J.Mycopathol.Res. 64(1) : 219-223, 2026, ISSN : 0971-3719 (Print), 2583-6315 (Online) 10.57023/JMycR.64.1.2026.219

First Report of Stem canker pathogen of dragon fruit in Nagaland

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Received : 21.01.2026 Accepted : 02.03.2026 Published : 30.03.2026

Dragon fruit (*Hylocereus* spp.) is one of the ornamental fruits and it is of immense important now a days because of highly nutritious and medicinal values. This is sensitive to disease infection but the crop has gained less attention in disease management practices which causes high yield reduction. So, knowledge on the causal organisms associated with the diseases of dragon fruit is the foremost priority to develop disease management practices. Stem canker disease has been found to be one of the most destructive and causes economic losses in various parts of the world. Based on the pathogen's morphological characteristics, theresults of a pathogenicity test, and the symptoms that developed on the hostplant, the pathogen was identified as *Neoscytalidium dimidiatum* using the species description and confirmed as causal organism of the stem canker disease of dragon fruit.

Keywords: Dragon fruit, *Neoscytalidium dimidiatum*, pathogenicity test

INTRODUCTION

Dragon fruit (*Hylocereus* spp.) is a quick growing herbaceous perennial climbing cactus and is indigenous to South America, Mexico, and Central America (Koli *et al*., 2022). The dragon fruit plant belongs to the Cactaceae family and it is well known across the world for its colorful fruits that are also highly priced for their strong antioxidant potential, high vitamin A and C contents, and other nutritional qualities. In India, dragon fruit was introduced in the late 1990's and its cultivation is expanding gradually (Karunakaran *et al*. 2019). The dragon fruit cultivating states in India includes Tamil Nadu, Karnataka, Mizoram, Andhra Pradesh, West Bengal, Nagaland, Maharashtra, Gujarat, Odisha, and the Andaman and Nicobar Islands.There are four *Hylocereus* species which are widely cultivated around the world *viz*. *Hylocereus polyrhizus* (red flesh, red rind), *Hylocereus undatus* (white flesh, red rind,), *Hylocereus megalanthus* (white flesh, yellow rind) and *Hylocereus costaricensis* (super red flesh, red rind) (Esquivel *et al*. 2007).

Dragon fruit is attacked by many diseases *viz*. anthracnose that is caused by *C.gloeosporioides* (Takahashi *et al*. 2008),*C*. *truncatum* (Pornpimon *et al*. 2014), *C. karstii* (Nascimento *et al*. 2019), *C. siamense* (Abirami *et al*. 2019) and *C. aenigma* (*et al*. 2015); fruit rot and stemrot that is caused by *cactivora* (Tarnowski *et al*. 2010); stem rot andbasal rot by *Fusarium semitectum, F. proliferatum, F. fujikuroi*(Hawa *et al*.2010, 2013, 2017); stem canker that is caused by *Neoscytalidium dimidiatum* (Chuang *etal*. 2012;Hawa *etal*.2013); Alternaria blight or stem canker caused by *Alternaria alternata* (Vilaplana *et al*. 2017);stem gray blight caused by *Diaporthe* spp. (Huda-Shakirah *et al*., 2021); stem necrosis caused by *Curvularia lunata* (Hawa *et al*. 2009); *Enterobactercloacae* and *Paenibacillus polymixa* causing soft and stem rot (Masyahit *et al*., 2009; Zhang *et al*., 2017) and Cactus virus X that cause a viral disease (Liou *et al*. 2001; Kim *et al.* 2016).

