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# Colletotrichum spp. associated with agricultural crops in Malaysia, causal pathogens and potential control methods

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#### **ABSTRACT**

Colletotrichum is one of the most significant and common plant pathogens, infecting many economically important crops worldwide. Colletotrichum is also one of the most studied fungal genera in Malaysia because it contains many species that infect various types of agricultural crop including fruit, vegetable and industrial crops. Studies on Colletotrichum spp. are mostly focused on the causal pathogens, the host range and disease management. Among the host crops, most studies on Colletotrichum spp. have been conducted on infection in chilli (Capsicum spp.), which, in Malaysia, first arose in the 1980s and still continues to be a problem to this day. Studies have also been conducted on Colletotrichum spp. associated with anthracnose in fruit crops including dragon fruit, papaya, banana and mango. Disease management of anthracnose pathogens postharvest has also been conducted to prolong the shelf life of these fruit crops. In this review, Colletotrichum spp. associated with chilli and several fruit crops as well as their disease management are highlighted. There have been many changes in Colletotrichum taxonomy and systematics in recent years, affecting the identification of anthracnose pathogens reported in Malaysia. Colletotrichum species identified solely by morphology or solely via the internal transcribed spacer (ITS) region should be referred to as sensu lato (in the broad sense) since many species belong to species complexes. Species within a species complex are closely related, and most are cryptic species particularly species in the C. gloeosporioides and C. acutatum complexes which are commonly associated with agricultural crops in Malaysia. Due to the importance of Colletotrichum spp. as plant pathogens, up-to-date identification methods should be used since accurate species identification of Colletotrichum is crucial for formulating suitable disease management programmes as well as for plant quarantine and biosecurity purposes. This review provides the current knowledge on the status of Colletotrichum spp. associated with agricultural crops in Malaysia and potential control methods on different types of agricultural crops.

Keywords: Colletotrichum, crops, fruit crops, chilli, control methods

## INTRODUCTION

Colletotrichum is one of the most significant plant pathogenic fungal genera whose species infect a wide range of crops in the tropical, subtropical and temperate regions. According to Dean et al., (2012), based on contributions to the Molecular Plant Pathology journal, Colletotrichum ranked eighth among the most important plant pathogenic fungi. Nearly every crop planted worldwide is infected by various Colletotrichum. The most common diseases caused by Colletotrichum are anthracnose or postharvest rot and blight of aerial plant parts (Dean et al., 2012). Colletotrichum is also the most important and major postharvest pathogen due to infection latency, with symptoms only appearing after harvest, during storage or marketing.

Life styles of many Colletotrichum are complex as they have the potential to cross infect various host plants and switch life styles modes (Phoulivong et al., 2012; De Silva et al., 2017b). Various life styles habits and infection mechanisms as well as colonisation of Colletotrichum species have been reviewed by De Silva et al. (2017b) who stated that differences depend on species, host physiological maturity of the host and environmental conditions. Nevertheless, infection by Colletotrichum typically begins when the conidia attach to the surface of the plant. The conidia germinate and then form appressoria, beneath which a penetration peg forms to provide entry to the plant to establish the biotrophic stage. At this stage, primary hyphae are formed in a few cells, but symptoms are not apparent. The pathogen then switches to the necrotrophic stage by colonising the host tissues using narrower hyphae and producing cell walldegrading enzymes (Mendgen and Hahn, 2002).

With the advent of molecular taxonomy and phylogenetic studies, many new Colletotrichum species have been identified. Using the multiple gene phylogeny approach, it is now established that the genus Colletotrichum consists of a number of complexes, namely, C. acutatum, C. boninense, C. dematium, C. destructivum, C. gloeosporioides, C. graminicola, C. orbiculare, C. spaethianum and C. truncatum (Cannon et al., 2012). Each complex comprises individual species that are pathogens of many plants with the potential for cross infection (Phoulivong et al., 2012; De Silva et al., 2016a). Due to the importance of Colletotrichum spp. as plant pathogens, accurate species identification is vital for disease management strategies and to improve biosecurity measures, particularly where species in the C. gloeosporioides and C. acutatum complexes are concerned as they are known to be common pathogens worldwide.

In Malaysia, *Colletotrichum* infects various crops including different types of fruit, vegetable and industrial crops. This review highlights the *Colletotrichum* spp. that have been reported to infect agricultural crops in Malaysia and the potential control methods against *Colletotrichum* spp.

## ANTHRACNOSE IN FRUIT CROPS

Fruit crops are susceptible to pathogens causing anthracnose, the most serious economic losses being incurred when infection affects crops during the fruiting stage. Anthracnose symptoms appear in both developing and mature fruits, affecting the fruit in the field as well as during storage. Infection is characterised by sunken necrotic tissues on the fruit surface, with orange conidial masses often produced in the lesion (Freeman, 2000).

Anthracnose not only affects fruit crops in tropical regions but also in subtropical and temperate regions, and the most common fungal genus causing anthracnose is *Colletotrichum*. A single fruit crop may be infected by multiple *Colletotrichum* spp., and multiple fruit crops may be infected by a single *Colletotrichum* species (Freeman, 2000). Before molecular identification became widely used, *C. gloeosporioides* and *C. acutatum* were identified as the most common species associated with fruit crop anthracnose, either on single or multiple hosts.

Molecular identification is part of polyphasic approach for identification of Colletotrichum spp. Polyphasic approach includes morphological characterization, phylogenetic analysis of multiple markers pathogenicity tests which are the most common methods applied for Colletotrichum spp. (Cai et al., 2009). Following these recommended methods, many new species have been described by Damm et al. (2009), Weir et al. (2012), Doyle et al. (2013), Manamgoda et al. (2013), Liu et al. (2015), Zhou et al. (2019) and Damm et al. (2019). By applying polyphasic approach, C. capsici is synonymous with C. truncatum (Shenoy et al., 2007; Damm et al., 2009) and has been used to resolve many species within C. gloesporioides, C. acutatum and C.

boninense species complexes (Weir et al., 2012; Damm et al., 2012a; Damm et al., 2012b).

