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PREVALENCE AND MOLECULAR CHARACTERISATION OF *Neoscytalidium* Spp. ASSOCIATED WITH STEM CANKER DISEASE OF DRAGON FRUIT IN CENTRAL KERALA

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ABSTRACT

Dragon fruit (*Hylocereus* spp.) cultivation is rapidly expanding in India, and stem canker has emerged as a major constraint to its production. A survey conducted from December 2024 to March 2025 across the major dragon fruit–growing regions of central Kerala revealed a widespread incidence of stem canker, with the highest disease incidence and severity levels recorded in Kottoppadam and Chirakkekode. Infected cladodes exhibited small chlorotic depressions with orange-centres that gradually enlarged into greyish-black cankerous lesions. Symptomatic samples were collected, surface sterilized, and plated on potato dextrose agar (PDA). All the isolates initially produced white colonies that later turned olive-green to grey with black pigmentation. Microscopic examination revealed brown, septate, branched hyphae and arthroconidia characteristic of *Neoscytalidium*. Pathogenicity tests conducted on three-month-old stems confirmed symptom development within 6–10 days after inoculation, with the Chirakkekode isolate identified as the most virulent. Molecular characterization based on ITS sequence analysis revealed that the isolates obtained from Thrissur and Ernakulam districts exhibited 100% sequence similarity with *Neoscytalidium dimidiatum*, whereas the isolates from Malappuram district showed 100% sequence similarity with *N. hyalinum*. Phylogenetic analysis further confirmed these identifications by clustering the Kerala isolates with the respective reference strains of the pathogens This study conclusively identifies *Neoscytalidium* spp. as the predominant causal agent of stem canker in Kerala and highlights the need for targeted disease management strategies to mitigate its impact on dragon fruit cultivation.

Keywords **:** Dragon fruit, ITS primers, *Neoscytalidium* spp.

Introduction

Dragon fruit (*Hylocereus* spp.) is a climbing cactus belonging to the family cactaceae and is considered as an important tropical fruit crop. It is also commonly known as pitaya, pitahaya, or strawberry pear. In India, it is referred to as *kamalam*, because the outer shape of the fruit resembles a lotus. The plant is a native of South and Central America and is cultivated in tropical and subtropical regions around the world. The most commonly cultivated species are *H. undatus* with red skin and white flesh, *H. monacanthus* (previously known as *H. polyrhizus*) with red skin and red flesh, *H. costaricensis* with red skin and purple flesh and *H. megalanthus* with yellow skin and white flesh (Wakchaure *et al*., 2020). Dragon fruit is usually propagated by seeds, micropropagation or by vegetative propagation methods (Balendres and Bengoa, 2019). The crop thrives in dry tropical climate with an average temperature range of 20-29 ºC, but can tolerate temperatures as high as 38-40 ºC, and as low as 0 ºC for short periods (Karunakaran *et al*., 2019). Regions receiving heavy rainfall are not suitable for the crop, as excessive rainfall can result in flower and fruit drop (Karunakaran and Arivalagan, 2019). Dragon fruit was introduced into India during the late 1990s and is currently cultivated in Karnataka,

