

Major diseases of dragon fruit (*Hylocereus* spp.) in coastal area of Pangandaran, West Java, Indonesia

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(Received : July 23, 2019/Accepted : August 19, 2019)

ABSTRACT

Pangandaran regency, a region located on the southern coast of West Java, Indonesia, has the potential to be developed as a centre for dragon fruit production. Dragon fruit farming has begun in this area but is currently experiencing some disease problems. Several diseases have been reported in dragon fruit in Indonesia, to mention some of them are stem canker and stem rot diseases. The purpose of this study was to examine the incidence of disease and to identify the main causal agents of dragon fruit disease that appeared in Pangandaran regency. Field observations showed that the incidence of the disease reached up to 100% of the sample plants, while the severity of the disease was actually high which occurred in stem canker and anthracnose caused by *Neoscytalidium dimidiatum* and *Colletotrichum gloeosporioides* reaching more than 50%. Other significant diseases found in the plantation were stem necrosis (*Curvularia lunata*), scab (*Pestalotiopsis* sp.) and soft rot (Bacteria X). Because there is no comprehensive review available, this study will provide information about dragon fruit diseases that will benefit further studies on dragon fruit disease management and the development of agro-tourism programs in the research area.

Key words : Dragon fruit, *Hylocereus* spp., major diseases, *Neoscytalidium dimidiatum*

INTRODUCTION

As an agricultural country, agriculture is one of the largest and most significant human activities in Indonesia. Therefore, agriculture is one of the main sectors in the Indonesian economy. Indonesian horticulture consists mostly of fruits and vegetables, one of which is dragon fruit (*Hylocereus* sp.). Dragon fruit is a perennial plant and is popular in Indonesia because of its expensive price in the market. Dragon fruit has a high economic value compared to other fruits so that it has good prospects in the fruit industry. Soeparjono (2015) reports that dragon fruit farming and cultivation in Indonesia is very profitable and feasible to be developed and can be designed

as a source of new growth in the national economy.

In Indonesia, dragon fruit has been cultivated in large-scale areas since the 2000s. However, in fact, the productivity of dragon fruit in Indonesia is still low. One of the main obstacles faced by farmers in dragon fruit production is the presence of plant diseases. The first report of dragon fruit disease in Indonesia was in 2010 in the Riau Islands Province and then disease outbreaks were reported in dragon fruit production centres in East Kalimantan about five years later. Factors supporting the incidence of disease in dragon fruit include environmental conditions, low soil nutrition, and a lack of knowledge about plant diseases among farmers which in many cases

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farmers abandon diseased plants (Yusuf *et al.*, 2017).

Some diseases cause significant damage to dragon fruit plants, especially stem canker, stem rot and anthracnose. Stem canker is referred to as the most destructive disease in dragon fruit in the world (Jumjunidang *et al.*, 2016). The pathogens that associated with stem canker are *Neoscytalidium dimidiatum*, *Alternaria* sp. and *Pestalotiopsis* sp. (Hawa *et al.*, 2013, Hawa *et al.*, 2015). The species *N. dimidiatum* is also reported to cause fruit internal brown rot (Yi *et al.*, 2015). Stem rot is reported to be associated with the genus of *Fusarium*, *Schlerotium*, *Alternaria*, *Acremonium*, *Rhizoctonia*, *Phytophthora*, *Pythium*, *Pseudomonas*, *Stenotrophomonas*, *Enterobacter* and *Erwinia* (Masyahit *et al.*, 2009a, Isnaini *et al.*, 2010; Wibowo *et al.*, 2011; Barthana *et al.*, 2013). Meanwhile, anthracnose or stem spotting is confirmed caused by the genus of *Colletotrichum*, the species of *C. gloeosporioides* and *C. truncatum* (Takahashi *et al.*, 2008; Masyahit *et al.*, 2009b; Wibowo *et al.*, 2011; Syafnidarti, 2013; Guo *et al.*, 2014). Other pathogenic species reported from dragon fruit include *Curvularia lunata*, *Bipolaris cactivora*, *Phomopsis* sp. and *Botryosphaeria dothidea* (Valencia-Botín *et al.*, 2003; Hawa *et al.*, 2009; Oeurn *et al.*, 2015).