Among all the diseases mentioned above, stem

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canker disease caused by *Neoscytalidium dimidiatum*has been found as one of the most destructive and causing significant economic loss in various parts of the globe (Chuang *et al*. 2012; Hawa *et al*. 2013). The initial symptoms start with a minute chlorotic sunken spot usually with red fleck at the center. Spots expand withthe center producing a hard brown scab with black pycnidia that are embedded in the surface followed by development of conspicuous yellow halos that surroundthe scab which results in the expansion of the lesions and may eventually lead storotting of the cladodes. The expansion of the lesions leads to formation of large zonate and gray scab like lesions that destroys large areas of the cladodes. At later stages,decaying of older lesions takes place that of ten drop sout resulting in large shot hole symptom in the cladodes. The damaged shoots deteriorate as the decay destroys the fibrous substance of the cladode. The long cladodes of dragon fruit are easily infected by the disease that causes stem rot.The infected plants are more prone to the risk suffering from environmental hazards, like drought and extreme heat, and they are more likely to be affected by other pathogenic fungus or bacteria. The yellowing and browning discolorations which is the result of the stem rot disease reduces the attractiveness and value ofthe dragon fruit plants and fruits which inturn lower the crop's market value (Fullerton *et al*. 2018). Considering it as an important emerging fruit crop, and as Dragon fruits are being attacked by many diseases, the present study reports a pathogen from Nagaland which has not been reported earlier.

MATERIALS AND METHODS

A field survey was conducted in four districts of Nagaland, India where dragon fruits were majorly cultivated *viz*. Chumukedima district, Dimapur district, Niuland district and Peren district.

Symptoms were recorded on the field and compared to the disease symptoms recorded in the recent literatures. Identification of symptoms was done on the field, by comparing with recorded photos and literatures. Suspected plant parts were collected for further confirmation in the laboratory. Identification of pathogen was done by isolating diseased plant parts in the laboratory which were collected. The infected Dragon fruit stems showing cankerous symptoms were cut into small blocks (5mm × 5mm × 5mm) and were surface sterilized with 1% sodium hypochlorite for three minutes followed by washing with sterile distilled water for three times (1 minute for each) and then they were transferred to sterilized potato dextrose agar medium (PDA) and incubated at 25±2°C for 7 days. The pure culture of the fungus was obtained by utilizing single hyphal tip culture method (Rangaswamy, 1972). The experiment was conducted in Department of Plant pathology, School of Agricultural Sciences (SAS), Nagaland University, Medziphema Campus.

Identification was done on the basis of their microscopic and macroscopic characteristics. The microscopic characterization was basically done on conidiogenous cells, and shapes and sizes of conidiaand the measurements of conidia were taken at 40x magnification with 50 structures randomly measured. For macroscopic characterization, colony appearance such as the colour and texture of aerial mycelium,and the grow thrate and pigmentation were examined. The colony appearances and pigmentation assessment were done after one week of incubation and the measurement of growth rate was taken daily until the mycelia were fully grown (3days) on the Petriplate (90 mm plate).

Confirmation of the causal organism was done by performing pathogenicity test on young dragon fruit stems. The stems were wounded using a sterile needle and the pathogens were inoculated to the wounded lesions using agar plug method. The controls were processed similarly but agar plug of pathogen was substituted to agar plug of non-colonize PDA.The emergence of external symptoms was observed everyday for two weeks. For pathogenicity test confirmation, morphological characterization of the re-identify after re-isolation of the funga lisolates was also studied.

