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FIRST REPORT OF WILT DISEASE CAUSED BY *RIGIDOPORUS VINCTUS* ON DRAGON FRUIT IN KERALA, INDIA

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ABSTRACT

This study investigated the prevalence and characteristics of fungal wilt disease in dragon fruit plants across two agroecological units (AEU) in Kerala, India. A purposive sampling survey was conducted in Kottayam AEU 09 and Thiruvananthapuram AEU 12. Wilt disease, characterized by yellowing, wilting, and death of plants, was particularly prevalent in the Vellavoor block of Kottayam district. Fungal pathogens were isolated from infected samples, and pathogenicity studies confirmed the rapid symptom development by the isolates. Among the isolates, *Rigidoporus vinctus*, identified through molecular characterization, showed significant pathogenicity. *In vitro* evaluations of various bioagents, fungicides and chemicals against *R. vinctus* revealed that *Trichoderma harzianum* and difenoconazole were highly effective in inhibiting fungal growth. Additionally, chemicals like potassium phosphonate, calcium chloride, and sodium bicarbonate achieved 100% inhibition of the pathogen. The study highlights the efficacy of specific bioagents, fungicides, and chemicals in managing *R. vinctus* infections in dragon fruit plants under in vitro conditions.

Key words : Agroecological units, *Rigidoporus vinctus*, Difenoconazole.

Introduction

Hylocereus undatus Haw., or dragon fruit, is a member of the Cactaceae family and is favoured for its unusual appearance and delicate texture (Nerd *et al.*, 2002). Due to its unique vegetative traits, it can be easily distinguished from the other plant kingdom families. According to Nobel (2002), *Hylocereus* species typically have elongated, three-angled, or three-winged stems with aerial roots on their branches. Due to their edible nature and more than 9,000 years of history as a component of the human diet, (*Hylocereus* sp.) are among the most significant members of this family, native to Mexico, Central America, and South America are *Hylocereus* sp. (Nobel, 2002).

Materials and Methods

Survey and collection of samples

In Kerala's principal dragon fruit-growing districts, Kottayam and Thiruvananthapuram, a purposive random survey was conducted under the Agro-Ecological Units (AEU) 09-(south-central laterite) and AEU 12-(Southern and central foothill), respectively. Each district had a field that was infected and to determine the disease incidence, 100 plants were randomly chosen from each field.

Isolation of the Fungal Pathogens and Pathogenicity studies

Partially healthy tissue and a small portion of freshly infected cladode were removed to isolate pathogens. The plant sections were surface sterilized for 60 seconds using 1% sodium hypochlorite, and then they were washed

three times with sterile water. The cladode bits were placed on sterile tissue paper to remove any excess moisture. Following sterilization, these bits were put on sterile Petri plates plated with potato dextrose agar medium (PDA). After three days of incubation at room temperature (28±2°C), the fresh mycelial strands were observed from the cut end of plant bits kept in the plates and were transferred to PDA slants.

Characterization and Identification of Pathogen

Cultural characterisation of the isolates

The isolates were grown on a PDA medium to examine their cultural traits (Hawa *et al*., 2013). Before being put into Petri dishes and allowed to solidify in an aseptic environment, the PDA medium was sterilized. Each isolate's five-day-old culture produced a five-millimetre mycelial disc, which was placed individually in the Petri dish's centre. The plates were incubated at 27 ± 20° C or room temperature. Every isolate was kept in five replications. For every isolate, observations were made regarding the radial growth, growth rate, growth pattern, colony colour and the number of days needed to cover 9 cm diameter Petri dishes.

Morphological characterisation of the isolates

Using a Leica-manufactured DM 750 microscope, the morphological characteristics of the various isolates, such as mycelial characters, sporulation, size and shape of conidia and appressoria formation were examined under 100X and 400X magnification using a microscopic slide staining technique with lactophenol cotton blue stain (Rolshausen *et al*., 2013).

Molecular characterisation of the isolates

Using the universal primers ITS 1, ITS 4, and Tef and the DNA barcoding technique, the virulent isolates were molecularly characterized (Chuang *et al.*, 2012).

***In vitro* evaluation of bioagents using dual culture technique**

The assay was conducted using sterile Petri plates plated with PDA medium. Five-day-old, five-mm discs of the bioagents (*Trichoderma asperellum*, KAU isolate) and *Trichoderma harzianum*, IISR isolate) were placed separately on the opposite side of each isolate. Mycelial discs of each isolate were placed separately on one side of a Petri plate. *Pseudomonas fluorescens* (KAU isolate) and *B. amyloliquefacians* (KAU isolate) are the bacterial biocontrol agents. A 24-hour-old antagonist bacterial culture was streaked on one side, while a seven-day-old fungal isolate with five-mm-diameter mycelial discs was placed on the other. For every combination of isolate and bioagent, the experiment was run independently. Plates were sealed and incubated at room temperature after the inoculation. Each isolate's mycelial growth in dual culture plates and corresponding control plates was measured on a regular basis and the percentage of each isolate's growth inhibition by each bioagent was calculated (Tiwari *et al*., 2021).