Dragon fruit production centres in Indonesia are located in West Sumatra, East Kalimantan and Java. In West Java, one of the centres of dragon fruit plantations is Wanayasa area in Purwakarta regency with at least five dragon fruit plantation points with each area not less than two hectares (Distan Jabar, 2016). Considering the characteristics of dragon fruit plants that are suitable for coastal areas, Pangandaran regency—an area on the south coast of West Java, has the potential to become the centre of dragon fruit production due to the suitability of temperature, climate and soil conditions. It is also reported that the Pangandaran area has a large number of unused or unproductive land suitable for planting dragon fruit (Sudarjat *et al.*, 2019). In addition, as a tourism area, there is a current program called the Pangandaran Agro-Tourism Program, where the development of dragon fruit plantations will create synergies between agriculture and tourism in the region (Sudarjat *et al.*, 2017). However, similar to the current

disease outbreaks in other locations, the disease has occurred in dragon fruit in the Pangandaran area. Decline in plant health is seen while many plants show some symptoms of the disease. Until now, there has been no research on dragon fruit disease in the study area. Therefore, information about the disease occurrence and the disease-causing agents of dragon fruit is very important to support the management of emerging diseases in the research location.

MATERIALS AND METHODS

Assessment of Disease Occurrence

This research was conducted from July to October 2018 at a local dragon fruit plantation located in Cintaratu area, Parigi district, Pangandaran regency, West Java, Indonesia. Pathogen identification was carried out at the Plant Protection Biotechnology Laboratory, Faculty of Agriculture, Universitas Padjadjaran. The research method was a field survey to investigate the incidence and severity of disease in dragon fruit plants in the study area. Dragon fruit plants were considered to be at the same stage of growth. Formulas for calculating the incidence and severity of the disease were :

$$\text{Percentage of disease incidence} = \frac{\text{Total number of infected plants}}{\text{Total number of plants assessed}} \times 100$$

$$\text{Percentage of disease severity} = \frac{\text{Number of individual ratings}}{\text{Number of plants assessed}} \times \frac{100}{\text{Maximum scale}}$$

The maximum rating scale (1-4) had been used for assessment of the disease severity was : 0 = no disease symptom, 1 = 1-20%, 2 = 21-40%, 3 = 41-60% and 4 = more than 60% of the plant part appearing disease symptoms.

The sampling method used a diagonal sampling design. Observations were made at five sampling points in the plantation area, as much as 20% of the total plants were randomly selected at each sampling point. The infected plant samples were collected in brown paper packages and transported to the laboratory for isolation and identification of pathogens.

Isolation and Pathogen Identification

Fungal isolation was carried out according to Agrios (2005). The symptomatic part of the plant was taken from the healthy and diseased border. The pieces were sterilized by dipping them in 70% alcohol for 15 seconds and suspended in 1% sodium hypochlorite solution for 5 min. The sterilized tissues were rinsed by three times used sterile distilled water and blotted to dry on sterile filter paper. After drying, four pieces were placed aseptically into a Petri dish containing Potato Dextrose Agar (PDA) medium and incubated at room temperature $\pm 28^{\circ}\text{C}$ for 3-6 days. Then the mycelium was transferred to the new PDA medium as pure culture and was used in the process of identification and pathogenicity testing. Fungal identification was carried out based on the characterization of colonies and also the presence of spores or conidia through microscopic examination.

Bacterial isolation was done through serial dilution techniques. Pieces of plant samples with symptoms taken from the branch or main stem were briefly disinfected with 70% alcohol and crushed with mortar and pestle with the addition of sterile distilled water. Serial dilutions were prepared at a 10^{-1} to 10^{-3} dilution. A total of 100 μl of bacterial suspension was plated into Nutrient Agar (NA) medium from the last dilution and incubated at room temperature for 3-6 days. Single colonies of several types of bacteria were transferred to separate NA medium to obtain pure culture. The isolates were then identified and used in the pathogenicity test.

Pathogenicity Test (Koch's Postulates)

The stem of the plant was washed and cut into 7 cm lengths. The stem pieces were disinfected using 70% alcohol by rubbing the pieces along the surface. Artificial wounds were made using a sterile needle on the tested stem. Pathogen inoculation was made by placing the agar plug of fungal pure culture (\varnothing 5 mm) or dropping 20 μl of bacterial suspension ($\pm 3 \times 10^7$ cfu/ml) onto the wounded sites. Bacterial inoculation was also done by dipping the cut ends of the stem into the bacterial suspension. In fungal inoculation, the agar plug was covered using sterile wet cotton and wrapped using a transparent plastic wrap for 24 h to maintain

moisture and prevent microorganism contamination. Inoculated pieces of stem were placed inside a closed plastic container with moistened filter paper. The container was then incubated and the development of symptoms was observed every day.

