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# FUNGAL SPECIES ASSOCIATED WITH BLACK SPOT DISEASE IN ROSE

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**Abstract:** Black spot disease is a significant worldwide disease on the rose plant. Due to this infection, the leaves become yellow and eventually fall off. The occurrence of this disease has become a major problem, especially in landscape purpose. Therefore, this research was conducted to isolate fungal species from black spot disease in rose and identify using morphological characteristics. Then, all the isolates were tested for pathogenicity to confirm Koch's postulates. In this study, four fungal isolates have been successfully isolated from black spot disease in rose namely *Rhizoctonia* sp. (one isolate), *Colletotrichum* sp. (two isolates) and *Penicillium* sp. (one isolate). Based on pathogenicity test result using potato dextrose agar (PDA) plug technique, fungus UMTT27R (*Penicillium* sp.) showed highly pathogenic on rose's leaves with disease severity (DS) = 88.89% followed by UMTT13R (*Colletotrichum* sp.) with DS=72.22%, UMTT21R (*Colletotrichum* sp.) with DS=66.67% and UMTT4R (*Rhizoctonia* sp.) with DS=61.11%. Correct identification of fungal pathogens is very important to strategize a proper method to control the black spot disease in rose cultivation.

Keywords**:** Rose, black spot, fungal pathogens

## Introduction

Rose is scientifically known as Rosa which belongs to family Rosaceae. It is a bisexual plant where the male and female organ is in the same plant with thorny stems (Shinwari & Shinwari, 2003). The rose plants have many varieties which are grown for their flowers' beauty, fragrance, symbolism, colour and sheer elegance of form. Rose plants are prone to many fungal pathogens that cause serious diseases problem such as black spot, powdery mildew, botrytis, downy mildew and stem canker (Debener & Bryne, 2014). During the fluctuated environmental condition, the plants became more susceptible to the plant diseases either planting in the greenhouse or at the open areas. Almost all rose varieties are susceptible to black spot disease caused by *Diplocarpon rosae* (Walker *et al.,* 1995). *D. rosae* causes the formation of purple to black spots on leaves and shoot of infected rose plants. The fungus grew on the upper side of leaves and stems which can be easily recognised from the white, and powdery patches form.

Among the fungal diseases, a black spot was considered as the main destructive disease on many rose plant cultivars. Typically, this disease was discovered on the rose plants cultivated in the exposed environment (Linde & Debener, 2003) such as for landscaping (Marchant *et al.,* 1998; Whitaker *et al.,* 2007). A fungus namely *D. rosae* was reported to be the causal pathogen for black spot disease. According to Gachomo *et al.* (2006) and Yasin *et al.* (2016), black spot disease not only attack the leaves but can be further attack the whole shoot including petioles, leaves, buds, petals, fruits and young stems. Presence of this fungal diseases in nurseries will degrade the rose quality appearance which eventually lowers the market price. Lack of study has been conducted on fungal identification from black spot disease in this country. Considering the importance of this disease, this study was aimed to isolate

the fungal species associated with black spot disease in rose and determine their pathogenic level. The data from this study will provide good information on black spot disease on rose in Malaysia.

## Material and Method

### *Isolation of Fungal Isolates*

Infected leaves of rose's plants with black spot symptoms were collected in Kuala Nerus, Terengganu. The leaves were cut into small pieces, surface sterilized using 3% of sodium hypochlorite and rinsed for three times. Then, the leaves tissues were blotted dried and plated on potato dextrose agar (PDA). All the plates were incubated at 27 + 2$^{o}$C until growing of mycelia. The mycelia were then subcultured and went through a single spore process before obtaining a pure culture.

### *Morphological Identification*

All fungal isolates obtained in this study were identified based on their macroscopic and microscopic characteristics such as colony colour and pigmentation. For microscopic characteristics, the shape of spore, hyphae and other related structures were determined under the light microscope.

### *Pathogenicity Test*

For the pathogenicity test, healthy rose's leaves were surface sterilized using 3% sodium hypochlorite and air-dried. All the leaves were placed in the petri dish before inoculated with PDA plug containing the fungal isolates. A total of nine leaves were used for each treatment. The appearance of symptoms was observed every day for 10 days. The disease symptoms were scored from 1 to 5 adopted from Yassin *et al.* (2016). Then, the percentage of disease severity was calculated using the following formula:

$$\text{Disease index} = \frac{\Sigma\,(v \times n)}{N \times Z} \times 100$$

Where,

v= disease severity

n= the number of infected plants with disease

N= total number of plants

Z= maximum disease severity

### *Statistical Analysis*

All the data were analysed using one-way ANOVA from SPSS version 20. Standard deviation and the significant value was based on the Turkey test at $p \leq 0.05$.

## Result and Discussion

Rose cultivation has increased a new interest to many farmers due to high demand. Besides that, it is typically known as the king of the flower which provides high-profit income and increases the employment opportunities (Boskabady *et al.,* 2011). In addition, this activity gives benefit to the floral industry and may expand this profitable enterprise in this country. The plants have been exploited for both ornamental and medicinal values (Ahmad et al., 2010). Among ornamental plants, roses have been considered as most sensitive plant toward changing environmental condition which leads to susceptible to plant diseases. The occurrence of potential fungal pathogens that cause plant diseases will give a negative impact to the rose industry in Malaysia. In this study, four fungal isolates have been isolated from rose's leaves that showed black spot symptoms.