Nowadays, although many publications still use both C. gloeosporioides and C. acutatum in their broad sense, it is well known that both comprise a large number of species and species complexes. By applying multilocus markers, a great number of species belonging to the acutatum and gloeosporioides species complexes have been identified (Damm et al., 2012a,b; Weir et al., 2012). Consequently, the identities of Colletotrichum spp. associated with anthracnose of various fruit crops have been reassigned. According to Udayanga et al. (2013), several species within the C. gloeosporioides complex were the main anthracnose pathogen in tropical Asia, although C. gloeosporioides sensu stricto has limited host range. In their study, C. gloeosporioides was only found in Citrus aurantifolia and Syzygium in Northern Thailand. C. gloeosporioides has also been found to be prevalent on Citrus leaves and fruits in China (Lijuan et al., 2012; Huang et al., 2013).

The fruit crop industry is economically important in Malaysia, contributing to the socio-economic well-being of the population and supporting many smallholder farmers. Although a wide variety of fruit crops are planted in Malaysia, studies on *Colletotrichum* spp. associated with anthracnose have been limited to banana, mango, papaya, dragon fruit and guava, covering mainly the anthracnose pathogens and their control methods. Table 1 shows the overview of *Colletotrichum* spp., fruit crops and potential control methods that have been reported in Malaysia and discussed in this review.

## Banana (Musa spp.)

Banana is often intercropped with rubber and oil palm and currently accounts for 24% of Malaysia's total fruit production (Khazanah Research Institute, 2019). Various local varieties are planted in Malaysia, which include Abu, Abu Keling, Awak, Berangan, Kelat, Mas, Nangka, Raja, Rastali, Susu and Udang, with production both for domestic consumption and export. The local Berangan is commonly planted for export to Singapore, Brunei, Japan, Hong Kong, the Middle East and Korea.

In Malaysia, research has shown that based on morphology and Random Amplified Polymorphic DNA (RAPD) analysis, C. musae was the most common species associated with anthracnose in different local banana cultivars (Latiffah et al., 2009). Based on ITS regions, β-tubulin sequences and phylogenetic analysis, C. musae and C. gloeosporioides were the most common species associated with anthracnose of local banana varieties, Mas, Berangan, Awak, Nangka and Rastali, with C. gloeosporioides more prevalent than C. musae (Intan et al., 2013; 2014a). Colletotrichum gloeosporioides has also been reported as the pathogen of anthracnose in banana fruit in Ecuador (Riera et al., 2019). However, Udayanga et al. (2013) and Vieira et al. (2017) have reported that C. musae is the most prevalent Thailand respectively. species in and Brazil,

**Table 1**: The agricultural crops, *Colletotricum* spp. and potential control methods reported in Malaysia.

Agricultural crop	Reported Colletotrichum spp.	Potential control methods
Banana ( <i>Musa</i> spp.)	- C. musae - C. gloeosporioides sensu lato	<ul> <li>Edible coating: 10% gum arabic and 1% chitosan (Maqbool et al., 2010)</li> <li>Edible coating: 10% gum arabic and 0.4% cinnamon oil (Maqbool et al., 2011)</li> <li>Hot water treatment (Mirshekari et al., 2013)</li> </ul>
Mango ( <i>Mangifera indica</i> )	<ul><li>C. dianesei</li><li>C. asianum</li><li>C. gloeosporioides sensu lato</li></ul>	<ul> <li>Thiophanate, benzimidazoles, difolatan and zincofol (Lim, 1980)</li> <li>10 mg/mL unripe Areca catechu nut extract (Rusdan et al., 2015)</li> <li>UV irradiation at 72.0 kJ m<sup>-2</sup> (Gunasegaran et al., 2018)</li> </ul>
Papaya (Carica papaya)	- C. capsici (syn. C. truncatum) - C. gloeosporioides sensu lato	<ul> <li>- 1.5% and 2.0% chitosan coating (Ali et al., 2010)</li> <li>- Sodium bicarbonate (baking soda) (Hasan et al., 2012)</li> <li>- Lemongrass oil vapour (Ali et al., 2015a)</li> <li>- Ozone treatment at 1.6 ppm for 96 h, 28 days of storage at 12 °C ± 1 °C and 80% relative humidity (Ong and Ali, 2015)</li> <li>- 2% ginger oil and 10% gum arabic coating (Ali et al., 2016)</li> </ul>
Dragon fruit ( <i>Hylocereus</i> spp.)	- C. gloeosporioides sensu lato - C. truncatum	<ul> <li>- 0.5% propolis extract (Zahid <i>et al.</i>, 2013)</li> <li>- 1.0% submicron chitosan dispersion (Asgar <i>et al.</i>, 2013)</li> <li>- Crude extract of "dukung anak" (5.0 g L<sup>-1</sup> or 10.0 g L<sup>-1</sup>) and turmeric (10.0 g L<sup>-1</sup>) after 28 days of cold storage at 11 ± 2 °C, 80% relative humidity (Bordoh <i>et al.</i>, 2020)</li> </ul>
Guava (Psidium guajava)	- C. gloeosporioides sensu lato	No available reports in Malaysia
Chilli ( <i>Capsicum</i> spp.)	<ul> <li>C. gloeosporioides sensu lato</li> <li>C. acutatum sensu lato</li> <li>C. scovillei,</li> <li>C. truncatum</li> <li>C. siamense</li> </ul>	<ul> <li>Ethanolic extract of propolis coating (Ali et al., 2015b)</li> <li>0.5% lemongrass essential oil and 1.0% chitosan coating (Ali et al., 2015c).</li> <li>Mancozeb and propineb (contact fungicides), benomyl and difenoconazole (systemic fungicides) (Mohd Noor and Zakaria, 2018)</li> </ul>
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	- C. fioriniae - C. fructicola	- Potential biocontrol agent: <i>Streptomyces</i> strain P42 (Shahbazi <i>et al.</i> , 2014), <i>Lactobacillus plantarum</i> (El-Mabrok <i>et al.</i> , 2012), <i>Lactococcus lactis</i> subsp. <i>lactis</i> (Fakri <i>et al.</i> , 2018).
Cocoa ( <i>Theobroma cacao</i> L.)	- C. gloeosporioides sensu lato	No available reports in Malaysia
Tomato (Solanum lycopersicum)	- C. boninense	No available reports in Malaysia
Rose apple (Syzygium samarangense)	- C. gloeosporioides sensu lato	No available reports in Malaysia
Bok choy ( <i>Brassica rapa</i> subsp. <i>chinensi</i> s L.)	- C. capsici (syn. C. truncatum)	No available reports in Malaysia
Legume crops (soybean, bean, pea, lima bean, lentil, chickpea, peanut, cowpea, winged bean and country bean)	- C. truncatum, - C. dematium - C. gloeosporioides	No available reports in Malaysia

