Faculty of Resource Science and Technology

Identification of Fungal Isolates Associated with Anthracnose Disease on Chilli

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Identification of Fungal Isolates Associated with Anthracnose Disease on Chilli

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A thesis submitted in partial fulfilment of the Requirement of The Degree Bachelor of Science with Honours

(Plant Resource Science and Management)

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Programme of Plant Resource Science and Management

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Identification of Fungal Isolates Associated with Anthracnose Disease on Chilli

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ABSTRACT

Chilli fruit is an important plant crop that can be easily infected by anthracnose disease, that caused severe yield losses at the post-harvest stage to chilli producers and farmers. *Colletotrichum* species infection is the most common disease concern in chilli production, leading to anthracnose disease. This study aims to identify the *Colletotrichum* species associated with chilli anthracnose from infected fruits collected from several markets in Kota Samarahan, Sarawak. Pathogenicity test was conducted for the fungal isolates to evaluate their pathogenic level. Based on the results that have been obtained, three fungal isolates were successfully obtained and morphologically identified as *Colletotrichum* sp. The three isolates were identified as *Colletotrichum* sp. 1, *Colletotrichum* sp. 2 and *Colletotrichum* sp. 3. Each isolate was labelled as C11R2, C12R3 and C1R1-1 respectively. From these, *Colletotrichum* sp. 2 had the highest level of pathogenicity among the *Colletotrichum* spp. with disease severity percentage of 44%. Meanwhile, the percentage of disease severity of *Colletotrichum* sp. 1 ranged from 22% to 29%, and the ranged of disease severity percentage for *Colletotrichum* sp. 3 ranged from 12% to 19%. Identification of fungal isolates and their pathogenic level from the infected chilli is essential in disease control and management, correlated to selection of suitable resistant cultivars and fungicides.

Keywords: Anthracnose, chilli, *Colletotrichum* sp.

ABSTRAK

Buah cili ialah tanaman tumbuhan yang mudah dijangkiti oleh penyakit antraknosa yang menyebabkan kehilangan hasil yang serius pada peringkat lepas-tuai kepada pengeluar cili dan petani. Jangkitan oleh spesis Colletotrichum yang menyebabkan penyakit antraknosa adalah kebimbangan paling utama dalam pengeluaran cili. Kajian ini bertujuan untuk mengenalpasti spesis Colletotrichum yang berkaitan dengan antraknosa cili daripada cili berjangkit yang telah dikumpul daripada beberapa pasar di Kota Samarahan, Sarawak. Ujian sifat patogenik dijalankan pada asingan kulat untuk menilai sifat patogenik mereka. Berdasarkan keputusan yang telah diperolehi, tiga asingan kulat berjaya diperolehi dan dikenalpasti secara morfologi sebagai Colletotrichum sp. Ketiga-tiga asingan dikenalpasti sebagai Colletotrichum sp. 1, Colletotrichum sp. 2 dan Colletotrichum sp. 3. Setiap asingan dilabel sebagai C11R2, C12R3 and C1R1-1 mengikut urutan. Dari sini, Colletotrichum sp. 2 mempunyai sifat patogenik yang tertinggi di antara Colletotrichum spp. dengan peratusan keterukan penyakit ialah 44%. Manakala, peratusan keterukan penyakit untuk Colletotrichum sp. 1 adalah dalam lingkungan 22% ke 29%, dan lingkungan peratusan keterukan penyakit untuk Colletotrichum sp. 3 pula adalah dalam 12% ke 19%. Pengenalpastian asingan kulat dan tahap patogenik daripada cili berjangkit adalah penting bagi pengawalan dan pengurusan penyakit yang berkait dengan pemilihan kultivar dan racun kulat yang sesuai.

Kata kunci: Antraknosa, cili, Colletotrichum sp.

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CHAPTER 1: INTRODUCTION

Chilli has become one of the economically important vegetables worldwide. The worldwide production of chilli in 2016 was 3.92 million tons for dry chilli and 34.5 million tons for fresh chilli (Ridzuan *et al.*, 2018). It is one of the main commercial spice crops and part of a vital ingredient in different cuisines around the globe as it integrates taste, flavour, colour, and pungency taste to the dishes (Saini *et al*., 2017).