RESULTS AND DISCUSSION

Occurrence of Diseases

A total of 120 dragon fruit plants were observed at the study site. Various symptoms of the disease were found in all sampling points and sample plants since the incidence of the disease in the study sites reached up to 100%. There were five main diseases in dragon fruit plants, namely, anthracnose, scab, stem necrosis, stem canker and soft rot. The two dominant diseases were stem canker and anthracnose with disease severities of 53.6 and 50%, respectively.

Description of Diseases and Pathogen

Anthracnose

Symptoms of anthracnose were characterized by circular, sunken and reddish brown lesions surrounded by brownish yellow haloes with orange masses of conidia in concentric rings (Fig. 1a). In PDA, the fungal colony was initially white and became greyish white with age, cottony texture and dense mycelium (Fig. 1b). Pathogenicity test using Koch's Postulate procedure produced early symptoms of circular brown spots then became sunken and yellow lesions (Fig. 1c and d). Conidia were cylindrical, straight, single-celled and hyaline (Fig. 1e). Pathogen was identified morphologically as *Colletotrichum gloeosporioides*.

Scab

Symptom of scab disease was raised, rusty reddish brown and circular lesions on the stem (Fig. 2a). The surface of the infected stem will become damaged and rough. Scab lesions had a lot of acervuli throughout the lesion surfaces. In PDA, the fungal colony had white and cottony mycelium with black conidial masses produced in concentric rings (Fig. 2b). Isolated fungal inoculation of healthy stems produced symptoms of scab even though

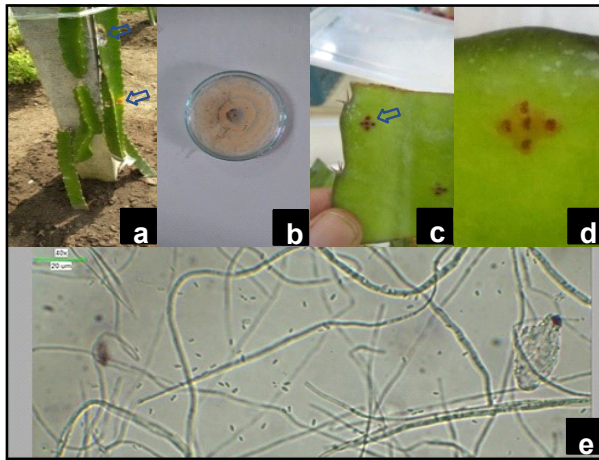


Fig. 1. Anthracnose disease of dragon fruit and morphological characteristics of *C. gloeosporioides*. (a) Disease symptom of blackish brown spots with yellow haloes (arrows); (b) *C. gloeosporioides* pure culture; (c and d) Sunken and yellow lesions on pathogenicity test and (e) Conidia of *C. gloeosporioides*.

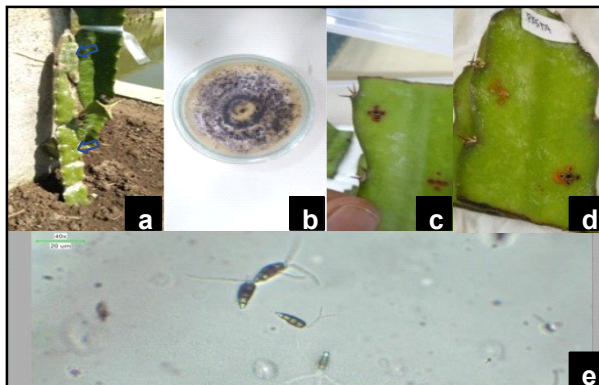


Fig. 2. Scab disease of dragon fruit and morphological characteristics of *Pestalotiopsis* sp. (a) Disease symptom of damaged and rough lesions; (b) *Pestalotiopsis* sp. pure culture; (c and d) Scabbing lesions on pathogenicity test and (e) Conidia of *Pestalotiopsis* sp.

symptom formation was limited to around the inoculation point (Fig. 2c and d). The conidia were five-celled with three appendages arising from the apical cell (Fig. 2e). Pathogen was identified as *Pestalotiopsis* sp.

