

# Identification of common fungal pathogens of dragon fruit (*Hylocereus undatus*) in Sarangani Province, Philippines

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## Abstract

Fungal disease is one of the major challenges that affects yield quality and profit in dragon fruit production today, as it causes a considerable amount of production loss. In this study, fungal pathogens of dragon fruit (*Hylocereus undatus*) plants in Southern Philippines were identified. Sample collection was conducted in selected farms in Sarangani Province, Philippines. Diseased parts of the dragon fruit plants were collected and brought to the laboratory to culture its associated fungi. Grown fungi were pure cultured and subjected to pathogenicity testing using the detached stem method. A total of eight distinct fungal colonies were isolated from the collected dragon fruit samples. The pathogenicity test revealed that three of the isolates caused lesions in a healthy dragon fruit plant tissue, which turned yellow soft and watery as the infection progressed. The three pathogenic isolates were molecularly identified as *Fusarium oxysporum* (FRI3), *Aspergillus minisclerotigenes* (FRI2), and *Fusarium incarnatum* (FLI3). Interestingly, *F. incarnatum* and *A. minisclerotigenes* were new recorded pathogens of dragon fruit. Identification of fungal pathogens on economically important crops is an essential step in the development of strategies to address problems related to fungal diseases which, in turn, will help farmers to enhance their production.

**Key words:** Cactaceae, dragon fruit, fungal diseases, *Hylocereus undatus*.

**Abbreviations:** DAI, days after inoculation; PDA, potato dextrose agar.

## Introduction

Like many other crops, cultivation of dragon fruit (*Hylocereus undatus*) is faced with production issues that greatly contribute to yield reduction. Short-storage life of fruits and non-stable fruit supply are exacerbated by the presence of diseases in the dragon fruit industry. In the recent years, several studies have identified some pathogens on dragon fruit plant, showing its susceptibility. These include organisms that can mummify stems and often reduce yield. The species include: *Nigrospora sphaerica*, which causes reddish-brown spot (Taguiam et al. 2020a); *Epicoccum sorghinum*, causing necrotic brown lesion (Taguiam et al. 2020b); and *Neoscytalidium dimidiatum*, causing stem canker (Taguiam et al. 2020c). In addition, *Collectrichum fruticola* causes anthracnose to dragon fruit species including *H. undatus* and also *Hylocereus monacanthus*, which was reported in the Philippines (Evallo et al. (2021). Associated diseases caused by these pathogens have been recorded in different parts of the Philippines including Laguna, Ilocos Norte, Cavite and Bukidnon. Taguiam et al. (2021) also indicated that these pathogens can also infect stem cuttings, which reduces the quality of planting material and can be an agent to the spread of

diseases. These diseases have significantly contributed to production and post-harvest losses, which affected farmers' income (Taguiam et al 2020a). Currently, management practices include cultural and physical approaches wherein infected parts are cut and burned, chemical approaches and biopesticides (Balendres, Bengoa 2019). These practices bear large costs to farm owners and managers, which affects production and income (Tepora 2019).

Sarangani had the third highest number of farms covering 150.8 thousand hectares of agricultural land in Soccsksargen. Dragon fruit farming is an emerging industry in Sarangani and is among the major crops within the region (PSA 2002). Furthermore, a comprehensive review has not been conducted for dragon fruit pathology in the region. A brief review on associated pathogens of dragon fruit in the Philippines was published by Valencia-Botin et al. (2013). There have been numerous novel and first country reports of dragon fruit diseases and associated infections over the past five years, which supplement the earlier literature (Balendres, Bengoa 2019). There have also been cultural management practices observed by farmers in order to limit the effects of diseases and associated pathogens (Ngoc et al. 2018).

Problems related to diseases have emerged in this

region which lower yield and production of several farms. This study aims to identify fungal pathogens causing diseases to dragon fruit, which will eventually help in developing strategies to combat and prevent the spread of these diseases.

## Materials and methods

### Study sites

The study was conducted in three selected dragon fruit farms in Sarangani Province (Brgy, Banate 6.3742003 N, 125.2865547 E; Brgy, Kawayan Bagiles 6.4255773 N, 125.3134046 E; Hill of Thorns Farm 6.4823770 N, 125.33621718 E). Sarangani is a Philippine Province located in the Region XII in Mindanao. Its capital is Alabel, and it is bordered to the north by South Cotabato and to the east by Davao del Sur. In Central Mindanao, Sarangani is located at the southern tip of Socckargen (<https://alabel.gov.ph/geography/>). The province encompasses 3601.25 km<sup>2</sup> and covers 360 125 thousand hectares. Sarangani has a tropical rainforest climate. It is usually very warm, humid, and rainy all year round. The average annual temperature for Sarangani is 29 °C with about 1280 mm rain annually. It is dry for 47 days a year with an average humidity of 80% and an UV-index of 7 (<https://alabel.gov.ph/geography/>).