Capsicum annuum is globally the most cultivated chilli among these five species, followed by *C. frutescens* (Ridzuan *et al.*, 2018). There are five species of chilli (*Capsicum baccatum, C. pubescens, C. chinense, C. frutescens,* and *C. annuum*) are generally cultivated and domesticated in different tropical and subtropical regions around the globe.

The quality of chilli is influenced by biotic factors, such as bacteria, viruses, fungi, and other pests, or abiotic factors, such as light, humidity, light, and temperature. However, chilli anthracnose has become one of the most devastating fungal diseases which disrupts the quality, production and marketability of chilli at post-harvest stage (Diao *et al.*, 2017). The damage caused by the chilli anthracnose has resulted in significant yield loss towards chilli production. Chilli anthracnose has been discovered in many plant crops, and the symptoms of the fungal diseases can be found on fruits, flowers, leaves, and stems (Ridzuan *et al.*, 2018). The disease is caused by fungi, known as *Colletotrichum* spp., which are capable of infecting more than 30 plant genera (Farr and Rossman, 2016).

More than 10 *Colletotrichum* spp. have been discovered infected chilli, with different distributions and locations among countries (Diao *et al*., 2015). For example, anthracnose on chilli in China is caused by *C. fioriniae, C. scovillei, C. endophytica, C. karstii,* and *C. viniferum* (Noor and Zakaria, 2018). Anthracnose on chilli in Indonesia is caused by *C. makassarense, C. tropicale,* and *C. siamense,* while anthracnose on chilli in Thailand is caused by *C. scovillei, C. fructicola, C. siamense,* and *C. plurivorum* (De Silva *et al*., 2019). In Malaysia, the notable *Colletotrichum* spp. causing chilli anthracnose on chili fruits are, *C. acutatum*, *C. capsici* and *C. gloeosporioides* (Yun *et al.*, 2009).

All of the *Colletotrichum* spp. can cause anthracnose symptoms on chilli fruit, but there are some differences in the severity and appearances of the symptoms (De Silva *et al.*, 2019). This makes identifying the fungi causing the disease important, which could contribute to the development of improved chilli cultivars and speed up the selection process. The proposed objectives for this study will be:

1. To identify the *Colletotrichum* species associated with chilli anthracnose from infected fruits
2. To evaluate the pathogenic level of *Colletotrichum* species on chilli fruits.

CHAPTER 2: LITERATURE REVIEW

2.1 Chilli anthracnose and its symptoms

Anthracnose, which means "coal" in Greek, is one of the most common fungal diseases that cause severe yield losses at the post-harvest stage. Chilli anthracnose is a fungal disease that has led to a chilli production loss of more than 50% in Malaysia, a 15% marketable yield loss in Korea, approximately 35% of chilli production in Indonesia, and 80% yield loss in Thailand (Ridzuan *et al.*, 2018). This disease also influenced chilli quality, which decreases their market value (Oo *et al*., 2017). The fungal infection caused by *Colletotrichum* spp. is one of the most damaging and devastating plant diseases in both pre-harvest and post-harvest stages in chilli-cultivating regions, especially in tropical Asia (Ridzuan *et al.*, 2018). The prevalence of chilli anthracnose depends on the environmental conditions with a high frequency of rainfall, relative humidity and temperature.

According to Ridzuan *et al.* (2018), the disease shows typical symptoms on chilli fruit such as dark spots, sunken lesions, or water-soaked necrotic tissues, with concentric rings of acervuli (Figure 1). The symptoms of anthracnose caused *Colletotrichum* spp. on chilli fruits are initially identified as a small dark spot before turning into a lesion (Liu *et al.*, 2016). After two weeks, the anthracnose disease become severe as the infected area of the chilli developed water-soaked lesions characterized by black acervuli on the chilli surface, creating dirty black or brown conidial masses (Rahman *et al.*, 2011). Besides lesions, chilli anthracnose also creates leaf spots, blight on seedlings, and stem dieback (Mistry *et al.,* 2010). On other occasions, the sunken lesions can be brown before turning black due to the formation of setae and sclerotia, especially when the infected parts spread quickly due to excessive irrigation or rain on young chilli pods (Hasyim *et al.*, 2014).