Maharashtra, Gujarat, Kerala, Tamil Nadu, Orissa, West Bengal, Andhra Pradesh, Telangana and Andaman & Nicobar Islands, as well as in Punjab, Haryana, Madhya Pradesh, Uttar Pradesh and North Eastern States. Gujarat, Karnataka and Maharashtra are the leading producers contributing about 70 per cent of country's dragon fruit production (Kakade *et al*., 2025). In Kerala, dragon fruit is cultivated over an area of 485 ha with a production of 2370 MT (Wakchaure *et al*., 2020). In the year 2024–25, dragon fruit (Kamalam) cultivation in India covers approximately 14,640 hectares, with an estimated annual production of around 55,780 tonnes (NEDFi, 2025). Diseases are among the most important constraints limiting dragon fruit production. Heavy rainfall, overirrigation, and waterlogging conditions predispose the crop to disease outbreak. Major diseases reported in dragon fruit include stem canker caused by *Neoscytalidium dimidiatum*, stem blight caused by *Fusarium oxysporum*, bacterial rot caused by *Xanthomonas campestris*, brown stem spot caused by *Botryosphaeria dothidea*, grey blight caused by *Diaporthe* spp., and anthracnose caused by *Colletotrichum* spp., particularly *C. siamense* (Valencia-Botín *et al*., 2013; Abirami *et al*., 2019).Recently a canker disease affecting both fruits and cladodes of dragon fruit was observed across various dragon fruit growing regions of central Kerala districts. The disease initially appeared as deep chlorotic leisons with little orange centre on the cladodes, which subsequently developed into characteristic canker symptoms. The first report of stem canker of dragon fruit was given by Salunkhe *et al*. (2023) from Maharashtra. In Kerala, the disease has emerged as serious threat, causing a disease incidence up to 78% (Suhaira *et al*., 2024). Although dragon fruit is considered a high-value crop, the prevalence of destructive diseases affecting the economic yield and quality of fruits has become a major constraint, leading many farmers to discontinue its cultivation in Kerala. Considering its increasing importance and economic impact, the present study was undertaken to estimate the losses caused by stem canker and to characterise the pathogens associated with the disease.

Materials and Methods

Survey, symptomatology and disease assessment

A sampling survey was conducted during 2024 December to 2025 March, in dragon fruit plantations across central Kerala, to record the incidence of stem canker disease. The geographical details of the orchards were recorded and the percent disease incidence and severity were calculated. Infected fields were located in each district, and 100 plants were randomly selected from each field to estimate the disease incidence. The prevalence of the disease in the surveyed area was expressed in terms of per cent infected plants in the plantation. Disease incidence (PDI) was recorded based on the number of diseased cladodes out of the total number of cladodes. The disease severity was worked out using the alternative scaling method proposed by Lazarin *et al*., (2014) in which the scale and description are as follows

Scale 0 = no symptom
Scale 1= Infection on 0-20% area of cladode
Scale 2= Infection on 20-40% area of cladode
Scale 3= Infection on 40-60% area of cladode
Scale 4= Infection on 60-80% area of cladode
Scale 5= Infection on 80-100% area of cladode/complete death of the plant

Disease severity (DS) was calculated using the formula given by Kranz (1988) as follows:

$$DS = \frac{\sum(a \times b)}{N \times Z} \times 100$$

Σ (a× b) = Sum of symptomatic plants and their corresponding score scale

N = Total number of sampled plants

Z =Highest score scale

Symptomatic stems collected from each orchard and was brought to laboratory for isolation of the pathogen.

Pathogen isolation

Surface sterilization of stem lesion was done by swabbing the symptomatic area with 70% ethanol and cutting into small blocks (0.5×0.5×0.5 cm). The blocks were soaked in 0.1% mercuric chloride for 2 min and washed three times with sterile distilled water (1 min for each). All the sterilized sample were placed onto potato dextrose agar (PDA) medium. The resulting culture was sub cultured on to fresh PDA medium and was later pure cultured using hyphal tip method (Mohd *et al*., 2013) on water agar. The pure culture was further maintained on PDA and was stored under refrigerated conditions for further studies.

Cultural and morphological characterization of the pathogen

The fungal isolates were identified based on the macroscopic and microscopic characteristics. The macroscopic characteristics examined were colony appearance (texture and colour of mycelium), pigmentation and growth rate onto PDA plates. The colony appearances and pigmentations were assessed after 1 week of incubation at 25°C while growth rate was measured daily until the mycelia were fully grown

(3 days) on the plate. For microscopic characteristics, the structure of conidiogenous cells, and the shapes and sizes of conidia were observed by using a light microscope (Rolshausen *et al*., 2013).

Pathogenicity tests

The inoculation was conducted on 3 months old dragon fruit stems. The 5 mm disc cut from actively growing regions of 4-day old culture of the isolated fungus was placed on the stem. Injuries were made using sterilized needle by pinpricking method (Rocha *et al*., 1998). Development of external symptoms on inoculated plants was observed every day for 2 weeks. Fungal isolates were re-isolated and re-identified using morphological characteristics for confirmation of Koch's postulates.

Molecular characterization of the pathogen

The molecular characterization was done by isolating the genomic DNA, followed by amplification of specific ITS regions using universal primers of ITS 1 and ITS 4 by PCR and obtained sequence was analyzed in BLASTn program of NCBI.