RESULTS AND DISCUSSION

Symptomology

The symptoms observed at the initial stages of

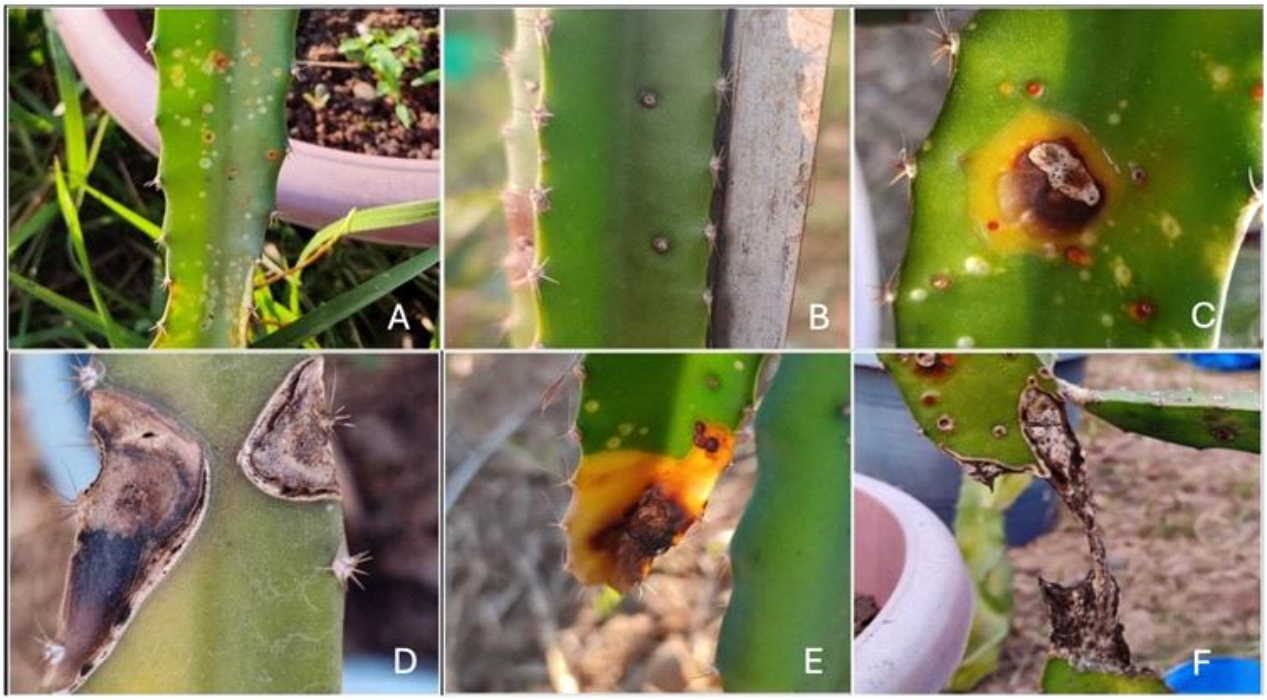

Fig 1 : Symptoms of stem canker on Dragon fruit caused by *Neoscytalidiumdimidiatum* A) Initial infection with minute chlorotic or orange spots B) Elevation of the center of the spotC)Spots expand with pycnidia on the surface which are then surrounded by yellow halos D) Expansion of spots leading to large, grey lesions E) Rotting of cladodes F) Decay of older lesions

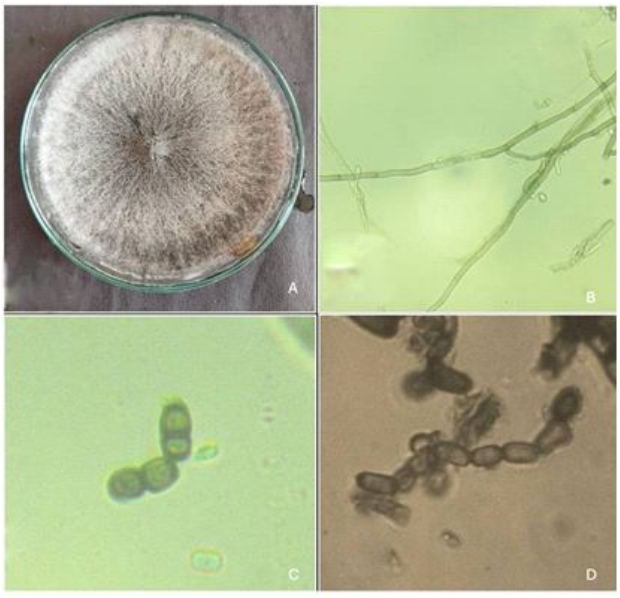

Fig 2 : Morphological characteristics of *Neoscytalidiu-mdimidiatum* A) Colony appearance on PDA B)

Fig 3: Pathogenicity test of the putative pathogen A) Symptom after 7 days of inoculation B) Symptom after 14 days of inoculation

the infection were minute chlorotic depressed whitish-yellow spots and turns orange to brown with age (Fig. 1A). The spots elevate, expand and coalesce to form larger spots and black pycnidia embedded on the spots can be seen which were then surrounded by yellow halos (Fig.1B and 1C). The expanded spots then lead to large grey lesions (Fig.1D). The final stage was a necrotic water-soaked lesions which led to rotting and decay of older lesions (Fig. 1 E and 1F).