In addition, the lesions on the fruits of the chilli can reduce their fruit weight and the quantities of capsaicin and oleoresin (Hasyim *et al.*, 2014), and the lesions do not progress until the fruits become mature and complete their colour change from green to red. Other dominant factors contribute to the prevalence of anthracnose diseases, such as the quality of seeds, climate, and plant genetics.

Figure 1: Appearance of anthracnose symptoms on a chili fruit (Ridzuan *et al*., 2018)

2.2 *Colletotrichum* spp. and occurrences

Colletotrichum spp. of the genus *Colletotrichum*, which belong to the kingdom fungi is responsible for causing chilli anthracnose disease (Ridzuan *et al*., 2018). The *Colletotrichum* species are the most devastating plant fungi by infecting and damaging a wide range of crop plants. Several *Colletotrichum* species have been discovered around the globe as being the major cause of anthracnose in chilli (Table 1). One of the *Colletotrichum* spp., known as *C. truncatum* has been generally found in chilli (*C. annuum*), and the species has a broad range of host of more than 460 species of plant (Farr & Rossman, 2016).

Table 1: *Colletotrichum* spp. with the host, location, and sources

***Colletotrichum* species**	**Host**	**Location**	**Sources**
***C. acutatum* species complex**			
C. fioriniae	*Capsicum annuum*	China	Noor and Zakaria, 2018
C. scovillei	*Capsicum annuum*	China	Noor and Zakaria, 2018
C. scovillei	*Capsicum annuum*	Malaysia	Noor and Zakaria, 2018
C. scovillei	*Capsicum annuum*	Thailand	De Silva *et al*, 2019
C. cairnsense	*Capsicum annuum*	Australia	Noor and Zakaria, 2018
***C. truncantum* species complex**			
C. truncantum	*Capsicum annuum*	India	Noor and Zakaria, 2018
C. truncantum	*Capsicum annuum*	Malaysia	Noor and Zakaria, 2018
***C. gloesporioides* species complex**			
C. conoides	*Capsicum annuum*	China	De Silva *et al.*, 2019
C. endophytica	*Capsicum annuum*	China	Noor and Zakaria, 2018
C. fructicola	*Capsicum sp.*	China	Noor and Zakaria, 2018
C. fructicola	*Capsicum annuum*	Thailand	De Silva *et al.*, 2019
C. fructicola	*Capsicum annuum*	Taiwan	De Silva *et al.*, 2019
C. grossum	*Capsicum sp.*	China	Noor and Zakaria, 2018
C. karstii	*Capsicum sp.*	China	Noor and Zakaria, 2018
C. siamense	*Capsicum sp.*	China	Noor and Zakaria, 2018
C. siamense	*Capsicum annum*	Malaysia	Noor and Zakaria, 2018
C. siamense	*Capsicum annuum*	Indonesia	De Silva *et al.*, 2019
C. siamense	*Capsicum annuum*	Thailand	De Silva *et al.*, 2019
C. siamense	*Capsicum annuum*	Sri Lanka	De Silva *et al.*, 2019
C. viniferum	*Capsicum sp.*	China	Noor and Zakaria, 2018
C. makassarense	*Capsicum annuum*	Indonesia	De Silva *et al.*, 2019
C. tainanense	*Capsicum annuum*	Taiwan	De Silva *et al.*, 2019
C. tropicale	*Capsicum annuum*	Indonesia	De Silva *et al.*, 2019
C. viniferum	*Capsicum sp.*	China	De Silva *et al.*, 2019
C. acutatum species complex			
C. brisbanense	*Capsicum annuum*	Australia	De Silva *et al.*, 2019
C. cairnsense	*Capsicum annuum*	Australia	De Silva *et al.*, 2019
C. karsti	*Capsicum sp.*	China	De Silva *et al.*, 2019