### Field sampling

Field sampling was conducted in three dragon fruit plantations in Sarangani Province. The farms were chosen based on the following inclusion criteria: plantations with more than 5000 m<sup>2</sup> (0.5 ha), at least two or more years of plant propagation, presence of disease symptoms, and with farmers encountering problems related to pathogen-causing diseases. Along with the reconnaissance and survey, assessment of infected plants in each farm was conducted. With the permission of farm owner and managers, infected fruit, stems, and flowers were collected for the isolation of potential pathogens. Three representative infected samples were collected on the same day and placed separately in sterile zip-locked bags, properly labelled, placed in a cooler/ice box, and transported to the Microbiology Laboratory of the University of Southern Mindanao for further processing.

### Sterilization of glassware and agar solution

Glassware was first washed with liquid detergent and rinsed with distilled water, air-dried, wrapped in a paper, and then dry-sterilized for 2 h at 180 °C. Sterile glassware were kept

in a cool dry place until utilization. Agar solutions were sterilized in a pressure cooker at 121 °C for 15 min.

### Isolation and purification of the isolated pathogens

The infected part of the plant was removed using a sterile scalpel, mixed with Phosphate Buffered Solution and homogenized thoroughly. The samples were then serially diluted up to 10<sup>-6</sup>. From the highest dilutions, 0.1 mL of homogenized samples were spread plated on Potato Dextrose Agar (PDA) supplemented with 2 mg ampicillin (antibacterial agent) per 20 mL, prepared in triplicates. The inoculated plates were incubated at room temperature (25 °C) for a maximum of 14 days and checked every three days. Distinct isolates were pure cultured using the same culture medium supplemented with antibacterial agent. Pathogen Safety Data Sheets provided information regarding hazards and handling for isolation processes.

### Morphological and cultural characterization of the isolated fungi

Stock cultures of pure culture pathogenic fungal isolates were grown in PDA plates and incubated inverted at room temperature (25 °C). Fungal growth was assessed and colony morphology characteristics such as shape, color, as well as the texture were recorded after 3 to 4 days of daily monitoring. Fungal cells were stained using methylene blue to observe its morphological structures under 100 to 1000 × light microscopy. The isolated pathogens were pre-identified based on their morphological characteristics up to the genus level using standard taxonomic manuals based on Barnett, Hunter (1998).

### Pathogenicity test

Pathogenicity tests were done to confirm the pathogenicity of isolated fungi collected from the infected stem of the *H. undatus*. The pathogen was inoculated using the detached stem method following the technique of Tangonan, Pecho (2009). The stem was collected and disinfected through sequential immersion in 10% sodium hypochlorite for 2 min, rinsed with sterile distilled water three times and allowed to dry in sterile tissue paper. Mycelial discs (5 mm) were taken from the edges of a 2-week-old pathogen culture growing on PDA plates. The mycelial discs were placed in the sub axial part of wounded and unwounded stem, with the mycelia facing downwards. Fresh sterile 5 mm PDA blocks placed in wounded and unwounded stem were used as the negative control. The set-up was maintained inside a plastic container with sterile distilled water at the bottom to provide moisture, and sealed with

**Table 1.** Primer sequences used in this study

Locus	Primer	Sequence (5'-3')	Annealing conditions	
			Temperature (°C)	Time (s)
rDNA ITS	ITS 1	TCCGTAGGTGAACCTGCGG	55 – 60	15
rDNA ITS	ITS 4	TCCTCCGCTTATTGATATGC	55 – 60	15

a clear container, ensuring that the area was disinfected to avoid contamination. The set-up was incubated for 7 to 10 days under room temperature or until appearance of infection was observed. The test stems were observed after every two days of monitoring. Furthermore, after the infection occurred, observation was extended up to 21 days. The test stem was marked positive when lesions such as rotting, swelling of tissue, and spots (necrosis) symptoms were observed.