Following taxonomic revision of the genus Colletotrichum using multiple markers, various species in the gloeosporioides complex have been identified as causal pathogens of banana anthracnose, including C. siamense, C. tropicale, C. chrysophyllum, C. theobromicola and C. scovillei (Kumar et al., 2017; Zhou et al., 2017; Vieira et al., 2017). Other Colletotrichum spp. have also been reported to be associated with banana anthracnose, which include C. karstii (Damm et al., 2012b) and C. paxtonii (Damm et al., 2012a), members of the C. boninense and C. acutatum complexes, respectively. Thus, further and detailed studies are needed to clarify the diversity of Colletotrichum species associated with banana anthracnose in Malaysia.

## Control of banana anthracnose

Due to the importance of banana as one of the important fruits crops for export, studies have been conducted on control methods to reduce incidence of banana anthracnose, with the main purpose to prolong the shelf-life of harvested banana fruits. The control methods including hot water treatment, edible composite coating and essential oils, as well as combinations of control methods.

Heat treatment is a simple method to kill or inactivate plant pathogenic microbes. A combination of heat or hot water treatment and fungicides is used to reduce the incidence of banana anthracnose. Hot water treatment, by dipping Berangan bananas in water at 50 °C for 10 or 20 min with or without fungicide, slowed respiration and ethylene production, indicating that the treatment delayed fruit ripening (Mirshekari *et al.*, 2013). The recommendation from this study was that for export of Berangan bananas, hot water treatment at 50 °C for 10 min without fungicide could be used to extend their shelf life. This control method provides an alternative that minimises the use of fungicides.

Although hot water treatment is preferable, this control method affects the nutritional quality of the bananas. Another approach is the use of a polysaccharide edible coating to inhibit the growth of the anthracnose pathogen and extend the shelf life of the bananas. Magbool et al. (2010) studied the effect of an edible coating using gum arabic and chitosan as a potential postharvest biofungicide against the anthracnose pathogen, C. musae. Results showed that a coating combination of 10% gum arabic and 1.0% chitosan had the potential to control the incidence of anthracnose in banana. Moreover, the combined coatings inhibited the mycelial growth and conidial growth of C. musae. In another study, Maqbool et al. (2011) tested the possibility of using gum arabic and essential oils to control anthracnose in banana. Results indicated that a coating of 10% gum arabic combined with 0.4% cinnamon oil was effective in controlling the incidence of anthracnose both in vitro and in vivo. Therefore, composite edible coating materials were suggested as a suitable treatment for use by banana growers and exporters to extend the shelf life of bananas.

## Mango (Mangifera indica L.)

Mango is one of the major fruit crops in Malaysia, cultivated both for local consumption and for export. Among popular mango cultivars grown in Malaysia are Harumanis, Chokanan, Masmuda, Sala, Siam Panjang and Nam Dorkmai. As in banana, anthracnose is one of the postharvest diseases that affect fruit quality, especially for marketing.

Studies on *Colletotrichum* spp. associated with anthracnose in mango are very limited, with most conducted on control methods. The causal pathogen has been referred to as *C. gloeosporioides*, though this may not have been accurate. Moreover, Phoulivong *et al.* (2012) stated that *C. gloeosporioides* was not the causal pathogen of mango in Laos and Thailand. It is possible that the anthracnose pathogen of mango is not *C. gloeosporioides* but species within the *C. gloeosporioides* complex.

Lima et al. (2013) reported that five species within the C. gloeosporioides complex, C asianum, C. fructicola, C. tropicale, C. dianesei and C. karstii, were associated with anthracnose of mangoes in Brazil. Thus, with the taxonomic revision of the genus Colletotrichum, more than one species of Colletotrichum may be involved in causing anthracnose in mango in Malaysia.

The only molecular identification study Colletotrichum spp. associated with anthracnose in mango in Malaysia was conducted by Latiffah et al. (2015). Colletotrichum isolates were recovered from anthracnose lesions of Chokanan and Harumanis mango cultivars. Although the study was based only on ITS regions and  $\beta$ -tubulin, phylogenetic analysis of combined sequences suggested that C. dianesei and C. asianum (associated with C. gloeosporioides sensu lato) were associated with anthracnose in mangoes. Further studies involving multiple genes are required to determine the causal pathogens of mango anthracnose in Malaysia, as several newly reported Colletotrichum spp. have been reported associated with mango anthracnose. In a study by Li et al. (2019) using actin (ACT), chitin synthase (CHS-1), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), ITS region, β-tubulin sequences, 13 species were associated with mango anthracnose in southern China, namely C. asianum, C. cliviicola, C. cordylinicola, endophytica, C. fructicola, C. gigasporum, C. gloeosporioides, C. karstii, C. liaoningense, C. musae, C. scovillei, C. siamense and C. tropicale. Therefore, there could be more species of Colletotrichum occur in Malaysia. Moreover, correct species identification is important when formulating suitable disease control methods and for quarantine purposes, especially as Malaysia also imports mangoes from other countries.

In addition to anthracnose in mango, there have also been reports of *Colletotrichum* spp. associated with leaf spot and blossom blight in mango (Nurul Husna, 2016; Nor Dalila *et al.*, 2017).