C. orchidearum complex			
C. plurivorum	*Capsicum annuum*	Thailand	De Silva *et al.*, 2019
C. plurivorum	*Capsicum annuum*	China	De Silva *et al.*, 2019
C. plurivorum	*Capsicum annuum*	Malaysia	De Silva *et al.*, 2019
C. sojae	*Capsicum sp.*	China	De Silva *et al.*, 2019

The *C. truncatum* has cause destructive damage to the production of chilli in India, China, Thailand, Australia, and other countries (Diao *et al*., 2015). *Colletotrichum acutatum* is also considered the most devastating, especially in the United States, as it caused chilli anthracnose to both ripe and unripe chilli fruit, while *C. gloesporioides* only infect ripe chilli fruit (Ridzuan *et al*., 2018). *Colletotrichum truncatum* has been reported as the most common occurrence among the *Colletotrichum* species causing chilli anthracnose in Australia, Indonesia, South Korea, Myanmar, Papua New Guinea, New Zealand, Taiwan, Thailand, United Kingdom, Vietnam, and United States (Garg *et al.*, 2014). Besides, *C. acutatum* is also discovered as the most critical among the anthracnose pathogens (Hasyim *et al.*, 2014). In Malaysia, *C. truncatum* and *C. scovillei* have the most occurrences in Malaysia (Noor and Zakaria, 2018). In contrast, *C. acutatum* and *C. cocodes* have very low occurrences in chilli fruits in Malaysia (Ridzuan *et al*., 2018).

Colletotrichum acutatum is one of the most infectious *Colletotrichum* spp., infecting ripe and unripe chilli fruit (Figure 2), followed by *C. gloeosporioides* and *C. truncatum* (Ridzuan *et al*., 2018). These three species are common pathogens of chilli anthracnose in the Southeast Asian region (Cueva *et al.*, 2018). *Colletotrichum gloeosporioides* infect unripe chilli while *C. truncatum* commonly attack ripe chilli (Ridzuan *et al*., 2018). The study of *Colletotrichum* spp. performed by Diao *et al.* (2017) on chilli in China, showed that the major species of *Colletotrichum* causing anthracnose on chilli are *C. fioriniae*, *C. fructicola*, *C. gloeosporioides*, *C. scovillei*, and *C.*

truncatum. Meanwhile in Malaysia, the major species of *Colletotrichum* associated with anthracnose of the chili fruits are *C. truncatum, C. fructicola, C. scovillei, C. fioriniae,* and *C. siamense* (Noor and Zakaria, 2018).

Figure 2: *Colletotrichum acutatum* infecting both green and red chilli fruits (Ridzuan *et al*., 2018)

2.3 Identification of *Colletotrichum* through morphological characteristics

The diversity of genus *Colletotrichum* make identification of *Colletotrichum* spp. as important through their morphological characteristics. According to Montri *et al*. (2009), morphological characteristics such as the colour of the colony, and the appearance of lesions are important factors in differentiating the *Colletotrichum* spp. For example, the colour of colonies produced by *C. gloeosporioides* ranges from pale grey to black, while *C. acutatum* is identified with their orange-coloured colonies with slight mycelium (Ridzuan *et al*., 2018). Another morphological characteristic that can be identified is the conidia shape of *Colletotrichum* spp.

through a light microscope. For example, the shape of conidia of *C. scovillei* is cylindrical to clavate with one end round and one end acute. In contrast, the shape of conidia of *C. acutatum* is cylindrical to fusiform, with both ends acute (Oo *et al.*, 2017). The major pathogen of anthracnose that is *C. truncatum*, has a falcate conidia and white to grey coloured colonies with a dark green center (Ridzuan *et al*., 2018).