#### Molecular identification of isolates

All fungal isolates showing a positive result from the pathogenicity test were sent to the Philippine Genome Center for the identification. The 2-week-old fungal isolates on petri-dishes were properly labelled, placed on sterile zip-locked bags, maintained in a cooler, and personally transported to the PGC Mindanao. Primer sequences used

in this study were from White et al. (1990) (Table 1).

#### Handling of fungal pathogens

Biosafety level-1 practices, facilities, and containment equipment based on the publication of Biosafety in Microbiological and Biomedical Laboratories was applied in the manipulation of the phytopathogen (National Institute of Health 2009).

Post-handling was done by chemically disinfecting the work surface of the inoculating chamber after the completion of every laboratory activity involving the pathogen with 70% ethyl alcohol and 4% formalin. Furthermore, before disposal, culture, stocks, and other potentially infectious materials were decontaminated in a pressure cooker at 121 °C for 30 min.

## Results

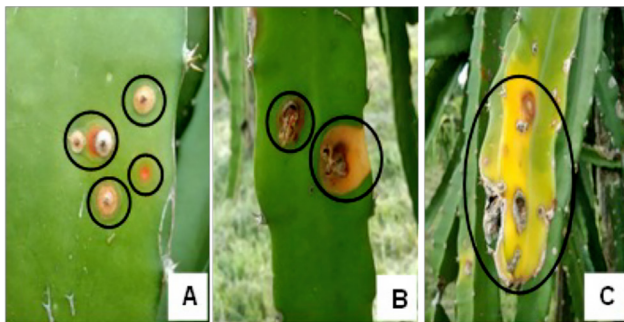
#### Common diseases of dragon fruit plants

Infected plants showed yellowish circular lesions which would then enlarge and spread through tissues. Infected tissues turned yellow and becomes soft and watery (soft rot) (Fig. 1). Other infected plants also showed circular brown to yellowish watery lesions, which were commonly observed in early stages of infection (Fig. 2). Later stages of infection showed brownish lesions that became dry and fell off from the parent plant. Farmers also observed that these two indicators of disease may occur simultaneously in one plant causing severe damage to the plant particularly the stem (Fig. 3). The causative fungal pathogens were identified as these were isolated from documented samples.

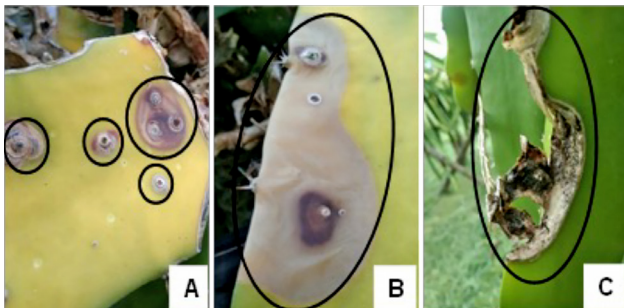
#### Isolated pathogens from fruit, stem and flower tissues

A total of eight distinct fungal isolates were obtained from fruit, stem, and flower of dragon fruit plants collected in three dragon fruit farms (Table 2).

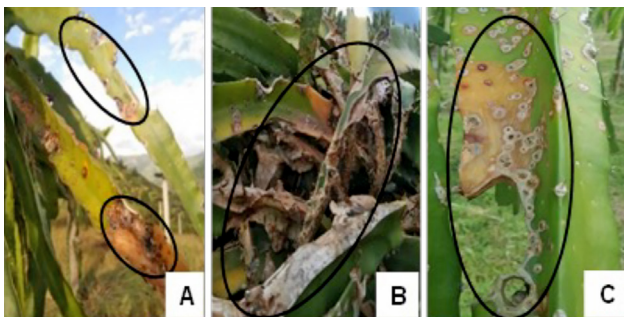
FLI3, FRI2, and FRI3 were isolated from infected flower, fruit, and stem of dragon fruit. Among these isolates, FRI3 was observed to occupy a large space in the culture set-up after 7 days of incubation. On the other hand, FLI2 and FRI1 were isolated from the infected flower and stem. FLI1 was isolated from infected fruit, and SI1 and SI2 were isolated from infected stem (Fig. 4).