$$PI = \frac{C- T}{C} \times 100$$

Where,

PI = Per cent Inhibition

C = Growth of the pathogen in control plates (cm)

T = Growth of the pathogen in PDA (cm)

***In vitro* evaluation of fungicides and chemicals against selected fungal pathogens by poisoned food technique**

Using the poisoned food technique, *In vitro* evaluation of fungicides against each pathogen was conducted at four different concentrations, *i.e.*, one-fourth the recommended dose, half the recommended dose, the recommended dose and double the recommended dose (Chuang *et al.*, 2012). The pathogen was grown in molten sterilized PDA, which served as the nutrient medium. To achieve the necessary concentration of each fungicide in the media, a separate amount of each fungicide was added to a 250 ml conical flask containing sterilized media. After thoroughly mixing the fungicide with the media and plating about 20 millilitres of the poisoned medium in each sterile Petri plate, the mixture was allowed to solidify. With a cork borer, a five mm mycelial disc of each isolate was cut off from the 10-day-old culture and aseptically placed in the middle of each Petri plate holding the poisoned solid medium. For every fungicide concentration, three replications were kept and the control plates were made with plain media. The incubation temperature of the inoculated plates was 27± 2°C. Every day, the radial growth of every pathogen was measured at various fungicide concentrations. Eraslan and Oksal's (2021) formula was used to calculate the per cent growth inhibition (PI) for each treatment.

$$\text{Per cent growth inhibition (\%)} = PI = \frac{C- T}{C} \times 100$$

Where,

PI = Percent inhibition (%)

C = Colony diameter in control plates (cm)

T = Colony diameter in corresponding treatment plates (cm)

Results

Survey and collection of samples

A purposive sampling survey was conducted in Kottayam AEU 09 (south-central laterite) and Thiruvananthapuram AEU 12 (southern and central foothill). The characteristic symptoms and the number of infected plants in each surveyed location were represented in Table 1 and Plate 1.

Wilt disease was prevalent in the Vellavoor block of Kottayam district, AEU 12. The plants affected by this disease exhibited rapid disease development, which initiated as yellowing of leaves, followed by wilting, and ultimately the death of the plants.

Isolation and pathogenicity studies of fungal isolates

Pathogenicity studies for the selected isolates were carried out separately and all the isolates reproduced the typical symptoms within a period of seven to ten days. The symptoms were initiated within 3–4 days after pathogen inoculation. The initial symptoms developed by the artificial inoculation of the isolate I_2 were the presence of white rhizomorphs on the stem. The disease was further advanced by rotting, which later turned the stem to black in colour (Table 2). The radial growth of isolate I_2 ranged from 2.2 cm at 3 DAI to 4.4 cm at 5 DAI and 8.0 cm at 7 DAI with dense white cottony mycelia (Table 3). It took 8 days for symptom development (Table 4). The surface of the fruit became brown in colour, but the infection was restricted to peripheral portions(Table 5).

Characterization and identification of the pathogen

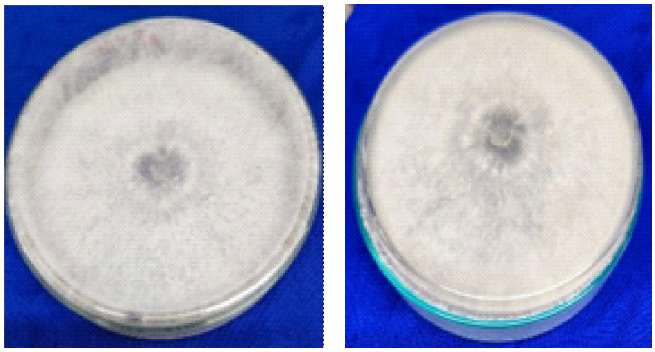
Cultural and morphological characterization of the isolates

Isolate I_2 produced white flattened mycelium when cultured on PDA (Table 6). The plates were fully covered with a milky white mycelium within 8–10 days at 28 ± 2°C. The width of the hyphae varied from 3.0 to 4.5 µm. They were thick-walled, hyaline, and septated with no clamp connections, and the hyphal system was monomitic (generative hyphae) (Table 7).

Vellavor (AEU12)

Plate 1 : Disease symptom observed in surveyed plots located in AEU 12.

Plate 2 : Symptom developed on cladode in response to artificial inoculation.



Front end **Rear end**

Plate 3: Pure cultures of the isolate

Molecular characterization of the isolates

Sequencing of the ITS region of each isolate was carried out for fungal identification. In this study, the DNA of the isolate I_2 was further amplified with large subunit ribosomal r RNA(LSU) gene-specific primers, and a PCR product of 480 bp was obtained.