These findings were also reported by Fullerton *et al*. (2018) who described the symptoms as minute chlorotic depressed spots which were followed by elevation at the centre of the spot that may turn red or grey. Spots may expand with the centre having hard and brown scab with black pycnidia on the surface followed by yellow hal o and expansion of the lesion. The expansion of the lesion leads to large, zonate and scab-like

lesions and the final stage is decaying of the older lesions which may dropout. Similar observation was also recorded by Dy *et al*. (2022) who observed the symptoms as whitish-yellow spots turning orange to brown. Coalescing of the spots leads to formation of largers pots which were surrounded by yellow halos (Fig.1C). The spots became water soaked and produced black pycnidia on the cladode.

Isolation and identification of the pathogen

The infected Dragon fruit stems showing cankerous symptoms were collected from the Dragon fruits farms in Chumukedima districts, Dimapur districts, Niuland districts and Peren districts of Nagaland. The fungus was isolated from cankerous stems of Dragon fruit and the causal organism was identified as *Neoscytalidium dimidiatum* using the species description given by Crous *et al*. (2006) andthose described in literature based on the microscopic and macroscopic characteristics.The macroscopic characteristics observed were hairy or woolly colonyand olive green to greyish colony which turns to dark to black pigmentation ona PDA medium (Fig.2A). *N. dimidiatum* grows rapidly on plates filled with PDA medium and colonized the Petriplate swith in 3days. Under the microscope,tehyphae appeared brown, branche, and septate while the arthroconidia were in chains, hyaline to dark brown in color,thick walled having 0 - 1 septation which were ellipsoid to ovoid, rod, round and capsule in shape (Fig. 2B and D). The conidia area septate, hyaline to dark brown in color with ellipsoid-cylindrical in shape and averaged size of 10.38 (8.21-12.25) × 6.13 (4.13-8.68) µm (Fig.2C).

The isolates were identified as *Neoscytalidium dimidiatum* (Penz.) Crous & Slippers based on their morphological characteristics (Crous *et al*. 2006).These findings also agree with Hawa *et al*. (2013) who observed the isolates having septate, branched with brown hyphae which disarticulated intoarthro spores with 0 to1 septation.

This result was in agreement with Danupat and Kasem (2016) who isolated *N. dimidiatum* and identified based on their morphological characters. The observation recorded was septate and brown, branching hyphae which disarticulated into arthrospores which are 0-1 septate with arthroconidia resembling *N. dimidiatum* which are ellipsoid to ovoid, rod and round shaped, thick walled, 0-1 septate and hyaline to dark brown. Similar observation was also noted by Salunkhe *et al.* (2022) who identified *N. dimidiatum* as brown branched septate hyphae disarticulating into chains of arthroconidia which are hyaline to dark brown, 0 to 1 septation, thick walled, ellipsoid to ovoid, rod, round, capsule shape. The conidia were aseptate, hyaline and ellipsoid to cylindrical in shape.

Pathogenicity test

Pathogenicity test was conducted on healthy stems of *Hylocereus polyrhizus* using agar plug method. On the inoculated plants, the development of symptoms was being observed every day for two weeks (Fig.3). Re-isolation and re-identification of fungal isolates proved that symptoms on infected cladodes were caused by *N. dimidiatum.*

These observations are in agreement with Danupat and Kasem (2016) who conducted pathogenicity test on *H. polyrhizus* using agar plug of pathogen and observed development of external symptoms for 2 weeks and confirmed Koch's postulates by re-isolation and re-identification using morphological characteristics. Similar observation was also noted by Dy *et al*. (2022) who conducted pathogenicity test in four replicates on *H. polyrhizu s*using agar plug method and proved pathogenicity.

CONCLUSION

It may be concluded from the present findings that stem canker disease of dragon fruit was caused by *Neoscytalidium dimidiatum* and it was also confirmed experimentally by performing its pathogenicity test were the same pathogen. *Neoscytalidium dimidiatum* was reisolated under artificial inoculation in the laboratory. Therefore, thorough knowledge on the causal organism associated with the stem canker disease of dragon fruit is the foremost priority to develop disease management practices.

ACKNOWLEDGEMENT

The authors would like to thank the Department of Plant Pathology, SAS, for permitting to carry out the research work in this topic.

DECLARATION

Conflict of interest. Authors declare no conflict of interest

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