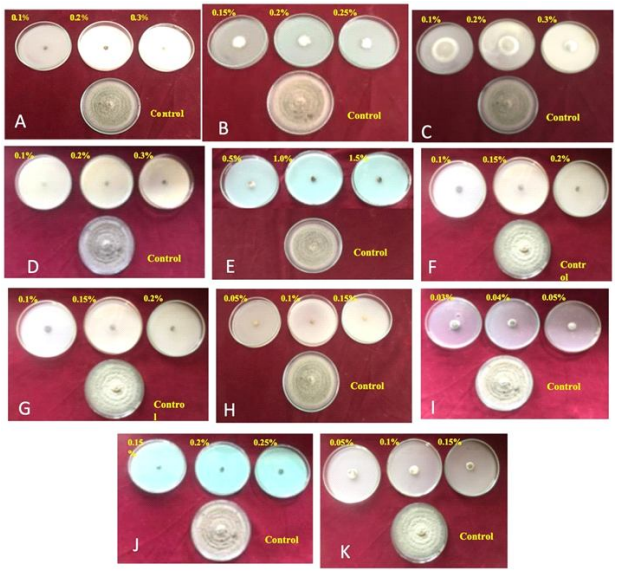

Fig.3: *In vitro* efficacy of fungicides against *C. siamense*. **A**-Chlorothalonil; **B**-Copper hydroxide; **C**-Propineb;**D**-Mancozeb;**E**-Bordeaux Mixture; **F**-Tebuconazole; **G**-Difenoconazole; **H**-Carbendazim; **I**-Tebuconazole (50%)+Trifloxystrobin (25%); **J**-Carbendazim (12%)+Mancozeb (63%); **K**-Azoxystrobin (18.2%)+Difenaconazole (11.4%)

of 966 with accession number MH151143.1 of *Colletotrichum siamense.*The nucleotide sequence of the isolate was deposited in GenBank with accession no.PP025517. These results confirmed the identity of the fungus as *C. siamense.*

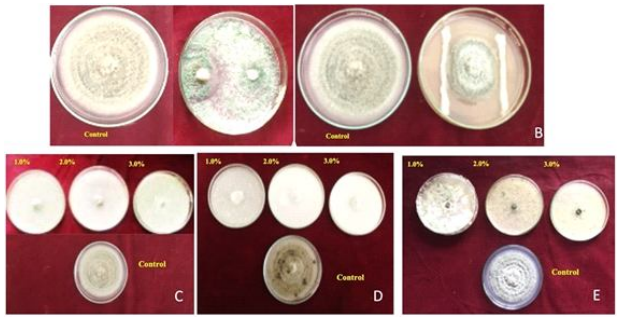

Fig.4 :*In vitro* efficacy of bioagents and their formulationsagainst *C. siamense*. **A**- *Trichoderma asperellum;***B**- *Pseudomonas fluorescens;***C-** Talc formulation of *T. asperellum*; **D**- Talc formulation of *P.fluorescens ;* **E**- Talc formulation of PGPM

In vitro evaluation of chemical fungicides and biocontrol agents against Colletotrichum siamense

The *in vitro* analysis was conducted in Completely Randomized Design (CRD) with three replications each. Among the contact fungicides tested, Chlorothalonil (75% WP) and Mancozeb(80 % WP) at all the three concentrations and Bordeaux mixture at medium and higher doses exhibited cent percentage inhibition against *Colletotrichum siamense* (Fig. 3).The least effective among them was Propineb (70 % WP) which showed an inhibition of 77.30 %, 56.52 % and 47.55 % at 3%, 2 % and 1 % concentrations respectively. In case of systemic fungicides, Tebuconazole (250 EC), Difenoconazole (25% EC)and Carbendazim (50 % WP) showed cent per cent inhibition at all the three doses tested. Among the combination fungicides evaluated, Carbendazim (12% WP) + Mancozeb (63% WP)showed complete inhibition at all three concentrations (Table 2).In the study of Zhang *et al*. (2020) similar kind of inhibition against *C. siamense* was observed in both Difenoconazole and Tebuconazole. Chen *et al*. (2016) recorded the similar inhibition of *Colletotrichum siamense* in the media incorporated with Tebuconazole.

The response of *Trichoderma asperellum* against *C. siamense* showed cent per cent inhibition of the pathogen through overgrowth mechanism and 54. 52 % inhibition was found in dual culture assay with *P. fluorescens* (Fig 5). When tested with talc formulation of *T. asperellum* (Table 3), the antagonist showed 80.06 %, 81.55 % and 88.29

% inhibition at 1 %, 2 % and 3 % respectively.However, the talc formulation of *P. fluorescens* showed 65.11 %, 75.17 % and 82.58 % inhibition only at 1%, 2% and 3% concentrations. Studies conducted by Fantinel *et al*. (2018) recorded inhibition of 44.2 %, 39.5 % and 43 % against *C.siamense* with *T. koningiopsis*, *T. asperellum* and *T. harzianum*. Talc based PGPM formulation was observed to show cent per cent inhibition at 2 % and 3 % concentrations respectively.

According to the review of the literature, *Colletotrichum siamense* has not yet been recorded from dragon fruit crop in Kerala. As a result, and hence, this appears to be the first record of *Colletotrichum siamense* anthracnose on dragon fruit in Kerala.

CONCLUSION

The paper describes the occurrence and symptoms of anthracnose associated with dragon fruit in Kerala. The pathogen was identified using cultural and morphological characteristics, and through molecular characterization,theidentity was confirmed as*Colletotrichum siamense*. According to the findings, contact fungicides such as Mancozeb (80% WP) and Bordeaux mixture systemic chemicals such as Tebuconazole (250 EC), Difenoconazole (25% EC) and Carbendazim (50 % WP) and the combination fungicide Carbendazim (12% WP) + Mancozeb (63% WP) were found effective in *in vitro* against *Colletotrichum siamense*. The microbial consortium PGPM was noticed to be extremely effective against *C. siamense* among the biocontrol agents tested.

DECLARATIONS

Conflict of interest: Authors declare no conflict of interest.

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