BLAST and phylogenetic analysis of DNA sequence of isolate I_2 with ITS primers 1 and 4 showed cent percent similarity to sequences of *Rigidoporus vinctus* available in GenBank. Hence, the isolate was identified as *Rigidoporus vinctus* with the Gen bank accession number OR501542.1 (Table 8)

In vitro evaluation of bioagents, fungicides and chemicals against selected fungal pathogens

In vitro evaluation of bioagents using dual culture technique

Four bioagents *viz.*, *Bacillus amyloliquefaciens*, *Pseudomonas fluorescens*, *Trichoderma harzianum*

Table 1 : Details regarding surveyed locations in AEU 9 and 12.

S. no.	Survey location	Agroecological unit (AEU)	GPS location	Nature of symptoms
1.	Vellavoor	AEU 09	9.48°N76.71°E	Rotting of the infected stem /cladode

Table 2 : Mycelial characteristics of different isolates and symptoms developed on plants in response to artificial inoculation.

S. no.	Isolates	Appearance of mycelia	Nature of symptoms
1	I_2	Concentric rings of white aerial mycelium	Yellowish, sunken brown lesions on cladodes

Table 3 : Variability in the radial growth of various fungal isolates obtained from dragon fruit plants.

S. no.	Isolates	Radial growth (cm)			Average growth rate (cm day^{-1})
		3 DAI*	5 DAI*	7 DAI*	
1	I_2	1.1 ± 0.04	2.2 ± 0.100	4.0 ± 0.050	0.733 ± 0.054

*Mean of 3 replications

Table 4 : Lesion size and number of days taken for symptom development on artificial inoculation by fungal isolates.

S. no.	Isolates	Lesion length (cm)**	Lesion width (cm)**	DTSD*
1	I_2	2.8 ± 0.048	2.5 ± 0.173	8

*DTSD – Days taken for symptom development.
**Mean of 3 replications

Table 5 : Nature of symptom and number of days taken for symptom development on fruits by various fungal isolates on artificial inoculation.

S. no.	Isolates	DTSD*	Nature of symptoms
1	I_2	6	The surface of the fruit became brown in colour, but the infection restricted to peripheral portions

*DTSD – Days taken for symptom development

Table 6 : Cultural characteristics of the isolates.

S. no.	Isolates	Cultural characters			
		Nature of mycelia	Pattern of growth	Front view	Rearview
1	I_2	White aerial mycelium with concentric rings	White, flattened	White	Had pinkish tinge at base

Table 7 : Morphological characteristics of the isolates.

S. no.	Isolates	Morphological characters				
		Hyphal Colour	Septation (present/not present)	Shape of spore	Size of spore	Hyphal width
1	I_2	White	Septate	Sickle-shaped	13.00 x 3.85 µm	3.25 - 4.8 µm

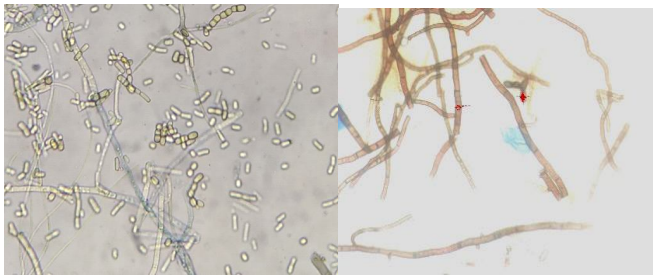
Table 8 : Sequence comparison of the isolate I_4 with the similar sequences available in the Gen Bank.

Scientific name	Query Cover	Accession No.	Country
Rigidoporus vinctus MEBP0032	100%	MT597859.1	Philippines
Rigidoporus vinctus	100%	LC269928.1	Thailand

and *Trichoderma asperellum* were evaluated under *in vitro* condition for their efficacy against *R. vinctus*. There had been significant differences in treatment effects with respect to reduction in radial diameter of fungal growth as influenced by various biocontrol agents. *In vitro* evaluation of biocontrol agents like *B. amyloliquefaciens* (VLY24), *P. fluorescens* (PN026), *T. harzianum* (IISR isolate) and *T. asperellum*. (KAU isolate) against *R. vinctus* revealed that all bioagents had an inhibitory effect on the pathogen. Among them, *T. harzianum* significantly inhibited the pathogen (58.33%) followed by *P. fluorescens* (26.19%) (Table 9).