All the isolates were morphologically identified as *Rhizoctonia* sp. (1 isolate), *Colletotrichum* sp. (2 isolates) and *Penicillium* sp. (1 isolate). For *Rhizoctonia* sp., the colony colour was white with yellow to green pigmentation at the centre. The branches of hyphae were 90$^{o}$ angle which similar to a study reported by Schisler *et al*. (1994). Two fungal isolates have been morphologically identified as *Colletotrichum* species. Both of the isolates appeared as white colony colour with dark green pigmentation at the centre. It

had a circular growth form. It also produces an abundance of cylindrical conidia with rounded at both ends under slide observation. Besides that, only one isolate has been morphologically identified as *Penicillium* sp. On the PDA, the colony appeared as white to dark green colour. Under microscopic observation, divaricate conidiophores of the hyphae were observed, and the terminal cell of the conidiophore was slightly swollen or vesiculate.

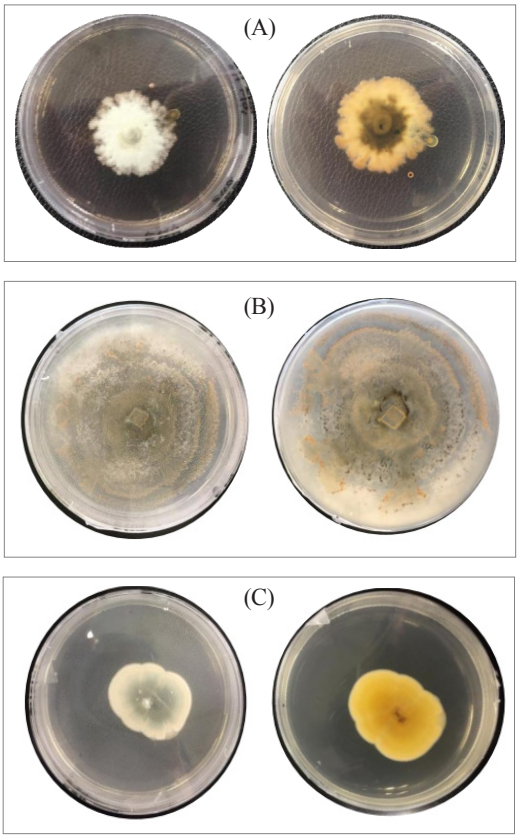


Figure 1: (A) Upper and a lower colony of *Rhizoctonia* sp.; (B) Upper and a lower colony of *Colletotrichum* sp. and (C) Upper and a lower colony of *Penicillium* sp.

Leaf spot diseases can cause by various fungal pathogens that become a major constraint to several plant species (Yasin *et al.,* 2016). In a rose plant, almost all varieties are susceptible to blackspot disease infection (Walker et al., 1995). The occurrence of this disease can be observed as a black spot on the leaves and shoot. The infected areas will turn yellow around the spots resulting in premature leaf fall. Severe infection can cause complete defoliation and dieback. According to Yasin *et al.* (2016), the fungal pathogen will initially attack the older leaves at the lower area of the plant which later spread upwards. The infected rose plants will become unattractive and lower the market price. Based on the previous studies, *Diplocarpon rosae* was reported as a fungal pathogen that causes black spot disease on rose worldwide (Gachomo *et al.,* 2006; Whitaker *et al.,* 2007). However, none of the isolates obtained in this study was identified as *D. rosae* which might be due to environmental factors that were not suitable for this species. According to Pinnschmidt *et al.* (1995), development of lesion on the leaves was significantly affected by the ontogenetic host and environmental factors.

Based on the pathogenicity test, all fungal isolates obtained in this study were pathogenic to rose's leaves with various level of pathogenicity. By using PDA plug technique, fungal isolates UMTT27R (*Penicillium* sp.) showed highly pathogenic level with disease severity (DS) =88.89% followed by UMTT13R (*Colletotrichum* sp.) (DS=72.22%), UMTT21R (*Colletotrichum* sp.) (DS=66.67%) and UMTT14R (*Rhizoctonia* sp.) (DS=61.11%) with p value<0.05 (Figure 1). The differences in pathogenic level in this study were attributed to different pathogenic races, varieties and environmental condition which result in different severity symptoms of black spot disease on rose (Gachomo, 2005). Besides, genetic variability and environmental condition play a big role in lesion development.

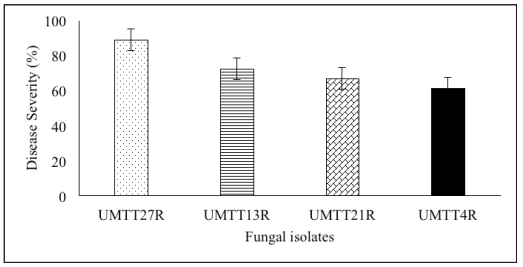


Figure 1: Pathogenic level of fungal isolates associated with black spot disease symptoms on rose's leaves

## Conclusion

As a conclusion, four fungal isolates have been successfully isolated from the infected rose leaves with black spot symptoms. All the isolates were morphologically identified as *Colletotrichum* sp., *Penicillium* sp., and *Rhizoctonia* sp. Although these species were not reported as the main pathogen on rose's leaves, all four isolates showed pathogenic on rose's leave in this study with various disease severity. This data will provide good information on black spot disease on rose in Malaysia and help many farmers to plan a strategic control measure to control the disease.

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