2.4 Pathogenicity level of *Colletotrichum* spp.

The shape and size of different chilli species vary considerably. Thus Shahbazi *et al.* (2014) standardise the pathogenicity level of different chilli species against *Colletotrichum* spp. with a disease severity scale. The scoring of the pathogenicity level is determined by measuring the size of the lesion as a proportion of the chilli fruit size (Hasyim *et al.*, 2014). According to Shahbazi *et al.* (2014), the development of disease severity scales for determining pathogenicity level that incorporated severity of infection would be suitable for helping in screening many isolates of *Colletotrichum* spp. The scales include lesion size, presence of acervuli and necrosis, or water-soaked lesion across different species of chilli (Oo *et al*., 2017). This approach would not only be efficient and accurate in measuring the severity of infection in a range of *Capsicum* spp., but this would also become useful in identifying *Colletotrichum* spp. based on qualitative analysis.

CHAPTER 3: MATERIALS AND METHOD

3.1 Collection and preparation of chilli samples

A total of 40 chilli samples of the same type with anthracnose symptoms was purchased from the markets in Kota Samarahan, Sarawak. Small sizes of the infected tissues were surface sterilised using 1% bleach for 3 minutes (Oo *et al.*, 2017). Next, the sterilised chillies were rinsed with sterilised distilled water and dried with sterilised tissue paper. Then, all the tissues were plated on the water agar. Finally, the plates were incubated under dark condition at 27^{o}C for 7 days. After incubation, the newly formed mycelia from the plates were transferred onto fresh PDA medium at 27^{o}C and followed by process of pure culture.

3.2 Morphological identification of fungal isolates

Morphological identification was conducted for chillies that show symptoms of chilli anthracnose. Morphological characteristics, including the conidia shape and color of colonies were observed under a light microscope with 10x and 100x magnification lens, and recorded every seven days. For this purpose, the colour of colonies and conidia shape were determined after two weeks (Oo *et al.*, 2017). Any morphological change on the chilli were recorded.

3.3 Pathogenicity level test

This method followed the procedures of Koch's postulates by inoculating healthy chillies with fungal isolated obtained from the infected chilli fruits (Segre, 2013). Healthy chillies were surface-sterilised with 1 % bleach for five minutes and wounded with a sterile scalpel at three different areas (De Silva *et al*., 2016). The healthy chillies were put in sterilised plastic containers. Three fungal isolates were chosen for the pathogenicity test. The inoculation with fungal isolates were performed by putting a small quantity of the isolate onto the wounded parts of the chillies. There were eight replicates for each isolate used in this method. Next, the plastic containers were sealed and maintained at high humidity in an incubator under dark condition with a temperature of 27 ºC (De Silva *et al.*, 2016). The appearance of symptoms on the chillies were monitored every three days for 15 days after inoculation. The disease severity scale (Table 2) of infected chilli fruits by *Colletotrichum* spp. is used to determine the pathogenicity level (Shahbazi *et al.*, 2014).

The calculation of disease severity is according to the equation:

$$\text{Disease severity} = \frac{Lesion\ length}{Fruit\ length} \times 100\%$$

Table 2: Disease severity scale (Shahbazi *et al.*, 2014)

Scale	**Disease description**
0	Fruit is healthy
1	10% of fruit surface is infected
2	25% of fruit surface is infected
3	50% of fruit surface is infected
4	75% of fruit surface is infected
5	100% of fruit surface is infected

CHAPTER 4: RESULTS

4.1 Collection and identification of isolates

Three isolate groups of *Colletotrichum* spp. were obtained from infected chilli fruits with anthracnose symptoms. Identification of the isolates was based on the morphological descriptions of *Colletotrichum* species outlined by Than *et al*. (2008). Morphological groups 1, 2 and 3 were identified as *Colletotrichum* sp. 1, *Colletotrichum* sp. 2, and *Colletotrichum* sp. 3, respectively.

4.2 Morphological examination

The differences in spore morphology and colony characteristics among the isolates resulted in morphological characteristics that correlated with the *Colletotrichum* species were identified (Table 3).