DNA isolation

About 100 mg of tissue or mycelium was frozen with liquid nitrogen and ground into a fine powder. The sample was mixed with buffer PL1, treated with RNase A, and incubated at 65 °C for 10 minutes. After filtering, buffer PC was added and the mixture was passed through a Nucleospin Plant II column. The column was then washed with buffers PW1 and PW2, dried, and the DNA was finally eluted using buffer PE after a short incubation at 65 °C. The purified DNA was stored at 4 °C. The quality of the extracted DNA was checked by running it on a 0.8% agarose gel. A small amount of DNA mixed with loading buffer was loaded into the gel, electrophoresed in TBE with ethidium bromide, and the bands were then visualized under UV light using a gel documentation system. PCR was performed using a GeneAmp PCR System 9700 with the following profile: initial denaturation at 98 °C for 1 min; 40 cycles of 98 °C for 5 s, 54 °C for 10 s, and 72 °C for 15 s; final extension at 72 °C for 2 min; at 4 °C.

Agarose Gel electrophoresis of PCR products and sequence analysis

The PCR products were checked on a 1.2% agarose gel prepared in TBE with ethidium bromide. Each sample, mixed with loading dye, was run at 75 V for about 1–2 hours along with a 2-log DNA ladder (NEB). The DNA bands were then visualized under UV light and recorded using a gel documentation system. Sequencing was performed on a GeneAmp PCR System 9700 using the Big Dye Terminator v3.1 Kit (Applied Biosystems) following the manufacturer's protocol. Resulting sequences were analyzed for similarity using the Nucleotide basic local alignment search tool (BLAST n) algorithm provided by the National center for biotechnology information by comparing them to the GenBank database. The ITS-5.8S rDNA sequence was utilized for phylogenetic analysis through the implementation of the Clustal-W function and neighbor-joining method using MEGA 11.0 (Tamura *et al*.,2011).

Results

Survey, symptomatology and disease assessment

A random sampling survey was conducted in the major dragon fruit cultivating areas of Kerala, including Vellanikkara, Chirakkakode, Varandarappilly, Anjur, Chiramanangad and Chalakuddy in Thrissur district; Karukutty, Mookkannoor, Airapuram, Varapetty and Ezhattumugham in Ernakulam district; Thuvvur, Kottoppadam and Vattaloor in Malappuram district and Kalpetta, Muttil in Wayanad district (Fig 1). Stem canker disease was identified as a major disease affecting dragon fruit in all the surveyed locations. On infected cladodes, the disease initially appeared as small, orange-centered, depressed chlorotic spots of 2-3 mm size which gradually turned brown. As the disease progressed, the lesions coalesced to form larger greyish-black cankerous lesions measuring approximately 2–3 cm, with numerous pycnidia of the pathogen appearing as minute black dots on the lesion surface. The pycnidia were dark brown to black, globose to sub globose, ostiolate, and measured approximately 180–250 µm. In advanced stages of infection, the lesions expanded and merged, causing yellowing of the surrounding tissues, which eventually led to extensive tissue necrosis. The entire cladode gradually transformed into a rotten black mass (Fig. 2). Microscopic examination revealed that the pycnidia produced abundant conidia, which were initially hyaline, unicellular, ellipsoidal to oblong, measuring 9× 4 µm. The disease incidence and severity were recorded at each surveyed location. The highest disease incidence and severity were observed Chirakkekode (38.0 %, 33.5%) and Kottoppadam (37.3%,31.2%) respectively (Table 1). Based on the disease incidence and severity from all the surveyed locations, one isolate from each district exhibiting highest incidence and severity were selected for further studies.