Stem necrosis

Symptomatic stems showed small, circular and reddish orange necrotic lesions (Fig. 3a). In PDA, this fungus had cottony and blackish grey colony (Fig. 3b). Orange necrotic lesions were produced in the pathogenicity test (Fig. 3c and d). Conidia were 3-5 cells,

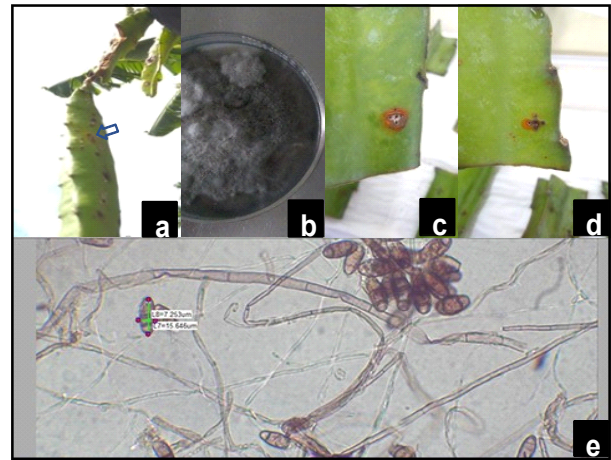


Fig. 3. Stem necrosis disease of dragon fruit and morphological characteristics of *Curvularia* sp. (a) Disease symptom of reddish orange necrotic lesions; (b) *Curvularia* sp. pure culture; (c and d) Orange necrotic lesions on pathogenicity test and (e) Conidia of *Curvularia* sp.

cylindrical or slightly curved with one of the central cells larger and darker (Fig. 3e). The fungus was identified as *Curvularia* sp. based on the morphological characteristics of conidia.

Stem canker

Symptoms of stem canker appeared as sunken and pale to dark brown necrotic lesions with the formation of black pycnidia on the surface of the canker (Fig. 4a). In PDA, the fungal colony was dense, cottony and dark grey with black pigmentation on the medium (Fig. 4b). Pathogenicity test showed sunken necrotic lesions with typical symptom of canker lesions (Fig. 4c and d). Conidia were one-celled, hyaline, straight, cylindrical to slightly curved (Fig. 4e). Mycelia were constricted into spore chains (Fig. 4e). The fungus was identified as *Neoscytalidium dimidiatum*.

Soft rot

The infected stem tissues became rotten and softened (Fig. 5a). Bacterial colonies on NA medium were circular, convex, creamy yellow and shiny (Fig. 5b). Pathogenicity test did not give a typical symptom of bacterial infection in dragon fruit plants. However, biofilm was formed in the inoculation point compared to condition that was not present in uninoculated wounds (Figs. 5c, d and e). Using the Gram staining technique, the colour of the bacterial

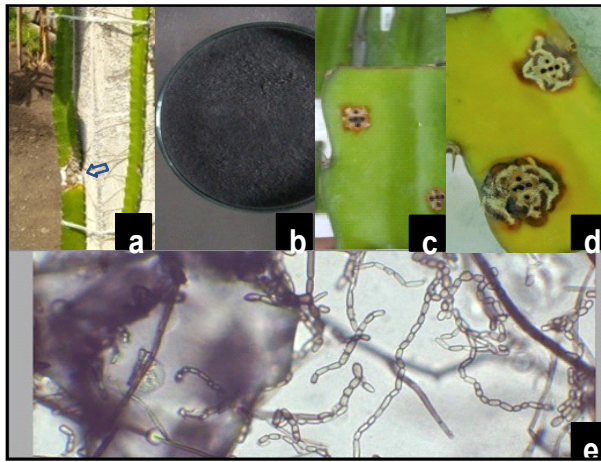


Fig. 4. Stem canker disease of dragon fruit and morphological characteristics of *Neoscytalidium dimidiatum*. (a) Disease symptom of brown sunken necrotic lesions (arrow); (b) *N. dimidiatum* pure culture; (c and d) Canker necrotic lesions on pathogenicity test and (e) Chains of arthroconidia of *N. dimidiatum*.

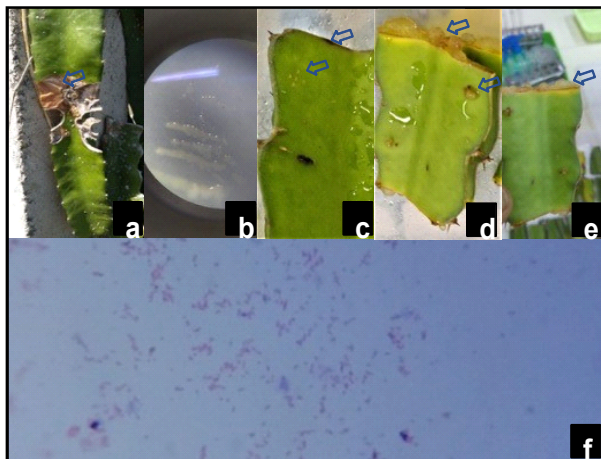


Fig. 5. Soft rot disease of dragon fruit and morphological characteristics of bacteria X. (a) Disease symptom of stem tissue soft rot; (b) Bacteria colony; (c) Control treatment without bacterial inoculation; (d and e) Mucus secretion on the inoculation points and (f) Red colour of bacterial cells in the Gram staining test.

cells was red, which was a characteristic strain of Gram-negative bacteria (Fig. 5f).