4.2.1 Characteristics of culture colony

Distinct colony characteristics on potato-dextrose agar dishes were identified after 14 days following subculturing. *Colletotrichum* sp. 1 produced greyish colonies with colorless ring in alternating concentric zones of orange at the centre (Figure 3(a)). Colonies produced by *Colletotrichum* sp. 2, varied from white to greyish with a few orange conidial masses around the centre (Figure 4(a)). Unlike *Colletotrichum* sp. 1, there is no colourless ring on the plate. Next, *Colletotrichum sp*. 3 produced pure black colonies with a colourless ring on the plate (Figure 5(a)).

4.2.2 Conidia shape

Oval-shaped conidia were identified in two out of three species of *Colletotrichum* (Table 3). *Colletotrichum* sp. 1 produced oval-shaped conidia (Figure 6(a)), and *Colletotrichum* sp. 2 isolates also produced mainly oval-shaped conidia (Figure 6(b)). However, the conidia of *Colletotrichum* sp. 3 were not identified but black setae were observed (Figure 6(c)).

4.3 Pathogenicity level test

Healthy chilli fruits inoculated with *Colletotrichum* sp. 1, *Colletotrichum* sp. 2 and *Colletotrichum* sp. 3 produced a small black spot after three days, before turning into a lesion in one week. The pathogenicity level of the three species of *Colletotrichum* were determined in Table 4. The scale of disease severity of *Colletotrichum* sp. 1, *Colletotrichum* sp. 2 and *Colletotrichum* sp. 3 were ranged from 1 to 2 because less than 50% of the chilli fruits' surface were infected.

Table 3: Summary of morphological characteristics for *Colletotrichum* species in groups 1, 2 and 3.

Morphological group	**Species**	**Characteristics of culture colony**	**Conidia shape**
1	*Colletotrichum* sp. 1	Greyish colonies with a colourless ring in alternating concentric zones of orange at the centre	Oval-shaped
2	*Colletotrichum* sp. 2	White to greyish colonies with a few orange conidial masses around the centre	Oval-shaped
3	*Colletotrichum* sp. 3	Pure black colonies with a colourless ring	Not visible

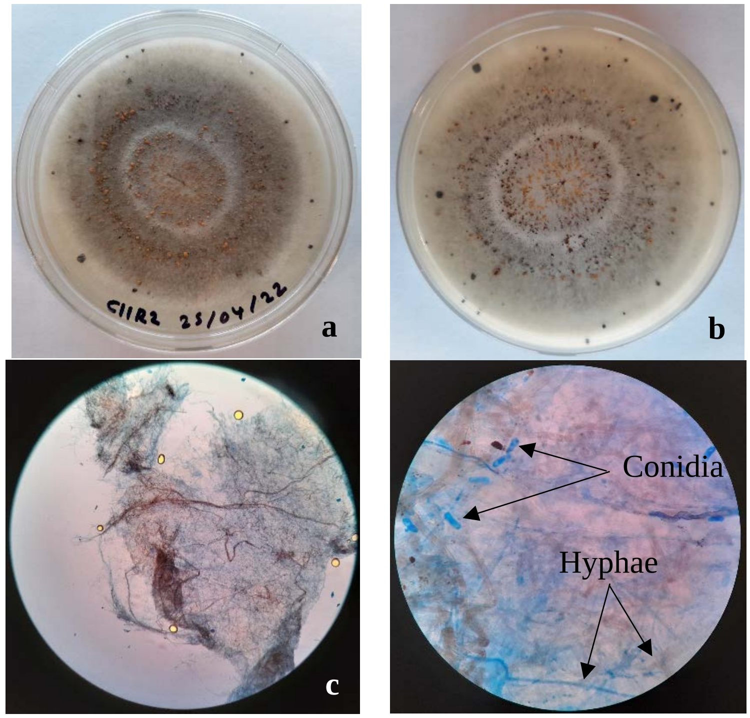

Figure 3: Top view(a) and bottom view(b) of *Colletotrichum* sp. 1. Fungal isolate under 10x magnification lens (c) and 100x magnification lens (d)

