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## Isolation, Identification, and Pathogenicity Test from *Neoscytalidium dimidiatum* Causing Stem Canker of Dragon Fruit

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The observed symptoms in the fields were small circular sunken orange spot, black pycnidia and rotted stem. Isolation of dragon fruit disease was done on PDA medium which isolate showed hairy colony and olive green to greyish colony with dark-grey to black pigmentation on PDA. The fungal isolates were identified based on morphological base under compound microscope which seen hyphae branched, septate, and brown and disarticulated into 0- to 1-septate arthrospores. The arthroconidia were ellipsoid to ovoid, rod shaped round shaped, hyaline to dark brown, thick walled and 0-1 septate. It is similar to *Neoscytalidium dimidiatum* caused stem canker on dragon fruit. The isolated fungus was tested for pathogenicity to confirm the same disease symptom and pathogen as the first record. It proved that stem canker of dragon fruit caused by *Neoscytalidium dimidiatum*.

**Keywords:** *Neoscytalidium dimidiatum*, stem canker, dragon fruit

### Introduction

Dragon fruit can be referred to as pitaya, pitahaya or strawberry pear, is a Cactaceous fruit crop cultivated widely in some tropical countries because of its high nutrient contents. Dragon fruit is becoming an emerging and important fruit plant in international markets. Its production could potentially create jobs and promote income for the nation that produces it. Dragon fruit is classified into three varieties. Many species of *Hylocereus* have been recognized but main two species grown for commercial production include *Hylocereus undatus*, which has fruit with bright pink skin and white flesh, and *Hylocereus polyrhizus*, which has fruit with bright pink skin and varying hues of pink flesh. One more varieties, *Selenicereus megalanthus* is another dragon fruit grown for commercial production, which has fruit with yellow skin and white flesh.

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All dragon fruit have black tiny edible seeds that are similar in appearance to kiwi seeds. The texture of dragon fruit is similar to kiwi or watermelon. (Frieda's, n.d.; Setzer, 2014; McEachran, 2015).

This present, diseases have been reported to infect dragon fruit such as Stem canker disease was considered as the most destructive disease on pitaya. It was found that the causal agent of stem canker was *N. dimidiatum*. The initial symptoms of stem canker were brown sunken lesion and the lesion became dark brown with age. Orange spot and black pycnidia were formed on the surface of the canker. As the disease progressed, the infected stem subsequently rotted (Masratul Hawa, *et al.* 2013b)

### ***Isolation the fungal pathogen***

Surface sterilization of stem lesion was done by swabbing the symptom at margin with 70% ethanol and cutting into small blocks (1.5×1.5×1.5 cm). The blocks were soaked in 1% sodium hypochlorite for 3 min and washed 2 times with distilled water (1 min for each). All the sterilized sample were placed onto water agar. Then hyphae growing from the tissue pieces onto water agar, subcultured to potato dextrose agar (PDA) until get pure culture and incubated at 25 °C for 7 day

### ***Morphological identification and characterization***

The fungal isolates were identified based on the macroscopic and microscopic characteristics. The macroscopic characteristics examined were colony appearance (texture and colour of aerial 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

### ***Pathogenicity tests***

The inoculation was conducted on 4 stems age of dragon fruit at 3 months were inoculated with agar plug of pathogen which the inoculated to wounded lesions on stem by sterilized needle. The controls were processed similarly but substituting agar plug of pathogen to agar plug of non-colonize PDA. Development of external symptoms on inoculated plants was observed

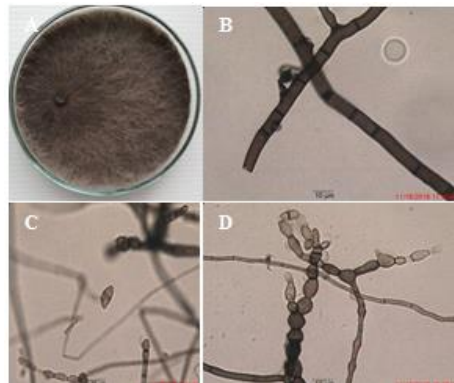
everyday for 2 weeks. Fungal isolates were re-isolated and re-identified using morphological characteristics for Koch's postulates confirmation.

## Result and discussion

The symptoms were observed as brown sunken lesion, which developed 3 days after inoculation. After 7 days of inoculation, the lesion became dark brown and sometimes produced orange spot. Black pycnidia formed on the surface of the canker after 10 days of inoculation. The infected stem became rotted after 14 days of inoculation. In control, the stem remained healthy.

The fungus reisolated from infected areas from Pathogenicity tests on dragon fruit was identified as *Neoscytalidium dimidiatum*. From macroscopic characteristics, hairy colony and olive green to greyish colony with dark-grey to black pigmentation on PDA (Fig. 1A). *Neoscytalidium dimidiatum* grows rapidly and colonized the plate within 3 days on PDA. The hyphae were branched, septate, brown which constricted into spore chains and disarticulated into arthroconidia (Fig. 1B). The arthroconidia were ellipsoid to ovoid, rod shaped round shaped, hyaline to dark brown, thick walled and 0-1 septate (Fig. 1C, Fig. 1D).

As result, stem canker of dragon fruit was isolation and identified as *Neoscytalidium dimidiatum* and proved as a pathogen isolate. This research finding is similar reported in Taiwan (Chuang *et al.* 2012), Malaysia (Masratul Hawa, *et al.* 2013) and Florida (Sanahuja G., *et al.* 2016)



**Fig. 1** Morphological characteristics of *Neoscytalidium dimidiatum* from stem canker of dragon fruit. (a) Colony appearance on PDA; (B) Branched, septate, and brown hyphae; (c) shapes of Conidia; (D) Contiguous arthroconidia

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