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IDENTIFICATION OF FUNGAL ISOLATES ASSOCIATED WITH ANTHRACNOSE DISEASE ON CHILLI AND ITS PATHOGENIC LEVEL

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Abstract: Many chilli producers in Malaysia are facing a huge problem of anthracnose disease that infect the chilli fruits either at pre- or post-harvest stage. Due to this infection, the chilli fruits become rotten and could be colonized by grey conidial masses which is unfit for human consumption and cannot be sold. This study was aimed to isolate the fungal species associated with anthracnose symptoms collected from several mini-markets near Gong Badak, Terengganu. Pathogenicity test was conducted for all these isolates to evaluate their pathogenic level. Based on the results, five fungal isolates were successfully isolated and morphologically identified as *Colletotrichum* spp. All these isolates were labelled as UMTT46C, UMTT48C, UMTT50C, UMTT52C and UMTT54C. From these, fungal isolate (UMTT54C) showed the highest pathogenic level with disease severity (DS), 68.9%. Other *Colletotrichum* spp. recorded percentage of DS ranged from 53.3% to 60%. Identification of fungal isolates and their pathogenic level from the infected chilli is very important to assist in control strategy which can further provide a good protection measure for many chilli producers.

Keywords: Chilli, anthracnose, *Colletotrichum* spp.

Introduction

Chilli is an economically important crop worldwide. It belongs to family Solanaceae and genus *Capsicum*. Major chilli producing countries are India (1.25 million), China (0.39 million), Bangladesh (0.27 million) and Peru (0.27 million) (Hussain & Abid, 2011). Among these, India has become the world's largest producer and exporter of chilli by exporting to countries such as the United States of America, Canada, United Kingdom, Saudi Arabia, Singapore, Malaysia and Germany (Musakhan & Zacharia, 2017). Throughout the planting and storage, chilli is reported to be susceptible to many fungal infections. Poor transportation practices and storage facilities contribute to severe post-harvest losses in developing countries (Saxena *et al.,* 2016). Infection by *Colletotrichum* spp. are the major disease problem in chilli production, which lead to anthracnose disease. The occurrence of this disease has caused quantitative and qualitative losses of the crop in the fields as well as in the storage every year (Musakhan & Zacharia, 2017). Typically, the anthracnose disease can be detected on mature fruits thus affecting pre-harvest and post-harvest quality of chilli fruits and its market values (Hadden, 1989; Bosland, 2003; Sahitya *et al.,* 2014). As a result, it causes major profit loss to farmers and seller which regarded as unfit for human consumption. Therefore, the objective of this study was to isolate the fungal species associated with anthracnose symptoms on chilli and evaluate its pathogenic level. Identification of pathogenic fungal isolates from plant disease is very important to assist in control strategy which can further provide a good protection measure for many chilli producers.

Materials and Method

Isolation of Fungal Pathogen

Several red chillies with anthracnose symptoms were purchased from the mini market around Gong Badak, Terengganu, before the isolation process. For this purpose, small parts of the

infected tissues were surface sterilized using 1% sodium hypochlorite, rinsed with sterile distilled and air-dried. Then, all the tissues were plated on the potato dextrose agar (PDA).

Morphological Identification of Fungal Isolates

Morphological identification was conducted after obtaining pure cultures. For this purpose, colony appearance and microscopic characteristics were observed under a light microscope and recorded.

Pathogenicity Test

All fungal isolates obtained in this study were put through a pathogenicity test. The healthy chilli pods were surface sterilised and wounded using a sterile scalpel at three different places. Then, PDA plugs containing fungal isolate were placed at each inoculated area. There were three replicates used in this study. Control chillies were only inoculated with PDA plugs without fungal isolate. All the chilli pods were arranged into a container and incubated at room temperature. The appearance of symptoms was evaluated at 3 days interval for 15 days after inoculation based on the following disease severity scale (Shahbazi *et al.,* 2014):

Scale	Symptoms description
0	Fruit was healthy
1	10% of fruit area infected
2	25% of fruit area infected
3	50% of fruit area infected
4	75% of fruit area infected
5	100% of fruit area infected

Where,

v= disease severity

n= the number of infected plants with disease

N= total number of plants

Z= maximum disease severity

Statistical Analysis

The data were analysed using analysis of variance (ANOVA; P=0.05). All the tests were computed by using Statistical Package for the Social Sciences (SPSS) version 20.