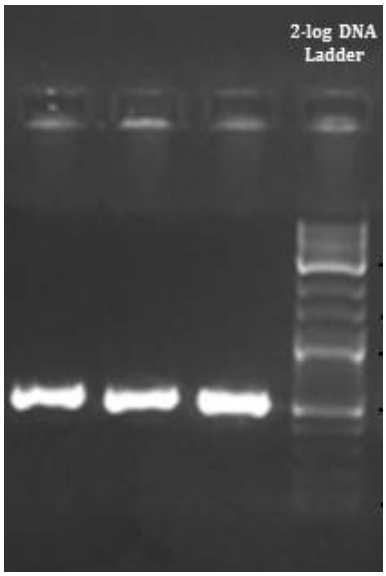
In vitro evaluation of fungicides against fungal pathogens by poisoned food technique

Against the pathogen *R. vinctus* under *in vitro* condition efficacy of three contact fungicides were evaluated. Four doses of each fungicide, namely copper hydroxide 53.8%w/w DF, copper oxychloride 50% WP,



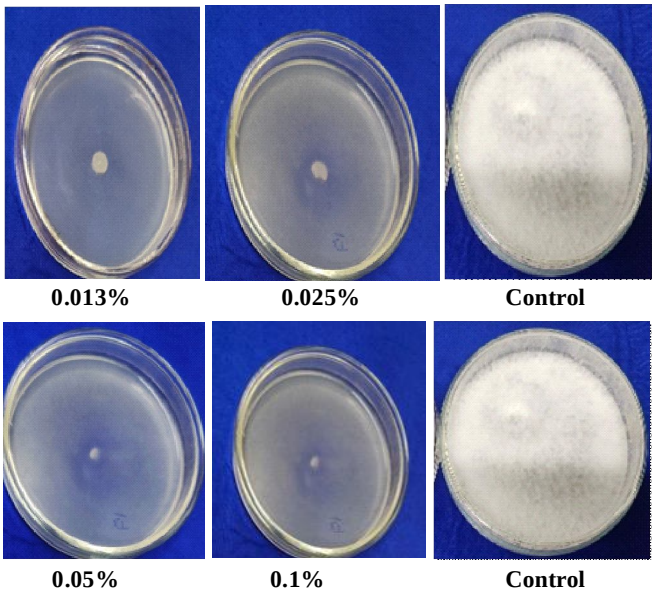
(a) Ellipsoid to ovoid spores **(b) Septate hyphae**

Plate 4 : Microscopic characteristics of isolate I_2.



(a) isolate with primer pairs ITS1 and ITS4 and 100 bp ladder (lane 4).

Plate 5 : Amplified products obtained in polymerase chain reaction.



Growth inhibition of *R. vinctus* by different concentrations of trifloxystrobin 25% WG+ tebuconazole 50%

Plate 6 : Percent growth inhibition of *R. vinctus* at different concentrations of various fungicides on PDA media at 7 DAI.

difenoconazole 25%EC, azoxystrobin 25%SC and propineb 70%WP. Among them all the concentrations of difenoconazole showed cent-per-cent inhibition of *R. vinctus*. This was closely followed by recommended and double dose of azoxystrobin which recorded an inhibition rate of greater than 97%. The least inhibition was observed for the least concentration of azoxystrobin evaluated (0.025%), *i.e.*, only 3.57% inhibition (Table 11).

Table 9 : *In vitro* evaluation of bioagents against *R. vinctus.*

Treatments	Bioagents	Growth of the pathogen *(cm)	Percent inhibition ** (%)
T_1	*P. fluorescens*	6.2 ± 0.087^{d}	26.19 (30.78)[b]
T_2	*T. asperellum*	7.4 ± 0.025^{c}	11.90 (20.18)[c]
T_3	*T. harzianum*	3.5 ± 0.087^{e}	58.33 (49.80)[a]
T_4	*B. amyloliquefaciens*	7.9 ± 0.038^{b}	5.95 (14.12)[d]
T_5	Control(mm)	8.4 ± 0.000^{a}	-
	SE(m)	0.0054	0.193
	CD (0.05)	0.0163	2.305

*Mean of three replications **Values in parenthesis are angular transformed values

**Values with the same alphabet are statistically not significant.

and BM were evaluated against the pathogen. Among them, all concentrations of BM contributed cent per cent inhibition. This was notably higher than the inhibition observed for the recommended dose of 0.2% *i.e.*, 89.28% In contrast, the inhibition was minimum for all the concentrations of copper oxychloride (Table 10). The systemic fungicides evaluated against *R. vinctus* were

Combination fungicides evaluated in the study were carbendazim 63%+ mancozeb 12% WP and trifloxystrobin 25% WG+ tebuconazole 50%, at four different concentrations. Among them, combination of trifloxystrobin 25% WG + tebuconazole 50% at all concentrations worked well with 100% inhibition of *R. vinctus* growth. The recommended and double doses of

Table 10 : *In vitro* evaluation of effect of contact fungicides against *R. vinctus* by poisoned food technique.