**Fig. 1.** Stages of disease development caused by pathogen 1 on dragon fruit plants.



**Fig. 2.** Stages of disease development caused by pathogen 2 on dragon fruit plants.



**Fig. 3.** Synergistic effect of pathogens 1 and 2 on dragon fruit plants.

**Table 2.** Coded fungi isolated from dragon fruit plant

Isolate number	Code name	Isolated from
1	FLI1	Flower, fruit
2	FLI2	Flower, stem
3	FLI3	Flower
4	FRI1	Fruit, flower, stem
5	FRI2	Fruit, flower, stem
6	FRI3	Fruit, flower, stem
7	SI1	Stem
8	SI2	Stem

**Pathogenicity test of fungal isolates**

Figures 5 to 8 shows the result of the pathogenicity test of the eight fungal isolates using a detached inoculation technique on healthy wounded and unwounded stem of dragon fruit plant. Among the eight fungal isolates obtained from the infected part of stem, fruit, and flower, three isolates tested positive in the pathogenicity test.

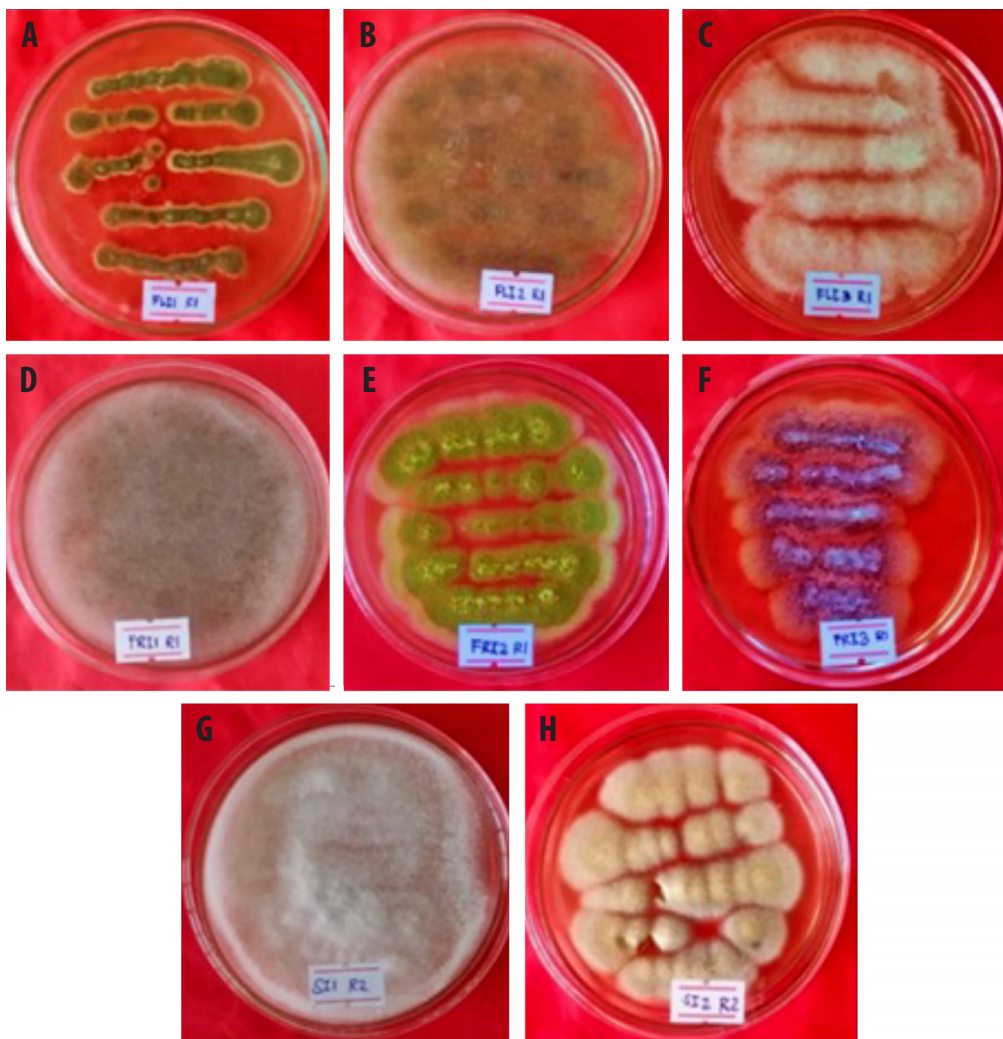
Isolates FLI3, FRI2, and FRI3 inoculated to wounded stem showed black to yellow lesions symptoms, which is an indicator that the isolate is pathogenic; however, no symptoms were observed in the unwounded stem where similar fungal isolates were inoculated. Symptoms of infection were observed two days after the inoculation of FRI3. This fungal pathogen spreads fast and was marked as the most devastating fungi among the three confirmed pathogens after 21 days of daily observation. Symptoms of infection were observed after three days of inoculation of FRI2 and FLI3. The control, which was inoculated with sterile PDA discs, remained uninfected.