## Control of mango anthracnose

In the 1980s, commercialisation of mango in Malaysia increased, accompanied by an expansion of the area planted with mango trees. This led to the occurrence of mango anthracnose which not only infected the fruits but also the leaves, blossom and peduncle, resulting in poor fruit set and low yield. Lim (1980) initiated a study to determine the efficacy of 15 fungicides against *C. gloeosporioides sensu lato*, conducted both in the greenhouse and in the field. The anthracnose pathogens were very sensitive to thiophanate, benzimidazoles and two of the N-trichloromethylthio group of fungitoxicants (difolatan and zincofol). Lim (1980) also reported that dithiocarbamates were relatively less toxic compared to benzimidazoles, thiophanate and the captan group of compounds.

According to Dirou and Stovold (2005), anthracnose fungicides are registered under three categories: mancozeb, copper hydroxide and copper sulphate (Group Y), prochloraz (Group C) and azoxystrobin (Group K). Although several fungicides are effective to control mango anthracnose, there is a risk of resistance or loss of sensitivity; therefore, fungicide rotation application is recommended.

In a study by Diedhiou *et al.* (2014), thiophanate methyl and azoxystrobin were effective anthracnose treatments during fruit development in the field. Mancozeb and myclobutanil were less effective. They concluded that the choice of product is the main factor in controlling mango anthracnose.

Aside from fungicides, UV irradiation and plant extracts are potential control methods. Some degree of success has been reported; however, more studies are required before these methods are applicable in the field.

The effects of UV-C irradiation on mycelial growth, sporulation and conidial germination were studied by Gunasegaran *et al.* (2018), and results show that UV irradiation at 72.0 kJ m<sup>-2</sup> produced significant inhibition of conidial germination and sporulation of *C. gloeosporioides senso lato*. Thus, UV-C irradiation is a potential treatment for reducing postharvest losses caused by anthracnose of mango in Malaysia.

The potential use of unripe *Areca catechu* nut extract to inhibit the growth of *C. gloeosporioides* and to treat mangoes against anthracnose has been reported by Rusdan *et al.* (2015). Chloroform extract of unripe *A. catechu* nuts at a concentration of 10 mg/mL inhibited 52% of mycelium growth and 100% of spore germination of the anthracnose pathogen. Dipping mango fruits in the extract solution at 52°C for 5 min reduced 51% of anthracnose infection. The authors considered that the potential of *A. catechu* nut extract as an antifungal agent might be associated with the presence of alkaloids and phenolic compounds.

## Papaya (Carica papaya L.)

Papaya is also an important fruit crop in Malaysia, widely planted as a smallholder's crop both for local

consumption and for export. Among the major papaya cultivars planted are Subang 6, Eksotika, Eksotika II and Sekaki. Along with the increasing demand for papaya, disease infection is also increasing. While bacterial dieback is currently the main disease affecting papaya, anthracnose still poses a threat, especially during storage.

As with mango and banana, studies on *Colletotrichum* spp. associated with papaya anthracnose in Malaysia are limited, and identification has been based mainly on morphological characteristics. All have reported *C. capsici* (syn. *C. truncatum*) and *C. gloeosporioides sensu lato* as anthracnose pathogens of papaya (Sepiah *et al.*, 1991; Rahman *et al.*, 2008). Both *C. truncatum* and *C. gloeosporioides sensu lato* have also been reported as anthracnose pathogens of papaya in the Yucatan peninsula, Mexico (Tapia-Tussel *et al.*, 2008), Miyako Islands, Okinawa, Japan (Yaguchi *et al.*, 1995), South Florida (Tarnowski and Ploetz, 2010) and Trinidad (Rampersad, 2011).

With the revision of *C. gloeosporioides* as a species complex, several species have been identified as anthracnose pathogens in papaya. In Brazil, *C. magna* and *C. brevisporum* were identified as causal pathogens in papaya fruit rot and papaya anthracnose, respectively (Nascimento *et al.*, 2010; Vieira *et al.*, 2013). In India, *C. fructicola* was identified as an anthracnose pathogen in papaya (Saini *et al.*, 2016). Thus, in addition to *C. truncatum* and *C. gloeosporioides sensu lato*, other species of *Colletotrichum* are also associated with anthracnose of papaya.

## Control of papaya anthracnose

Although studies on the *Colletotrichum* spp. associated with papaya anthracnose are limited, a number of studies have been conducted on control of the pathogens, with most investigating alternative control methods to reduce reliance on chemicals and heat treatment. Although heat treatment of papaya is a conventional and safe method for postharvest treatment of fruit crops, it often leads to nutrient deterioration.

Sodium bicarbonate (baking soda) is categorised as 'generally recognised as safe' (GRAS) by the United States Food and Drug Administration (US FDA). Hasan et al. (2012) reported the use of sodium bicarbonate against C. gloeosporioides sensu lato anthracnose in papaya. Mycelial growth and spore germination of the pathogen were affected even at 1% concentration. Anthracnose disease severity was also reduced, with complete absence of symptoms up to 14 days of storage at 12°C. The authors indicated that treatment with 2% sodium provided control bicarbonate effective against anthracnose in papaya.

The effectiveness of chitosan to inhibit growth of anthracnose pathogens in papaya and as fruit coatings was studied by Ali *et al.* (2010). Chitosan at 1.5% and 2.0% showed a fungistatic effect, inhibiting 90%–100% of mycelial growth. For chitosan coatings, the same concentrations not only controlled rot on the fruits but also

delayed the appearance of anthracnose symptoms by 3–4 weeks during 5 weeks of storage at 12°C ± 1°C and slowed anthracnose development. The authors suggested that as chitosan was able to control anthracnose on papaya fruits, it had the potential for commercial application on other fresh produce as well.