Cultural and morphological characterization of the isolates

On PDA media, all the isolates initially produced white mycelial growth. As the colonies matured, the mycelia gradually turned olive green to grey colour with black pigmentation (Fig 3). The pathogen attained full growth after three days of incubation. Microscopic examination revealed brown, septate hyphae with profuse branching and the production of arthroconidia (Fig 3, Table 2). The Hyphae were brown, septate, and branched and fragmented to produce chains of arthroconidia. The Arthroconidia were dark brown, thick walled, ellipsoid to ovoid measuring 10.1 ± 1.4 × 4.88 ± 1.1 µm with zero to one septum. The pathogen failed to produce pycnidia *in vitro.* The arthroconidia were ellipsoid to ovoid, initially hyaline with round apex and truncate base, single celled with few of them developing a single septum at maturity (Fig. 4). The conidia were disarticulating, cylindrical to truncate, dark brown in colour with zero to one septum upon maturation.

Pathogenicity tests

The selected isolates were subjected to pathogenicity testing, and each isolate successfully reproduced typical symptoms on inoculated plants within six to ten days after inoculation (Table 3). Initial symptoms appeared 3-4 days after inoculation as minute, circular, depressed chlorotic spots with reddish flecks, As the disease progressed, the lesions become necrotic, followed by yellowing and rotting of the stems (Fig. 5). Among all the isolates, Chirakkekode isolate from Thrissur was found to be the most virulent, followed by Kottopadam isolate from Malappuram producing symptoms within 6,8 days of inoculation. Therefore, these isolates were selected for further studies. The resemblance in these characteristics suggests that all the isolates may belong to a fungal species closely related to *Neoscytalidium* sp.

Molecular characterization of the pathogen

The stem canker pathogen of dragon fruit (Chirakkekode, Anjur, Karakutty, Mookanoor samples) showed 100 per cent sequence similarity with *Neoscytalidium dimidiatum*, corresponding to Gen bank accession number PZ506349, whereas (Kottopadam, Vattaloor samples) showed 100 per cent sequence similarity with *Neoscytalidium hyalinum*, corresponding to Gen bank accession number PZ506348 (Fig. 6). A phylogenetic tree was constructed using the Maximum Likelihood method with MEGA 11 software. Phylogenetic analysis of the ITS sequence obtained from Kerala isolate, together with ITS sequences of *Neoscytalidium* spp. associated with dragon fruit from different geographical regions, confirmed its clustering with *N. dimidiatum*. This result further validated the identity of the pathogen responsible for stem canker of dragon fruit in Kerala.

Discussion

Survey, symptomatology and disease assessment

Similar symptoms of stem canker disease caused by *N. dimidiatum* have been reported by Mohd *et al*. (2013), Sanahuja *et al*. (2016), Dy *et al*. (2022) from Malaysia, Florida, and Thailand respectively. The symptoms appeared as small, sunken, orangish brown spots that subsequently developed into cankerous lesions. Likewise, Salunkhe *et al*. (2023) reported that *N. dimidiatum* infection in dragon fruit initially produced sunken chlorotic spots, which later became necrotic and eventually led to stem rot.

Cultural and morphological characterization of the isolates

Similarly, Salunkhe *et al*. (2023) observed that the stem canker fungus *N. dimidiatum* produced white colonies on PDA, which later turned olive green to greyish with black pigmentation. Mohd *et al*. (2013) also described *N. dimidiatum* as producing olive green to greyish colonies with dark grey to black pigmentation on PDA. Lan *et al*. (2012) also isolated *N. dimidiatum* from dragon fruit and reported the production of dark grey to black coloured aerial mycelium on PDA.

Pathogenicity tests

Similar pathogenicity results have been reported by earlier researchers. Chuang *et al.* (2012) evaluated the pathogenicity of *N. dimidiatum* by placing a 5 mm culture disc on dragon fruit stems and incubating the inoculated plants under 100% relative humidity using plastic covers. Typical symptoms developed on the inoculated stems within 6–14 days after inoculation. Likewise, Sanahuja *et al.* (2016) reported the development of characteristic stem canker symptoms seven days after inoculation with *N. dimidiatum.* Salunkhe *et al*. (2023) conducted pathogenicity tests using a 7 mm mycelial disc of *N. dimidiatum* placed on dragon fruit stems where the initial symptoms appeared within two days of inoculation, and characteristic canker lesions surrounded by chlorotic halos developed within 15 days after inoculation