**Identification of the fungal pathogens**

Based on cultural characteristics, the majority of the fungal colonies have circular shape (Figs. 9, 10, 11). FRI3 and FRI2 had convex elevation while FLI3 was raised. It was observed that fungal colonies varied in terms of color/pigmentation. FRI3 had purple pigmentation, FRI2 colonies had a yellow green color and FLI3 had pinkish-white mycelium. Average growth diameter of the fungal colonies ranged from 80 to 90 mm seven days after the inoculation on the PDA plate.

In terms of morphological characteristics, it was observed that conidial structure varied among fungal colonies. FRI3 microconidia were oval to ellipsoid or kidney shaped. The spores of FRI2 were flask-shaped or cylindrical phialides, and FLI3 had canoe-shaped spore. FRI3 and FLI3 were pre-identified as belonging to the species of *Fusarium* and FRI2 under the genus of *Aspergillus*, which were subjected to molecular identification.

Based on molecular identification, FRI2 was identified as *Aspergillus minisclerotigenes*, FRI3 as *Fusarium oxysporum*, and FLI3 as *Fusarium incarnatum*.



**Fig. 4.** Cultures of fungi isolated from infected part of fruit, flower, and stem of dragon fruit plants collected from the three dragon fruit farms after seven days of incubation. A, FLI1; B, FLI2; C, FLI3; D, FRI1; E, FRI2; F, FRI3; G, SI1; H, SI2.

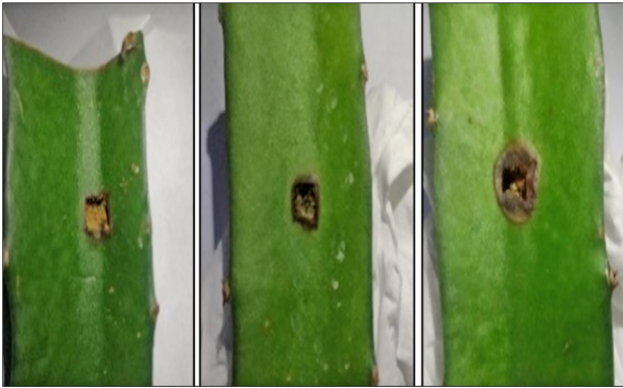


Fig. 5. Dragon fruit plant stem subjected to pathogenicity test against *Aspergillus minisclerotigenes* in triplicates.



Fig. 6. Dragon fruit plant stem subjected to pathogenicity test against *Fusarium oxysporum* in triplicates.



Fig. 7. Dragon fruit plant stem subjected to pathogenicity test against *Fusarium incarnatum* in triplicates.



Fig. 8. Negative control in triplicates.

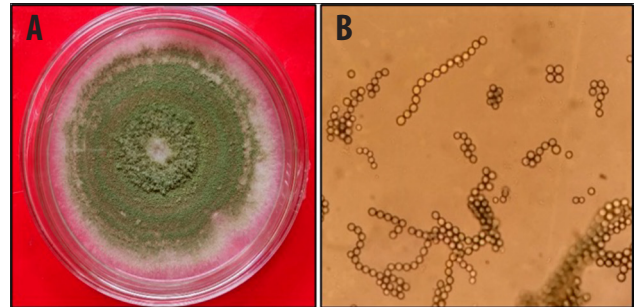


Fig. 9. Morpho-cultural characteristics of FRI2. A, pure culture; B, photomicrograph under light microscope (400 ×).

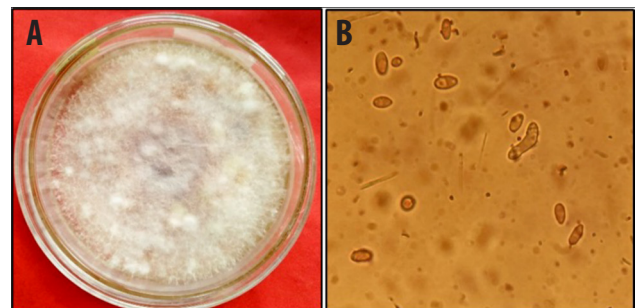


Fig. 10. Morpho-cultural characteristics of FRI3. A, pure culture; B, photomicrograph under light microscope (400 ×).