Another method tested to control papaya anthracnose is the use of lemongrass essential oil vapour. In an *in vitro* study, Ali *et al.* (2015a) showed that lemongrass oil vapour at concentrations of 33, 66, 132, 264 and 528 µL L<sup>-1</sup> inhibited mycelial growth and conidial germination of *C. gloeosporioides sensu lato.* In an *in vivo* study, Sekaki papaya exposed to lemongrass oil fumigation at a concentration of 28 µL L<sup>-1</sup> incubated at room temperature for 9 days was the most effective against anthracnose, and the quality of the tested papaya was not significantly affected. Ali *et al.* (2015a) stated that lemongrass oil vapour can be used to control papaya anthracnose without affecting the ripening process and that lemongrass oil vapour has the potential to be developed as a biofumigant for commercial application.

Antifungal properties of plant extracts have also been tested against anthracnose pathogens in papaya in tandem with the ability of the extracts to maintain the fruit quality during storage. Ali et al. (2016) evaluated ginger oil and ginger extract or gum arabic for postharvest control of papaya anthracnose during cold storage. In the study, a combination of 2% ginger oil and 10% ginger extract inhibited 93% of spore germination and delayed the ripening of papaya. Ali et al. (2016) also reported that a coating with a combination of 2% ginger oil and 10% gum arabic was effective in controlling anthracnose and maintaining papaya fruit quality. The findings also showed that higher antifungal activity was observed in treatments combining ginger oil with gum arabic and that this combination could be used as a biofungicide to protect papaya fruit during storage.

Another approach to ensure the safety and improve the shelf life of papaya postharvest is the potential application of ozone. Ozone is effective at controlling microbes and was granted GRAS status by US FDA. Ong and Ali (2015) studied the effect of several ozone concentrations (0.04, 1.6 and 4 ppm) and exposure time (48, 96 and 144 h) on the growth of anthracnose pathogens in papaya as well as the effect on anthracnose incidence on the fruits. Ozone treatment showed a fungistatic effect, with inhibition of mycelial growth and spore germination of C. gloeosporioides sensu lato being observed at all tested concentrations. Ozone treatment at 1.6 ppm for 96 h on papaya fruit reduced 40% of anthracnose after 28 days of storage at 12°C ± 1°C and 80% relative humidity, indicating that ozone treatment is another potential method to overcome infection by anthracnose pathogens in papaya.

## Dragon fruit (Hylocereus spp.)

Red-fleshed dragon fruit (*Hylocereus polyrhizus*) is an introduced fruit crop widely planted in Malaysia, preferred

over other species due to its attractive red colour, pleasant texture and sweet taste.

Anthracnose caused by *Colletotrichum* is one of the most serious diseases of dragon fruit, including *H. polyrhizus*, in Malaysia, infecting the stem and the fruit. *Colletotrichum* spp. associated with anthracnose of *H. polyrhizus* are poorly studied in Malaysia, with only *C. gloeosporioides sensu lato* reported as the anthracnose pathogen (Masyahit *et al.*, 2009).

Before the taxonomic revision of *C. gloeosporioides* as a species complex, several studies indicated *C. gloeosporioides sensu lato* as the causal anthracnose pathogen in *Hylocereus* spp. Anthracnose of *H. undatus* in Okinawa Prefecture, Japan and in South Florida, USA, and of *H. megalanthus* in Botucatu region, Brazil, was shown to be caused by *C. gloeosporioides sensu lato* (Taba *et al.*, 2006; Palmateer *et al.*, 2007; Takahashi *et al.*, 2008).

The only report of *C. gloeosporioides* as the anthracnose pathogen in dragon fruit was done by Ma *et al.* (2014) on the stem of *H. undatus*. The identification was based on ITS region and actin and glutamine synthetase sequences. By using molecular identification, species within the *C. gloeosporioides* complex have also been reported to be associated with anthracnose of *Hylocereus* spp. *C. siamense* identified using ITS regions and the translation elongation factor-1α gene was the causal pathogen of stem anthracnose on *H. polyrhizus* in China (Zhao *et al.*, 2018). Meetum *et al.* (2015) reported that *C. aenigma* and *C. siamense*, identified based on ITS regions, were the anthracnose pathogens of the stem and fruit in *H. undatus* in the Thai provinces of Pathum Thani, Nakhon Pathom and Samut Sakhon.

Based on a preliminary study using ITS regions, GAPDH and  $\beta$ -tubulin sequences, *C. gloeosporioides sensu lato* from stem anthracnose in *H. polyrhizus* form several clades, suggesting the occurrence of a subpopulation of species within the *C. gloeosporioides* complex (unpublished data) and indicating the possibility that more than one of the species in the complex are associated with stem anthracnose in *H. polyrhizus* in Malaysia.

Molecular identification using ITS regions, ß-tubulin, actin and GAPDH sequences revealed the occurrence of *C. truncatum* causing stem anthracnose of *H. polyrhizus* in Malaysia (Vijaya *et al.*, 2015). *Colletotrichum truncatum* has also been reported as an anthracnose pathogen of *H. undatus* in Yuanjiang County, Yunnan Province, China (Guo *et al.*, 2014).

Cross infection testing of *C. truncatum* and *C. gloeosporioides sensu lato* showed that *Colletotrichum* isolates from stem anthracnose can also cause anthracnose on the fruits of *H. polyrhizus*. Inoculated fruits produced brown lesions with conidial masses in concentric rings, surrounded by water-soaked areas, indicating that the stem and fruit of *H. polyrhizus* may possibly be infected by the same pathogens (Vijaya, 2013).

## Control of dragon fruit anthracnose

Studies on control methods for anthracnose in dragon fruit are limited, despite dragon fruit being widely planted in Malaysia.

The potential of ethanolic extract of propolis to control and manage *C. gloeosporioides sensu lato* anthracnose in dragon fruit has been reported by Zahid *et al.* (2013). Extracts at 0.75% and 1.0% produced 60% inhibition of radial mycelial growth and conidial germination of the fungus and also delayed the commencement of anthracnose symptoms and disease development. The extract treatment also raised the activity levels of the enzymes related to anthracnose resistance, such as peroxidase, polyphenol oxidase and phenylalanine ammonia lyase. However, treatment of the fruits at these concentrations (0.75% and 1.0%) showed some phytotoxic effects; thus, to control anthracnose in dragon fruit, the authors suggested a concentration of 0.5% propolis extract as suitable.