Treatments	Fungicides	Growth of mycelium (cm)*	Percentage of inhibition (%) **
T_1	Copper hydroxide (0.05%)	5.6 ± 0.100^{f}	33.33 (35.26)^{e}
T_2	Copper hydroxide (0.1%)	3.4 ± 0.100^{g}	59.52 (50.49)^{d}
T_3	Copper hydroxide (0.2%)	0.9 ± 0.058^{h}	89.28 (70.89)^{c}
T_4	Copper hydroxide (0.4%)	0.8 ± 0.058^{i}	90.47 (72.02)^{b}
T_5	Copper oxychloride (0.05%)	8.2 ± 0.058^{b}	2.38 (8.87)^{i}
T_6	Copper oxychloride (0.1%)	7.2 ± 0.058^{c}	14.28 (22.20)^{h}
T_7	Copper oxychloride (0.2%)	6.4 ± 0.058^{e}	23.80 (29.20)^{f}
T_8	Copper oxychloride (0.4%)	6.5 ± 0.058^{d}	22.61 (28.39)^{g}
T_9	Bordeaux mixture (0.25%)	0.00 ± 0.000^{j}	100.00 (90.00)^{a}
T_{10}	Bordeaux mixture (0.5%)	0.00 ± 0.000^{j}	100.00 (90.00)^{a}
T_{11}	Bordeaux mixture (1%)	0.00 ± 0.000^{j}	100.00 (90.00)^{a}
T_{12}	Bordeaux mixture (2%)	0.00 ± 0.000^{j}	100.00 (90.00)^{a}
	Control	8.4 ± 0.000^{a}	-
	Mean	0.322	-
	SE(m)	0.0406	0.220
	CD (0.05)	0.120	2.064

*Mean of three replications **Values in parenthesis are angular transformed values
**Values with the same alphabet are statistically not significant.

Table 11 : *In vitro* evaluation of effect of systemic fungicides against *R. vinctus* by poisoned food technique.

Treatments	Fungicides	Growth of mycelium (cm)*	Percentage of inhibition (%)**
T_{13}	Difenoconazole (0.025%)	0.00 ± 0.000^{j}	100.00 (90.00)^{a}
T_{14}	Difenoconazole (0.05%)	0.00 ± 0.000^{j}	100.00 (90.00)^{a}
T_{15}	Difenoconazole (0.1%)	0.00 ± 0.00^{j}	100.00 (90.00)^{a}
T_{16}	Difenoconazole (0.2%)	0.00 ± 0.00^{j}	100.00 (90.00)^{a}
T_{17}	Azoxystrobin (0.025%)	8.1 ± 0.100^{b}	3.571 (10.89)^{i}
T_{18}	Azoxystrobin (0.05%)	3.2 ± 0.058^{f}	61.90 (51.88)^{e}
T_{19}	Azoxystrobin (0.1%)	0.2 ± 0.000^{h}	97.61 (81.11)^{c}
T_{20}	Azoxystrobin (0.2%)	0.1 ± 0.000^{i}	98.80 (83.71)^{b}
T_{21}	Propineb (0.05%)	6.8 ± 0.100^{c}	19.04 (25.87)^{h}
T_{22}	Propineb (0.1%)	6.4 ± 0.058^{d}	23.80 (29.20)^{g}
T_{23}	Propineb (0.2%)	5.4 ± 0.058^{e}	35.71 (36.70)^{f}
T_{24}	Propineb (0.4%)	3.1 ± 0.058^{g}	63.09 (52.56)^{d}
	Control	8.4 ± 0.00^{a}	-
	Mean	0.242	-
	SE(m)	0.048	0.176
	CD (0.05)	0.139	2.056

*Mean of three replications **Values in parenthesis are angular transformed values
**Values with the same alphabet are statistically not significant.

Table 12 : *In vitro* evaluation of effect of combination fungicides against *R. vinctus* by poisoned food technique.

Treatments	Fungicides	Growth of mycelium (cm)*	Percentage of inhibition (%)**
T_{25}	Carbendazim + mancozeb (0.05%)	3.08 ± 0.100[b]	64.28 (53.30) [c]
T_{26}	Carbendazim +mancozeb (0.1%)	2.02 ± 0.058[c]	76.19 (60.79)[b]
T_{27}	Carbendazim +mancozeb (0.2%)	0.00 ± 0.00[d]	100.00 (90.00)[a]
T_{28}	Carbendazim +mancozeb (0.4%)	0.00 ±0.00 [d]	100.00 (90.00)[a]
T_{29}	Trifloxystrobin + Tebuconazole (0.013%)	0.00 ±0.00[d]	100.00 (90.00)[a]
T_{30}	Trifloxystrobin + Tebuconazole (0.025%)	0.00 ±0.00[d]	100.00 (90.00)[a]
T_{31}	Trifloxystrobin+ Tebuconazole (0.05%)	0.00 ±0.00[d]	100.00 (90.00)[a]
T_{32}	Trifloxystrobin + Tebuconazole (0.1%)	0.00 ± 0.00[d]	100.00 (90.00)[a]
T_{33}	Control	8.4 ± 0.00[a]	-
	Mean	0.322	-
	SE(m)	0.039	0.208
	CD (0.05)	0.118	2.120

*Mean of three replications **Values in parenthesis are angular transformed values
**Values with the same alphabet are statistically not significant.

Table 13 : Evaluation of four different doses of chemicals on mycelial growth of *R. vinctus* by poisoned food technique.

