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Diversity of Airborne Fungi at Pepper Plantation Lembah Bidong, Kuala Terengganu

NUR AINU FARHAH RABAE, SALMAN AZIZ, ASAMOAH FREDERICK OSEI & SITI NORDAHLIAWATE MOHAMED SIDIQUE*

Laboratory for Pest, Disease and Microbial Biotechnology (LAPDiM), Faculty of Fisheries and Food Sciences, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia
*Corresponding author: dahliasidique@umt.edu.my
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ABSTRACT

Piper nigrum L. is well-known as the king of spices and widely used in various field such as food and medicines. In Malaysia, 98% of pepper production comes from the state of Sarawak. The National Commodity Policy (2011-2020) targets to increase the pepper plantation area from the current 16,331 ha to 20,110 ha by year 2020. However, pepper diseases remain as a major challenge in the pepper industry. A great number of airborne fungi pathogen may contribute to a significant economic loss in pepper production. Therefore, this study aims to morphologically identify the diversity of fungi obtained from air-borne samples in a pepper planation that are capable of causing pepper plant diseases. This experiment was conducted at a pepper plantation near Lembah Bidong, Kuala Terengganu. An Andersen spore sampler was used to collect the fungi spores. Culture based identification were then made. The study resulted in the identification of four genus of fungi such as *Fusarium* sp, *Fusarium semictectum Fusarium oxysporum*, *Curvularia* sp., *Penicillium* sp. and *Trichoderma* sp. (Ascomycetes). Further molecular identification will confirm the species of fungal pathogens and more understanding of their population as well as severity.

Keywords: Pepper, *Piper nigrum* L., air-borne, fungi, Andersen spore sampler

INTRODUCTION

In Malaysia, pepper plant is identified as one of the national commodities (Chen *et al.*, 2010). Malaysia is the fifth largest pepper producing country in the world with 98% of the country's annual production coming from the State of Sarawak (Adam *et al.*, 2018). Domestic pepper consumption increased from 12,000 tons in 2014 by 11% to 13,500 tons in year 2015 as reported by International Pepper Community (IPC) (2014). However, the production of black pepper started falling due to pest and disease occurrence since the early 1980s and it is the main problem faced by growers in Malaysia (Akinsanmi & Drenth, 2009).

Crop loss due to pests and diseases have resulted in a yearly reduction of about 2% of the total pepper area (Adam *et al.*, 2018). Several listed diseases of pepper plant such as anthracnose, *Phytophthora* foot rot, stem rot, fruit rot, mosaic viruses and and *Fusarium* wilt have been reported and are known to cause economic losses (Shahnazi *et al*., 2012; Farhana *et al*., 2013; Farith *et al*., 2015). In India, *Phytophthora* foot rot also known as quick wilt is recognized as one of the major causes of low productivity (Thomas, 2017). Additionally, the number of newly described *Phytophthora* species causing diseases in pepper plants have increased and *P. palmivora* has been identified as pathogen causing foot rot pepper vines in Malaysia (Brasier, 2008; Farhana *et al*., 2013; Farith *et al*., 2015; Habetewold *et.al*, 2017).

Some fungal pathogens such as *Fusarium*, *Penicillium* and *Aspergillus* which are known to cause stem rot, fruit rot, and wilt can be transferred by air-borne spores or survive in crop debris (Rivka, 2001; Shahnazi *et al*., 2012). Fungi of the genera *Cladosporium*, and *Penicillium* have the ability to produce a lot of spores that they can be found in virtually every cubic meter of air (Wyatt

et al., 2013).

Dispersal in air is one of many mechanisms by which plant pathogens can spread to new susceptible plants either within the same field or even in a completely different continent (Pady & Kapica, 2007; West & Kimber, 2015). Studies available on air-borne fungi pathogen sampling and identification in Malaysia and other Asian countries have mainly been carried out using dust collection methods (Cai *et al*., 2011; Norbäck *et al*., 2014), settle plate method (Shams-Ghahfarokhi *et al*., 2014) and the use of the single-stage viable cascade air sampler (SKC) (Er *et al*., 2015).

However, the single-stage thermo Scientific Andersen N6 Microbial Sampler used in this study has been reported to be very effective in trapping viable fungi pathogens in polluted air aerosol onto a 100 x 15 mm petri dish with agar because of the precision-drilled orifices in its impactor stage, its adjustable stage and the relatively higher flow rate of its pump (Gentry *et al.*, 2012). This study was thus set up to use the Andersen N6 microbial sampler to trap air-borne fungi spore in a pepper plantation near Lembah Bidong, Kuala Terengganu to determine the diversity of air-borne fungi that may cause diseases in the pepper plantation.