Chitosan (in the form of submicron chitosan dispersion) as a coating for dragon fruit also has the potential to delay anthracnose and to maintain the fruit quality during storage (Ali *et al.*, 2013). A study by Ali *et al.* (2013) showed that a 1.0% submicron chitosan dispersion with 600 nm droplet size delayed the commencement of anthracnose and maintained the quality of fruit during storage up to 28 days at  $10^{\circ}\text{C} \pm 2^{\circ}\text{C}$  and  $80\% \pm 5\%$  relative humidity. However, the quality of the treated fruit was reduced, possibly due to the low viscosity of the submicron chitosan dispersion (Ali *et al.*, 2013).

Recently, Bordoh *et al.* (2020) conducted a study to evaluate antifungal effect of rhizome and medicinal herbs, namely ginger, turmeric and 'dukung anak' against *C. gloeosporioides sensu lato* in dragon fruits. The *in vitro* studies indicated all plant extract significantly controlled growth of *C. gloeosporioides* at an optimal concentration of 10.0 g L<sup>-1</sup>. However, phytoxcity occurs at certain concentration. Treatment using ginger extract at 5.0 g L<sup>-1</sup> and above highten anthracnose incidence due to phytotoxicity. For 'dukung anak' and turmeric treatments phytotoxicity occurred at 15.0 g L<sup>-1</sup>.

## Guava (Psidium guajava)

Guava is one of the fruit crops given priority for cultivation in Malaysia for both local consumption and export. One of the common diseases affecting guava is anthracnose which can reduce the quality and appearance of the fruit.

The only report of an anthracnose pathogen infecting guava fruit in Malaysia is done by Intan Sakinah *et al.* (2014b). Based on ITS regions and ß-tubulin sequences, isolates from anthracnose lesions on guava fruit were identified as *C. gloeosporioides sensu lato.* Results suggested that there might be distinct species within the species complex specifically associated with anthracnose in guava, though analysis of more genes would be required to accurately identify the species causing anthracnose in guava in Malaysia.

Colletotrichum gloeosporioides sensu lato has also been reported to be associated with anthracnose of guava in Brazil (Soares et al., 2008), Egypt (Abdul Wahid, 2001) and Nigeria (Amusa et al., 2005). Besides C. gloeosporioides sensu lato, some studies have also reported C. acutatum sensu lato associated with anthracnose in guava (Peres et al., 2002; Soares et al., 2008). Thus, there could be several species within the C. gloeosporioides and C. acutatum complexes that cause anthracnose in guava. Moreover, with the taxonomic revision of the genus Colletotrichum using multigene phylogeny, several species have been isolated from guava, namely, C. abscissum, C. guajava, C. psidii and C. siamense, which are also accepted Colletotrichum species listed by Jayawardena et al. (2016). C. abscissum and C. guajava belong to the C. acutatum complex, whereas Č. psidii and C. siamense belong to the C. gloeosporioides complex.

## Control of guava anthracnose

To date, there are no reports on control methods for anthracnose in guava in Malaysia. Nevertheless, the control methods currently applied are similar to those used for other tropical fruits, fungicide use pre- and postharvest, hot water treatment and edible coatings.

## Chilli (Capsicum spp.)

Anthracnose pathogens cause chilli fruit rot at both green and red stages in various *Capsicum* spp. According to Saxena *et al.* (2016), anthracnose is seed-borne, soilborne, water-borne and airborne; thus, chilli plants are susceptible to anthracnose infection at any stage of development, from seed to maturity. Anthracnose of chilli not only causes the fruit to rot but also affects other parts of the plant, particularly aerial parts.

In Malaysia, *Colletotrichum* spp. associated with chilli have been studied since the 1980s. Early comprehensive studies on anthracnose of chilli (*Cap. annuum*) in Malaysia were conducted by Mah (1987). The causal pathogen was identified as *C. capsici* whose infection characteristics differed in local and introduced chilli varieties. *In vitro* fungicide testing indicated that the growth of *C. capsici* was highly inhibited by Brestan 10 (maneb + fentin acetate). However, none of the fungicides tested in the field were effective at controlling anthracnose on ripe chilli fruits (Mah, 1987).

Meon and Nik (1988) conducted histological studies on the development of *C. capsici* as a seed-borne anthracnose pathogen in chilli. Mycelia were found in the outer and inner layers of the testa and in the endosperm. Acervuli were present below the seed coat and in the endosperm. When the acervuli mature, they emerge onto the surface, disrupt the seed tissues and spread the conidia which become the primary source of inoculum.

As above, prior to the application of multigene markers, chilli anthracnose in Southeast Asia was mainly associated with *C. capsici* and *C. gloeosporioides* (Mah, 1987; Khor, 2013). *C. acutatum* was not commonly

reported as the pathogen in chilli anthracnose. Following the extensive revision of Colletotrichum taxonomy and systematics using multigene markers, many species within Colletotrichum species complexes were broadly defined which affected the identification of the anthracnose pathogen in chilli in Malaysia. Using two markers, ITS regions and GAPDH, Nasehi et al. (2016) reported C. nymphaeae as the causal pathogen of chilli (Cap. annuum) from the Cameron Highlands, Pahang State, Malaysia. Mohd Noor and Zakaria (2018) reported that five species (C. scovillei, C. truncatum, C. siamense, C. fioriniae and C. fructicola) were associated with chilli anthracnose in peninsular Malaysia for which the identification was based on ITS regions,  $\beta$ -tubulin, actin and GAPDH genes. Hence, in Malaysia, species within the C. gloeosporioides and C. acutatum complexes are associated with anthracnose in chilli. However, recently, De Silva et al. (2019) reported C. plurivorum (belonging to the C. orchidearum complex) as an anthracnose pathogen in chilli in Malaysia.