Treatments	Chemicals	Radial growth of mycelium* (cm)	Percentage of inhibition** (%)
T_1	Potassium phosphonate (0.075%)	0.00 ± 0.00 [b]	100.00 (90.00) [a]
T_2	Potassium phosphonate (0.15%)	0.00± 0.00 [b]	100.00(90.00) [a]
T_3	Potassium phosphonate (0.3%)	0.00± 0.00 [b]	100.00(90.00) [a]
T_4	Potassium phosphonate (0.6%)	0.00± 0.00 [b]	100.00(90.00) [a]
T_5	Calcium chloride (0.1%)	0.00± 0.00 [b]	100.00 (90.00) [a]
T_6	Calcium chloride (0.2%)	0.00± 0.00 [b]	100.00 (90.00) [a]
T_7	Calcium chloride (0.4%)	0.00± 0.00 [b]	100.00 (90.00) [a]
T_8	Calcium chloride (0.8%)	0.00± 0.00 [b]	100.00 (90.00) [a]
T_9	Sodium bicarbonate (0.075%)	0.00± 0.00 [b]	100.00 (90.00) [a]
T_{10}	Sodium bicarbonate (0.15%)	0.00± 0.00 b	100.00 (90.00) [a]
T_{11}	Sodium bicarbonate (0.3%)	0.00 ± 0.00 [b]	100.00 (90.00) [a]
T_{12}	Sodium bicarbonate (0.6%)	0.00 ± 0.00 [b]	100.00 (90.00) [a]
T_{13}	Control	8.90 ± 0.00[a]	

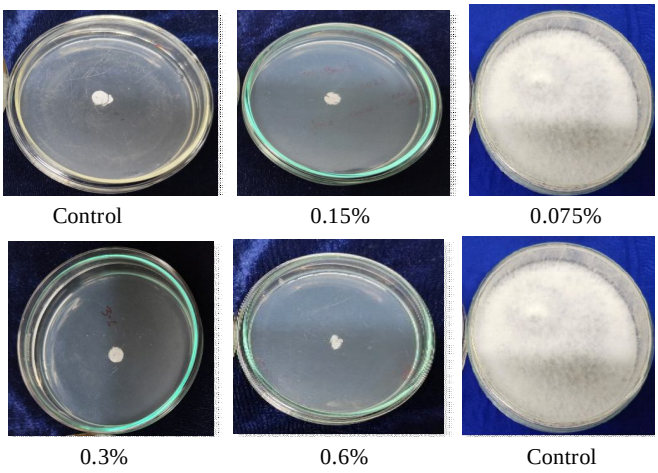
*Mean of three replications **Values in parenthesis are angular transformed values
**Values with the same alphabet are statistically not significant.

carbendazim 63% + mancozeb 12% WP (0.2% and 0.4%) also gave 100% inhibition, whereas its half dose also gave more than 75% inhibition of the fungal growth (Table 12).

Considering the overall performance of all the fungicides, the least concentration of BM (0.25%), difenoconazole (0.025%) and trifloxystrobin 25% WG+ tebuconazole 50% (0.025%) and recommended dose of carbendazim + mancozeb (0.2%) were identified as effective treatments against *R. vinctus* under *in vitro* condition

In vitro evaluation of chemicals against selected pathogens

Under *in vitro* conditions, three different chemicals



Growth inhibition of *R. vinctus* by different concentrations of Potassium phosphonate

Plate 7 : Per cent growth inhibition of fungal pathogen at different concentrations of various chemicals on PDA media at 7DAI.

were tested at varying concentrations *i.e.*, potassium phosphonate (0.075, 0.15, 0.3, 0.6%), calcium chloride (0.1, 0.2, 0.4, 0.8%) and sodium bicarbonate (0.75, 1.5, 3, 6%). to assess their efficacy against selected fungal pathogens isolated from dragon fruit. The results, presented in Table 13 highlighted the differences in treatment effects as the reduction in the radial diameter of each fungal growth, corresponding to the concentrations of the chemicals used. Against *R. vinctus*, all three chemicals *i.e.*, potassium phosphonate, calcium chloride and sodium bicarbonate, showed exceptional efficacy irrespective of their doses and achieved 100% inhibition of the pathogen (Table 13).

Discussion

Survey and collection of samples

A wilt disease that affected dragon fruit plants was common in the Vembayam region (AEU 12). The disease began as a yellowing of the cladodes and progressed to browning. The plants eventually began to wilt as a result of this disease (Plate 1). Ann *et al*. (1999) noted comparable symptoms, such as yellowing of leaves, wilting, and defoliation in rubber by a fungus (*Rigidoporus* sp.), even though wilt disease was not reported in dragon fruit. The current study found that this disease was present in dragon fruit plants that were grown on plantations that had previously been home to rubber trees. Thus, it is possible to suspect cross-infection of the pathogen linked to rubber root rot disease (*Rigidoporus* sp.) in this instance. The similarity in the way that dragon fruit wilting diseases progress.