Molecular characterization of the pathogen

Mohd *et al*. (2013) carried out the molecular characterization of *Neoscytalidium* sp. isolated from dragon fruit through amplification of the ITS region

and found that the isolates showed 99 per cent similarity with *N. dimidiatum* (Genbank accession number FJ648577). Similarly, for confirming the molecular identity of *Neoscytalidium*, the internal transcribed spacer (ITS), translational elongation factor 1- alpha (TEF1--tubulin (TUB2) was amplified using primer pairs (ITS1/ITS4, EF1-728F/EF1-986R, and BT2A/BT2B) by Salunkhe *et al*. (2023). These results revealed that sequence of the isolate showed 99 to 100 % similarity with *N. dimidiatum*

Conclusion

Stem canker disease caused by *Neoscytalidium dimidiatum* was found to be widely prevalent across the major dragon fruit–growing regions of Kerala with disease incidence (38.0 %), and severity (33.5%). The isolates showed characteristic cultural, morphological, and pathogenic features similar to previous reports of *Neoscytalidium* sp. Pathogenicity tests confirmed their ability to reproduce typical stem canker symptoms within a period of 5-6 days after inoculation. Molecular characterization further validated the identity of the Chirakkekode, Anjur, Karakutty and Mookanoor, revealing sequence similarity with *N. dimidiatum* &whereas Kottopadam, Vattaloor isolates showed similarity with *N. hyalinum*. Overall, the combined survey, cultural, pathogenic, and molecular analyses confirmed *Neoscytalidium* spp. as the predominant causal agent of stem canker in surveyed regions of Kerala.

Fig. 1 : Disease symptoms observed in surveyed plots located in central Kerala
a. Chirakkekode b. Ezhattumugham c. Kottoppadam d. Muttil