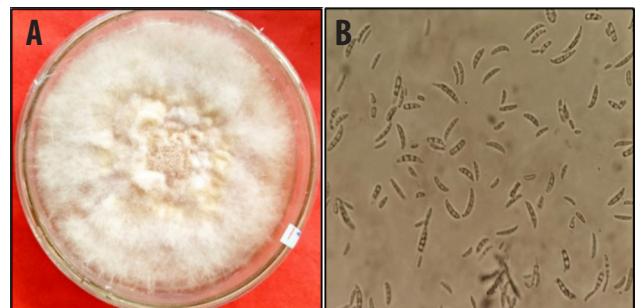


Fig. 11. Morpho-cultural characteristics of FLI3. A, pure culture; B, photomicrograph under light microscope (400 ×).

## Discussion

In this study, three fungal pathogens were isolated from fruits, stems and flowers, which is in agreement with other studies on pathogens of dragon fruit (Balendres, Bengoa 2019). Among the identified plant pathogens affecting the stem, flowers and fruits of dragon fruit plant, 21 are fungal species.

On wounded stem, FRI2 showed yellow to black lesions at an early stage (3 DAI) of infection. This indicates that this fungal isolate spread and infected tissues, which then turned into brownish to black lesion. Based on molecular analysis, this isolate was identified as *Aspergillus minisclerotigenes*. It is noteworthy that this species of *Aspergillus* had not been yet reported as a fungal species that causes infection to dragon fruit plants in Mindanao. There is species of *Aspergillus* known to infect dragon fruit plants reported

by Li et al. (2001), namely *Aspergillus oryzae*, which is an opportunistic fungus that causes black spot disease. Based on a previous study, *A. minisclerotigenes* was observed to be a post-harvest pathogen of soybean seeds causing seed rot disease (Awan et al. 2016). One species of the genus, *Aspergillus flavus*, has been identified as a post-harvest fungal pathogen of dragon fruit, which causes fruit decay (Yao et al. 2022). Moreover, *A. oryzae* has been identified as post-harvest fungal pathogen of dragon fruit in China (Li et al. 2022).

FRI3 showed brown-yellowish lesions at an early stage (2 DAI) of infection in wounded stem. In the later stage of infection, lesions were enlarged and spread through tissues. The infected part turned yellow and was soft and watery. It was identified as *Fusarium oxysporum*. A previous study reported that *F. oxysporum* causes stem blight to other species of dragon fruit plants (*H. polyrhizus*; Mohd et al. 2019), but the symptoms observed were not similar to those recorded in this study. 20% of dragon fruit plants expressed stem blight symptoms in a 2-ha commercial farm in Malaysia caused by the pathogen. A similar species of fungal pathogen was isolated from dragon fruit in South Korea (Choi et al. 2007) and Argentina (Wright et al. 2007) and stem and basal rot were observed, which is similar with the observation of this study. *F. oxysporum* is a causative agent of Panama wilt in banana; thus, the pathogen isolated in this study may have originated from nearby banana plantations surrounding the dragon fruit farms. Fungal spores can spread with the help of wind or insects to infect fruits.

The FLI3 isolate also gave a positive result in the pathogenicity test. Observed symptoms included swollen tissues at an early stage (3 DAI), which then became a black rot lesion. This fungal isolate was identified as *Fusarium incarnatum*. Similar with *A. minisclerotigenes*, there has been no previous report on infection caused by *F. incarnatum* on dragon fruit plants in Mindanao. Thus, this is a new record on the occurrence of this fungal species to dragon fruit plants. This fungal pathogen, on the other hand, was observed to cause fruit rot on muskmelon, reported by Wonglom, Sunpapao (2020).

Previous studies have suggested that a variety of *Fusarium* can also cause post-harvest soft rot disease to dragon fruit, but a report on *F. proliferatum*, which was recently found for the first time in China, suggested that *F. proliferatum* could be a regional pathogen (Li et al. 2022).

## Conclusions

This study isolated three fungal pathogens in dragon fruit plants (*Hylocereus undatus*) from fruit, stem and flowers – *Fusarium oxysporum*, *Aspergillus minisclerotigenes*, and *Fusarium incarnatum*. Two of these species, *A. minisclerotigenes* and *F. incarnatum*, were new records as pathogens of dragon fruit plants in the Philippines.

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