Among the species reported by Mohd Nor and Zakaria (2018), *C. scovillei*, *C. truncatum* and *C. siamense* were the most common species causing anthracnose in chilli (*Cap. annum* and *Cap. frutescens*) in Malaysia, which is in agreement with Mongkolporn and Taylor (2018). These three species are the main *Colletotrichum* species infecting chilli in Southeast Asia and South America. A recent report by De Silva *et al.* (2019) on anthracnose pathogens in Asia indicated that ten species were associated with anthracnose in chilli fruits, those being *C. endophyticum*, *C. fructicola*, *C. karstii*, *C. plurivorum*, *C. scovillei*, *C. siamense*, *C. tropicale*, *C. javanense*, *C. makassarense* and *C. taiwanense*. The three latter species were newly described as anthracnose pathogens in chilli

Following the extensive taxonomy revision of many Colletotrichum species complexes, the use of C. capsici, C. gloeosporioides and C. acutatum when referring to Colletotrichum species associated with chilli anthracnose needs to be treated with caution. C. capsici is regarded as a synonym of C. truncatum (Damn et al., 2009); the correct name is C. truncatum. C. acutatum is a species in the C. acutatum complex, and many varieties of C. acutatum have been defined. The only report of C. acutatum sensu lato in chilli (Cap. annuum) was in Sri Lanka (Damn et al., 2012). Among the species within the C. acutatum complex, C. scovillei, C. fioriniae, C. simmondsii, C. brisbanense and C. cairnsense are causal pathogens of chilli anthracnose in many countries (De Silva et al., 2017a; Diao et al., 2017; Nasehi et al., 2016; Liu et al., 2016; Katoch et al., 2016; Mohd Noor and Zakaria, 2018).

As with the *C. acutatum* complex, many species in the *C. gloeosporioides* complex have been described (Weir *et al.*, 2012). *C. gloeosporioides* was reported as the pathogen of chilli anthracnose in China (Liu *et al.*, 2016; Diao *et al.*, 2017) and India (Katoch *et al.*, 2016). Several species in the *C. gloeosporioides* complex have been found to be associated with chilli anthracnose, and the species most commonly reported is *C. siamense* in

Malaysia, Australia, Brazil, China, Thailand and India. Other species in the *C. gloeosporioides* complex associated with chilli anthracnose are *C. fructicola*, *C. conoides*, *C. grossum*, *C. kahawae*, *C. queenslandicum*, *C. tropicale* and *C. viniferum*.

Correct identification of Colletotrichum spp. causing anthracnose in chilli is important because different species cause anthracnose of varying degrees of severity related to their aggressiveness. Different species may also exhibit pathotype variation among isolates of the same species. Pathotype identification is part of the genetic studies on anthracnose resistance and breeding of new chilli varieties resistant to anthracnose. Identification is based on qualitative differential reactions and infection of inoculated chilli at different maturity stages on a set of differential Capsicum genotypes (Montri et al., 2009). Pathotype differences between Colletotrichum spp. have been reported by Mongkolporn et al. (2010) among isolates of C. scovillei, C. truncatum and C. siamense at different stages of physiological maturity in four Capsicum spp. (Cap. annuum, Cap. baccatum, Cap. chinense and Cap. frutescens).

Preliminary pathotype identification in Malaysia was conducted by Khor (2013) using infected mature chilli fruits from which several pathotypes from three Colletotrichum spp. were identified. One pathotype of C. scovillei was highly virulent on Cap. annuum (locally known as Kulai chilli), and three pathotypes of C. truncatum on Kulai and bell pepper were also identified. Four pathotypes were also identified within isolates grouped in the C. gloeosporioides complex. Khor (2013) also reported that isolates of C. scovillei and C. truncatum were able to infect the resistant Cap. chinense (genotype CO3865), indicating that resistance was stronger in mature green fruit compared to immature fruit.

## Control of chilli anthracnose

Integrated disease management comprising cultural practices, chemical control, biological control and use of resistant varieties is often applied to control anthracnose in chilli. Chemical control using fungicides is widely used, because they are effective at controlling the spread of anthracnose. However, chemical residues on the chilli fruits can affect both farmers and consumers, as well as affect the value of the fruits for export. Relying heavily on fungicides can lead to resistance especially if only a single chemical component is regularly applied (Staub, 1991). In Malaysia, there are reports (although not comprehensive) on in vitro studies using fungicides and biological agents to manage Colletotrichum associated with chilli anthracnose. However, further studies in the field are required to determine the efficacy of the fungicides as well as the biocontrol agents to control the pathogens of chilli anthracnose.

Studies on the effectiveness of contact and systemic fungicides to inhibit mycelial growth of *Colletotrichum* spp. causing chilli anthracnose were conducted by Mohd Noor and Zakaria (2018). Two contact fungicides, mancozeb and propineb, and two systemic fungicides, benomyl and

difenoconazole, were able to inhibit mycelial growth of *C. fructicola*, *C. siamense*, *C. truncatum*, *C. scovillei* and *C. fioriniae*. The study showed that the four fungicides can be applied in the field to manage anthracnose caused by several species of *Colletotrichum*.

In general, for chemical control, copper-based compounds, including benzimidazole, dithiocarbamates and triazole, are often recommended to manage anthracnose (Waller, 1992). In addition, strobilurin fungicides such as azoxystrobin, pyraclostrobin and trifloxystrobin are newer fungicides which are also recommended to control anthracnose in chilli (Chen et al., 2009; Saxena et al., 2016). Effective use of fungicides depends on timely application, usually during the earlier stages of plant development, to limit pathogen colonisation (Wharton and Diéguez-Uribeondo, 2004).

In vitro studies using bacteria as biocontrol agents to inhibit anthracnose pathogens have shown positive results. However, these studies still used the old species names when referring to *Colletotrichum* spp. Problems arise when the pathogen has been identified solely based on morphological characteristics for species referred to as *C. acutatum* or *C. gloeosporioides*. It is well known that many species within a species complex show overlapping morphological characteristics which leads to incorrect species identification. *C. capsici* is synonymous with *C. truncatum* which gives an indication that the species tested in the study is actually *C. truncatum*.