Fig. 2 : Disease score chart for *Neoscytalidium* sp. in dragon fruit

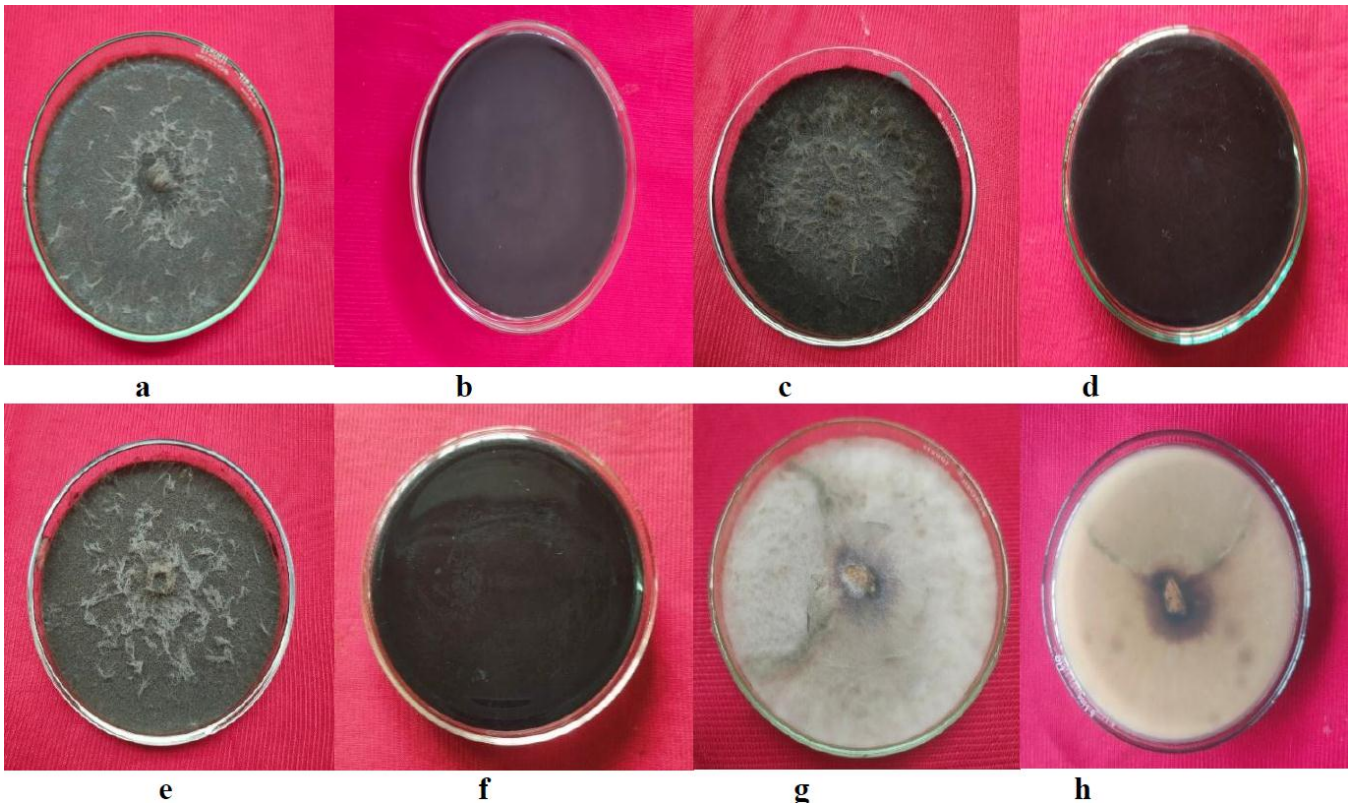

Fig. 3 : Mycelial growth of the pathogen on PDA medium a, b. Front and Rear view of Chirakkekode Isolate c, d. Front and Rear view of Isolate Ezhattumugham Isolate e, f. Front and Rear View of Kottoppadam Isolate g, h. Front and Rear View of Muttil Isolate

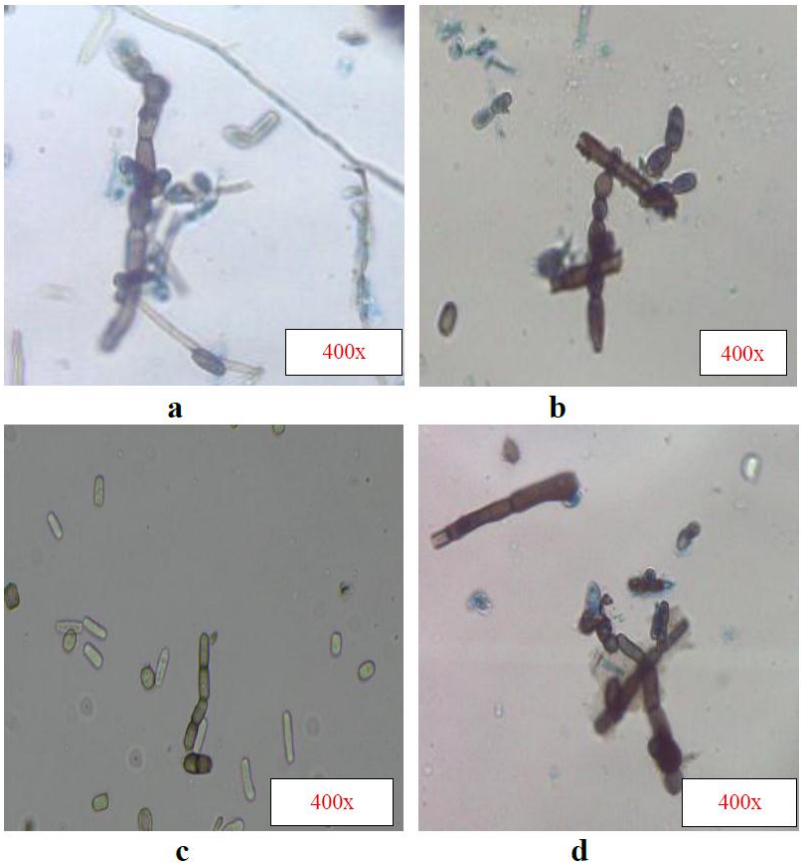

Fig. 4 : Microscopic characteristics of isolates under magnification of 40 X a. Chirakkekode b. Ezhattumugham c. Kottoppadam d. Muttil

a **b** **c** **d**

Fig. 5 : Symptom developed on cladode in response to artificial inoculation 6 days after inoculation
a. Chirakkekode isolate b. Ezhattumugham isolate c. Kottoppadam isolate d. Muttil isolate