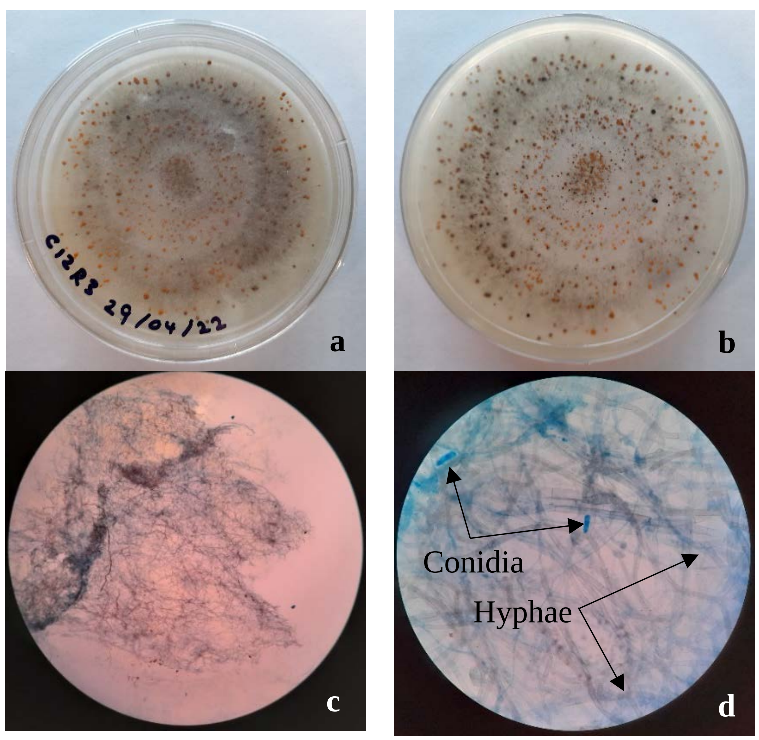

Figure 4: Top view(a) and bottom view(b) of *Colletotrichum* sp. 2. Fungal isolate under 10x magnification lens (c) and 100x magnification lens (d)

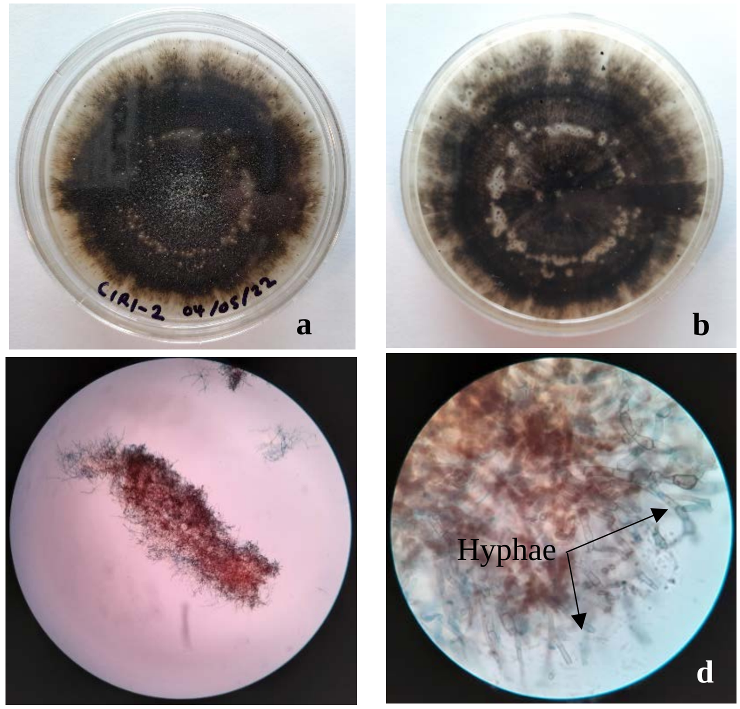

Figure 5: Top view(a) and bottom view(b) of *Colletotrichum* sp. 3. Fungal isolate under 10x magnification lens (c) and 100x magnification lens (d)