In a study by Shahbazi et al. (2014), a Streptomyces strain P42 isolated from soil inhibited the growth of three anthracnose-causing Colletotrichum spp. referred to as C. acutatum, C. capsici and C. gloeosporioides. El-Mabrok et al. (2012) reported chilli seeds treated with Lactobacillus plantarum cells or supernatant resisted infection by C. gloeosporioides as shown by a good percentage of germination. In another study by Fakri et al. (2018), seven strains of Lactococcus lactis subsp. lactis isolated from soil from a rice field exhibited antifungal activities against C. capsici with minimum inhibition concentrations at 10% (v/v).

Edible coatings have also been applied to control postharvest anthracnose in chilli. Ethanolic extract of propolis tested by Ali et al. (2015b) inhibited the mycelial growth of *C. capsici* (syn. *C. truncatum*) and delayed development of anthracnose for 28 days on bell peppers stored at 10°C and 90% relative humidity. The application of the propolis extract as a coating both reduced incidence of anthracnose and retained moisture in bell peppers.

Another edible coating with the potential to control postharvest anthracnose in chilli is a combination of lemongrass essential oil with chitosan. *In vitro* and *in vivo* studies showed that the combination enhanced the antimicrobial activity of the coating to control postharvest anthracnose in bell pepper. Incorporation of 0.5% lemongrass essential oil and 1.0% chitosan maintained the fruit quality of green bell pepper after 21 days at room temperature. Chitosan alone can extend bell pepper shelf life (Ali *et al.*, 2015c).

## Cocoa (Theobroma cacao L.)

After palm oil and rubber, cocoa is another major industrial crop in Malaysia. Due to a decline in production in the 1980s and the expansion of the area planted with oil palm, Malaysia began to import cocoa beans from Ghana, Ivory Coast, Ecuador and Papua New Guinea (Khazanah Research Institute, 2018).

In Malaysia, leaf spot and pod rot of cocoa, caused by *C. gloeosporioides sensu lato*, were first reported by Lin and Liew (1975) during the development of the cocoa industry. The symptoms observed were severe leaf blight and rotting of cherelles and immature pods. At that time, the identity of the pathogen was confirmed by the International Mycological Institute. Nair (2010) listed several *Colletotrichum* spp. associated with cocoa pod rot, namely, *C. gloeosporioides* (Penz.) Sacc., *C. theobromae*, *C. lurcificum*, *C. eradwickii*, *C. incarnatum*, *C. fructitheobromae* and *C. thobromicolum*.

Leaf spot and pod rot of cocoa are not regarded as serious diseases of cocoa plants; therefore, few studies have been conducted on these diseases and the causal pathogens. The major cocoa diseases are black pod, caused by *Phytophthora* spp., and witches' broom disease, caused by *Moniliophthora perniciosa*.

## Tomato (Solanum lycopersicum)

Tomato is one of the vegetable crops listed in Malaysia as a National Key Economic Area for development. Tomato is planted in the highland areas in the Cameron Highlands, Pahang and Kudasang, Sabah, as these areas provide a suitable environment for cultivating vegetable crops. Vegetable crops are mainly infected by wilt and root rot caused by both bacterial and fungal pathogens. Rashid *et al.* (2015) reported leaf anthracnose in Cameron Highlands, and the pathogen was identified as *C. boninense*. It is the only report of *Colletotrichum* spp. associated with tomato in Malaysia.

## OTHER CROPS INFECTED BY Colletotrichum spp.

Rose apple or wax apple (*Syzygium samarangense*) is planted by smallholders for local consumption. Fruit rot has been observed on wax apple in many farms in the state of Johor, Malaysia, with the causal pathogen being *C. gloeosporioides* identified using ITS region and the pectate lyase gene (Al-Obaidi *et al.*, 2017).

Anthracnose in bok choy (*Brassica rapa* subsp. *chinensis* L.) also has been observed in nursery fields in the Cameron Highlands. Based on ITS sequencing, the anthracnose pathogen was identified as *C. capsici* (syn. *C. truncatum*) (Mahmodi *et al.*, 2013).

Colletotrichum spp. associated with legume crops, namely, soybean, bean, pea, lima bean, lentil, chickpea, peanut, cowpea, winged bean and country bean, have been reported by Mahmodi *et al.* (2014), isolated from legume crops planted in various experimental and commercial farms in the states of Selangor and Pahang. Using ITS regions, ACT, β-tubulin, CHS-1, GAPDH and

histone genes, three species (*C. truncatum*, *C. dematium* and *C. gloeosporioides*) were found to be associated with rot in legume crops (Mahmodi *et al.*, 2014).

## **CONCLUSION AND FUTURE PROSPECTS**

As one of the most important genera of plant pathogenic fungi, more studies on *Colletotrichum* are required in Malaysia, particularly those associated with agricultural crops. *Colletotrichum* spp. not only cause anthracnose but other plant diseases as well, including leaf spot and pod rot. Cross infection studies are also needed since many species of *Colletotrichum* have a broad host range and because the potential for cross infection among crops is high.

Many Colletotrichum species associated with plant diseases in Malaysia belong to the *C. gloeosporioides* and *C. acutatum* complexes. In these complexes, the individual species are closely related, and most are cryptic species having similar modes of infection and colonisation (Damm *et al.*, 2012a; Jayawardena *et al.*, 2016). Thus, *Colletotrichum* species that were identified solely based on morphological identification in older publications should be regarded as sensu lato (in the broad sense). Moreover, species referred to as *C. gloeosporioides* are now known to occur much less often in the environment than previously reported (Phoulivong *et al.*, 2012; Weir *et al.*, 2012).

Accurate identification of *Colletotrichum* spp. associated with plant diseases is important for plant pathological and plant quarantine purposes (Jayawardena *et al.*, 2016). Proper identification of the causal pathogen and associated diseases is required to formulate effective disease management programmes. For plant quarantine to be effective, it is vital that the distribution and exact name of a particular species are known to avoid potential threat of pathogen introductions to any country (Hyde *et al.*, 2010).

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