Isolation of the fungal pathogens and pathogenicity studies

The initial symptoms developed by the artificial inoculation of isolate I_2 were the presence of white rhizomorphs on the stem. The disease was further advanced by rotting, which later turned the stem black in colour (Plate 2). Results were in agreement with the findings of Ann *et al*. (1999), where they reported that *Rigidoporus* cause rotting in rubber plantations.

Characterization and identification of pathogen

Cultural and morphological characterisation of the pathogen

As the white, fluffy mycelium from the isolate spread over the PDA plate, it finally showed signs of suppressed growth. After being incubated at 28 ± 2°C room temperature, a milky white mycelium covered the entire plate (Plate 3). This implies that the fungus is growing quickly on the artificial media. This isolate's hyphae showed a range of widths from 3.0 to 4.5 µm. They were transparent, had septations, and thick walls. I_2's hyphal system was found to be monomitic and made up of generative hyphae (Plate 4). In terms of *R. furcalus* fungal growth and hyphal characteristics, these results are consistent with those of earlier published studies by Silveira and Guerrero (1989)and Cui *et al.*, 2009. They indicate that *R. furcalus* has a monomitic to pseudo-dimitic hyphal system, which can have cystidia or not. For *R. furcatus*, forked cystidia have been reported (Nunez and Ryvarden, 2001).

Molecular characterisation of the pathogen

The sequences of *R. vinctus* deposited in the Gen bank (Gen bank no: OR501539.1) and isolate I_2 exhibited 100% similarity and at 480 bp clear bands were observed (Plate 5). According to Ahmad *et al*. (2021), there was 98–100% similarity between the pathogen that causes rubber to white rot and amplified sequences of *R. microporus*. This finding supports the likelihood of the pathogen spreading from rubber to dragon fruit.

In vitro evaluation of bioagents, fungicides and chemicals against the pathogens

In vitro evaluation of bioagents using dual culture technique

Trichoderma harzianum and *P. fluorescens* demonstrated the highest percentage of inhibition against *R. vinctus* (58.33 and 26.19%, respectively) (Plate 6). Similar findings were reported by Wonglom *et al.* (2022), who found that the *Trichoderma* formulation harbouring varying percentages of inhibition against *Rigidoporus* (60.25 to 76.19%) was incubated at a cool temperature.

In an *in vitro* study, *T. koningii*, *T. harzianum* and *T. reesei* were found to inhibit the growth of two unidentified *Ganoderma* sp. isolated from infected *Acacia mangium* plants in Indonesia (Widyastuti, 2006). The inhibition potential of *T. harzianum* (42.73%) against rubber root rot was also reported by Terna *et al.* (2016). When he found that the root rot pathogen of physic nuts experienced a 53.00% reduction in mycelial growth.

In vitro evaluation of fungicides by poisoned food technique

The contact fungicides Bordeaux mixture (BM) (100%), copper hydroxide (89.28%), and lower doses of copper oxychloride (*i.e.*, 2.38%) showed the highest and lowest percentages of inhibition for *R. vinctus* mycelial growth, respectively. According to Srinivasalu *et al.* (2002), BM is effective against fungi that rot roots and stems (*G. lucidum*). They speculate that the large copper ion particle size in BM, which caused its quick release and consequent suppression of spore germination, may be the cause of the inhibitory effect. The systemic fungicide difenoconazole completely inhibited the growth of the pathogen at all recommended, lower, and higher concentrations; the lowest amount of inhibition was seen at a lower concentration of azoxystrobin (3.57%) (Plate 6). According to Yousaf *et al.* (2018), difenoconazole's effectiveness against *Ganoderma* may be caused by inhibition of the pathogen cell wall's demethylation and sterol biosynthesis. The two combination fungicides that were tested, carbendazim + mancozeb and trifloxystrobin 25% + tebuconazole 50%WDG, both demonstrated 100% inhibition at the recommended dose, indicating a promising control against the pathogen (Plate 6). Tebuconazole 50%WDG plus fungicide trifloxystrobin 25% demonstrated a promising combination against the pathogen. According to Da Silva *et al.* (2022), the fungicide trifloxystrobin effectively prevents the white rot pathogen from affecting cinnamon by influencing the formation of cell walls.

In vitro evaluation of chemicals by poisoned food technique

At all concentrations, potassium phosphonate, calcium chloride, and sodium bicarbonate completely inhibited *R. vinctus* (Plate 7). In their research, Abdel *et al.* (2012) documented sodium bicarbonate's ability to inhibit the pathogen responsible for rubber's white rot. They concluded that the chemical changed the pathogen's cell wall composition and the hyphae's morphology.