Result and Discussion

Chilli is an important spice crop that heavily infected by fungal diseases such as damping off, anthracnose or fruit rot, powdery mildew and leaf spots. Among these, anthracnose is a major problem to chilli producers that can cause severe losses at both pre- and post-harvest stage. In this study, five fungal isolates successfully isolated from the infected chilli with anthracnose symptoms. All these fungi have been morphologically identified as *Colletotrichum* spp. and labelled as UMTT46C, UMTT48C, UMTT50C, UMTT52C and UMTT54C. In the plate containing potato dextrose agar, the colony appeared as white to grey colour with dark green at the centre (Figure 1(a)). It is also produced diurnal zonation of dense and sparse development of aerial mycelium. Under microscopic characteristics, the conidia were abundant and have a falcate shape (Figure 1(b)).

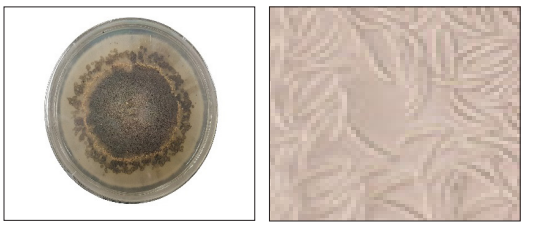

Figure 1: Colony appearance of *Colletotrichum* sp. cultured on potato dextrose agar plate in (a), and abundance conidia of *Colletotrichum* sp. with falcate shape in (b)

Based on the pathogenicity test, all fungal isolates were pathogenic to detach chilli pod with various percentage of disease severity (DS). On the inoculated area, the symptoms were observed as small brown, necrotic spots which became darker and larger. The infected areas were sunken, sometimes appeared grey conidial masses with several black acervuli on

the surface. Among the *Colletotrichum* spp., fungal isolate UMTT54C showed the highest pathogenic level with DS, 68.9% (Figure 2). Other *Colletotrichum* spp. recorded percentage of DS ranged from 53.3% to 60%. Differences in the pathogenic level of these fungal pathogens were suggested due to genetic variability in *Colletotrichum* genus. According to McDonald (1997), many factors contribute to genetic variabilities such as mutation, mating systems, gene flow or migration, population size and selection.

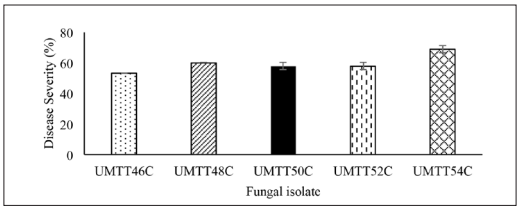

Figure 2: Pathogenicity level of *Colletotrichum* spp. isolated in this study

There are many species in this *Colletotrichum* genus such as *C. acutatum* (Simmonds), *C. capsici* (Syd.) Butler and Bisby, *C. gloeosporioides* (Penz.) Penz. and Sacc., and *C. coccodes* (Wallr.) S. Hughes (Simmonds, 1965; Johnston & Jones, 1997; Kim *et al.,* 1999; Nirenberg *et al.,* 2002; Voorrips *et al.,* 2004; Sharma *et al.,* 2005; Pakdeevaraporn *et al.,* 2005; Than *et al.,* 2008). In this study, all the isolates were only identified based on morphological characteristics. Therefore, it is difficult to separate the isolates into species-level since most of them have similar characteristics. This genus is a well-known plant pathogen worldwide that cause economic losses to a wide range of hosts including cereals, legumes, vegetables, perennial crops and tree fruits. According to Noor and Zakaria (2018), at least five species of *Colletotrichum* namely *C. truncatum, C. scovillei, C. siamense, C. fructicola, and C. fioriniae* are associated with anthracnose disease on red and green chilli in Peninsular Malaysia. Among these, *C. truncatum* has been reported as the most prevalent species in Malaysia, Thailand, India, and China (Noor & Zakaria, 2018; Than *et al.* 2008; Sharma *et al*. 2014). The prevalence of this species was suggested due to genetic differentiation and recombination of this species. In addition, Ranathunge *et al.* (2012) reported that this species showed high adaptive to overcome host resistance that might contribute to its prevalence. The occurrence of anthracnose disease due to *Colletotrichum* sp. was higher in higher humid condition especially during the fruits started to ripen (Thind & Jhooty, 1985). These problems are major issues among the farmers which reported to be losing up to 84%. According to Cannon et al. (2000), many *Colletotrichum* species can be associated with anthracnose of the same host (Simmonds, 1965; Freeman *et al.,* 1998). Therefore, accurate species identification is important in disease control and management which may relate to selecting appropriate fungicides or resistant cultivars.

Conclusion

The major limitation of chilli production in Malaysia is the occurrence of anthracnose disease either at the pre- or post-harvest stage which leads to the profit loss. In this study, five *Colletotrichum* spp. have been isolated and identified as pathogenic fungal pathogens. Further separation at the species level is difficult by using only morphological characteristics. Although there are many fungal species under this genus, several species might potentially infect the same host. Thus, identification of this genus is needed to develop a control strategy to protect major economic loss of chilli plantation.

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