Farmers notified that the existence of plant diseases has significantly reduced the productivity of dragon fruit even though until now there have been no data on the amount of losses caused by plant diseases in this area. Before the research was carried out, the symptoms of the disease which later diagnosed as stem canker were the most feared disease

by farmers. Results of this study showed that the incidence of stem canker reached 100% with highest disease severity of 53.6%. Therefore, stem canker became the most potential disease to destroy dragon fruit plants in the Cintaratu area. The incidence of stem canker in dragon fruit reported in Malaysia was 60% with disease intensity of 55% (Hawa *et al.*, 2015).

Stem canker is potentially the most damaging disease of dragon fruit in Asia that can destroy the canopy, disfigure fruit and make the orchards uneconomical (Fullerton *et al.*, 2018). This disease has been reported in several Asian countries such as Taiwan, Vietnam, Thailand, Malaysia and China, especially when farmers use imported plant material (Pascual *et al.*, 2016). Morphological and molecular identification determines that dragon fruit stem canker is caused by the fungus *Neoscytalidium dimidiatum* (Hawa *et al.*, 2013). The fungus can also infect fruits and cause fruit internal brown rot disease (Yi *et al.*, 2015).

The next highest damaging disease was anthracnose. The severity of anthracnose disease was 50%. With the severity of around 50%, the symptoms of stem canker and anthracnose were easily observed in the field in the study site. Anthracnose was reported to be the most common disease found in dragon fruit plants (Masyahit *et al.*, 2009; Hawa *et al.*, 2015). In Malaysia, the incidence and severity of anthracnose were 52 and 47%.

Anthracnose was reported to be the most common disease in dragon fruit. The disease can cause major losses especially when pathogens infect fruits so that fruits become inappropriate for consumption (Hawa *et al.*, 2015). There are at least two species of *Colletotrichum* causing anthracnose in dragon fruit, namely, *Colletotrichum gloeosporioides* and *Colletotrichum truncatum*. Conidia of both species are different, the straight shape belongs to *C. gloeosporioides*, while the conidia of *C. truncatum* is falcate. Therefore, the pathogen of anthracnose disease in this study was identified as *C. gloeosporioides*. These two *Colletotrichum* species have been reported to infect all types of dragon fruits (Takahashi *et al.*, 2008; Masyahit *et al.*, 2009b; Safnidarti *et al.*, 2013; Guo *et al.*, 2014).

Considering the incidence of two other fungal diseases of scab and stem necrosis,

Wibowo *et al.* (2011) reported *Pestalotiopsis* sp. as a causative agent of scab symptoms in dragon fruit. The fungus is a weak pathogen that usually requires wounds for infection to occur and mainly infects unhealthy hosts. Meanwhile, Hawa *et al.* (2009; 2015) reported that *Curvularia lunata* associated with stem necrosis in dragon fruit with disease incidence reached up to 41 and 25% for the disease intensity.

In addition to fungal pathogens, the bacteria X that cause stem soft rot has also become an important disease in the study site with a disease severity of 32.1%. This disease can be potentially damaging especially in environmental conditions that support the development of diseases such as excessive irrigation or during the rainy season (Masyahit *et al.*, 2009b).

The high incidence and severity of disease in dragon fruit plants at the study site can be caused by several factors. Chemical control using fungicides has never been done on this plantation. Likewise, poor sanitation, unhealthy use of dragon fruit plant material, and land conditions that were originally flooded rice fields could be factors that influence the development of disease in this plantation. However, the origin of the inoculum source of the disease is not yet known.

CONCLUSION

Several important diseases in dragon fruit such as anthracnose, scab, stem necrosis, stem canker and stem soft rot were found infecting dragon fruit plants in Cintaratu area. Meanwhile, stem canker disease was potentially the most destructive disease of dragon fruit in the Cintaratu area, Pangandaran regency.

ACKNOWLEDGEMENTS

The authors thank all members of our laboratory for their helpful discussions throughout the project. This study was funded by Universitas Padjadjaran for funding research by RKDU (research competency of lecturers of UNPAD) for the year 2019.

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