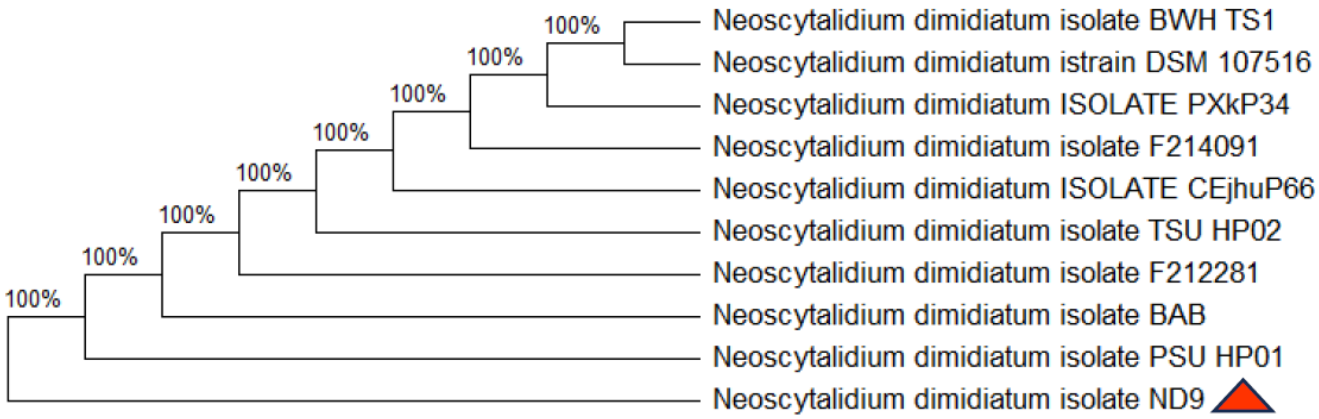

Fig. 6 : Phylogenetic tree by the maximum likelihood method based on the internal transcribed spacer sequences from the present isolate and other references strains of *N. dimidiatum*

Table 1 : Survey and collection of samples from central districts of Kerala

S. No.	Survey location	District	Latitude	Longitude	Disease incidence (%)	Disease severity (%)
1	Kottoppadam	Malappuram	10.99 ºN	76.37º E	38.00	33.35
2	Thuvvur		11.10 ºN	76.29º E	31.20	28.30
3	Vattaloor		10.99 ºN	76.12º E	23.50	19.50
4	Chirakkekode	Thrissur	10.56 ºN	76.28º E	37.30	31.20
5	Vellanikkara		10.56 ºN	76.29º E	33.60	27.3
6	Varandarappilly		10.42 ºN	76.33º E	35.10	29.6
7	Anjur		10.60 ºN	76.16º E	33.60	28.0
8	Chiramanangad		10.69 ºN	76.11º E	34.40	26.2
9	Chalakudy		10.30 ºN	76.32º E	33.60	27.3
10	Mookkannoor	Ernakulam	10.22 ºN	76.41º E	33.20	22.00
11	Karukutty		10.22º N	76.37º E	29.00	12.60
12	Varapetty		10.42º N	76.33º E	36.20	34.30
13	Airapuram		10.04º N	76.50º E	27.50	21.50
14	Ezhattumugham		10.22ºN	76.45º E	35.00	24.00
15	Kalpetta	Wayanad	11.63º N	76.07º E	23.5	18.50
16	Muttil		11.65ºN	76.11º E	28.0	23.35

Table 2 : Cultural and morphological characteristics of isolates

S.no	Isolate	Front end	Rear end	Average growth rate (cm per day)	Colour of conidia	Hyphae	Shape of conidia	Dimension of conidia (µm)
1	Kottoppadam	White mycelium turned to greyish black	Black	3.26	Brown,	septate	Arthroconidia – ellipsoid to ovoid	7.21-9.16 X 3.45-5.40
2	Chirakkekode	White mycelium turned to greyish black	Black	2.61	Brown,	Septate	Arthroconidia – ellipsoid to ovoid	6.44-10.63 X 4.70-5.28

Table 3 : Days taken for symptom development on artificial inoculation with various fungal isolates on dragon fruit

S. No.	Survey location	District	DTSD
1	Kottoppadam	Malappuram	8
2	Chirakkekode	Thrissur	6

DTSD* -Days taken for symptom development

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Conflicts of Interest: None

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