Conclusion

This study comprehensively examined the prevalence and management of fungal wilt disease in dragon fruit plants across two agroecological units in Kerala. The survey identified significant disease presence in the Vellavoor block of Kottayam district, where rapid disease progression was observed. Isolation and pathogenicity studies confirmed *Rigidoporus vinctus* as the primary pathogen. Molecular characterisation further substantiated this identification. The *in vitro* evaluation of various bioagents, fungicides and chemicals demonstrated effective management strategies against *R. vinctus*. *Trichoderma harzianum* and difenoconazole emerged as the most potent bioagent and fungicide, respectively. Additionally, chemicals such as potassium phosphonate, calcium chloride and sodium bicarbonate showed complete inhibition of the pathogen. These findings provide valuable insights into effective disease management practices for dragon fruit cultivation, offering potential solutions to mitigate the impact of fungal wilt disease.

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References

Abdel-Mageed, M.H., Mohamed F.G., Soltan H.H., Hafez M.S. and Abdel-Rahman F.A. (2012). Controlling the grey mould and white rot diseases on bean pods under storage conditions using some safe chemical compounds. *Environ. Sci.*, **7(2)**, 617-634.

Ahmad, K. and Ismail S.I. (2021). Disease prevalence and molecular characterisation of *Rigidoporus microporus* associated with white root rot disease of rubber tree (*Hevea brasiliensis*) in Malaysia. *J. Rubber. Res.*, **24**, 175–186.

Ann, P.J., Lee H.L. and Huang T. (1999). Brown root rot of 10 species of fruit trees caused by *Phellinus noxius* in Taiwan. *Plant Dis.*, **83(8)**, 746-750.

Chuang, M.F., Ni H.F., Yang H.R., Shu S.L., Lai S.Y. and Jiang Y.L. (2012). First report of stem canker disease of pitaya (*Hylocereus undatus* and *H. polyrhizus*) caused by *Neoscytalidium dimidiatum* in Taiwan. *Plant Dis.*, **96(6)**, 906-906.

Cui, B.K., Dai Y.C. and Li B.D. (2009). Notes on the genus *Rigidoporus* (Basidiomycota, *Polyporaceae*) in China. *Nova Hedwigia*, **88**, 189–197.

Da Silva Brito, F., da Costa D.P., de Souza and de Medeiros E.V. (2022). Selection and control efficacy of *Trichoderma* sp. against *Fusarium solani* and *Lasiodiplodia theobromae* causing root rot in forage cactus. *Physiol. Mol. Plant Pathol.*, **122**, 101-900.

Eralsan and Oksal E. (2021). *In vitro* Efficiency of Some Fungicides Against *Neocystadilium* (Penz.) causing Sudden Shoot Dry on Apricot Trees. *Turkjans*, **9(4)**, 797-802.

Hawa, M.M., Salleh B. and Latiffah Z. (2013). Characterization and pathogenicity of *Fusarium proliferatum* causing stem rot of *Hylocereus polyrhizus* in Malaysia. *Ann. Appl. Biol.*, **163**, 269–280.

Nerd, A., Tel-Zur N. and Mizrahi Y. (2002). Fruits of the vine and columnar cacti. 185-197.

Nobel, P.S. (ed.) (2002). Biology and uses. *Cacti*, 185-187.

Nunez, M. and Ryvarden L. (2001). East Asianpolypores. *Syn. Fungorum*, **14 (2)**, 167-522.

Rolshausen, P.E., Akgül D.S., Perez R., Eskalen A. and Gispert C. (2013). The first report of wood canker caused by *Neoscytalidium dimidiatum* on grapevine in California. *Plant Dis*., **97(11)**, 511-1511.

Silveira, R.M.B. and Guerrero R.T. (1989). The genera *Ritidoporus* Murr. and *Flaviporus* Murr. (Basidiomycetes) from aparados da serra national park, Rs. *Acta Botanica Brasilica*, **3**, 29-45.

Srinivasulu, B., Sabitha D., Aruna K., Rao D.V.R. and Rabindran R. (2002). Efficacy of biocontrol agent, chemicals and botanicals on *Ganoderma* sp. the coconut basal stem rot pathogen. *J. Plant. Crops*, **30**, 57-59.

Terna, T.P., Odebode A.C., Okogbaa J.I. and Waya J.I. (2016). *In vitro* screening of *Trichoderma* isolates for biological control of a post-harvest rot fungus. *J. Agric. Vet. Sci*., **9(4)**, 46-51.

Widyastuti, S.M. (2006). The biological control of Ganoderma root rot by *Trichoderma*. In: *Aciar proceedings*, **124**, 67.

Wonglom, P., Dy K.S. and Sunpapao A. (2023). Development of a Novel Emulsion Formulation of *Trichoderma asperelloides* PSU-P1 Conidia against Stem Canker on Dragon Fruit caused by *Neoscytalidium dimidiatum*. *Microbiol. Res.*, **14(3)**, 1139-1149.

Yousaf, Q., Subhani M.N., Iqbal M.Z. and Chattha M.B. (2018). Effectiveness of fungicides, methanol extracts and antagonists against invasive *Phytophthora palmivora* inciting leaf spots of the Chinese fan palm. *Bionature*, 376-385.