MATERIALS AND METHODS

Field Sampling

This study was conducted on 6th November 2019 from 3 pm to 6 pm at the only commercial pepper plantation in Lembah Bidong, Terengganu. The plantation follows a strict two weeks fungicide application scheduled hence sampling was carried out one week after fungicide application. A plot of 1.4 hectares was selected for the air-borne fungi sampling to be carried out. The zigzag method of point selection was chosen and in total of fifteen points were sampled (Figure 1). The blocks of pepper plants selected for sampling were 16 m apart and each block was 220 m long.

An Andersen N6 Microbial Sampler (Andersen Instruments Inc., USA) was used for the fungi spore sampling. The single stage was adjusted to a height of 1.5 m and potato dextrose agar (PDA) was exposed on the metal stage. Three sampling replicates were collected at each point and the pump of the sampler was turned on for three minutes.

Isolation of Fungi

After the air sampling, all the agar plates were incubated at room temperature for 2 to 7 days (27±2 °C). Different morphology from the fungi colonies such as mycelia formation and pigmentation were isolated. Then spores suspension was prepared and adjusted to concentration of 10^6 by using hemocytometer spores counting. The pure cultures were obtained by growing the single colony of the fungi isolated from the spore suspension prepared. Only pure single colony of fungi were selected for identification (Siti Nordahliawate *et al.*, 2012).

Identification of Fungi

After 7 to 10 days of incubation, morphological characteristics such as pigmentation and colony formation as well as microscopic characteristics such as conidia spores were observed under the microscope (Klich, 2002; Leslie & Summerell, 2006; Ellis, 1971). For the microscopic identification, slides were prepared and some small pieces of the pure cultures were cut as well and, observed at 100 x 10 magnification using Olympus CX22 (Olympus Corp., Japan) compound microscope.

Diversity of fungi

Colony-forming units (CFU) from the pure cultures were counted after which fungi diversity was determined. Fungi species diversity was calculated using the Shannon-Weiner Index as shown in Eq. (1) (Spellerberg, 2008).

$$H' = -\sum_{i=1}^{s} Pi \ln Pi\binom{n}{k} \quad (1)$$

Where: ∑ refers to "the sum of" there are s species in the community. H' is the value of Shannon-Weiner Index. *Pi* is the relative abundance (proportion) of the i species in the community and ln is the natural log.

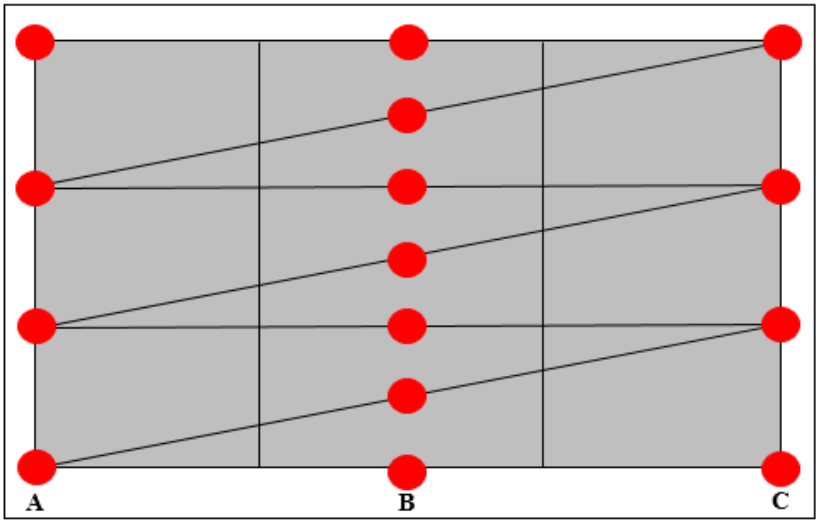

Figure 1. The dots show the zig-zag sampling points where the Anderson spore sampler was located for air sampling in a 1.4 hectares of pepper plantation

RESULTS

Fungi Identification

The results showed that a total of four fungi genus of the Ascomycota phylum were identified morphologically. They were *Fusarium* sp. *Curvularia* sp. *Penicillium* sp. and *Trichoderma* sp. All isolates were easily distinguished by the pigmentation and growth of the pure cultures through visual observation (Figure 2). Isolates identity were confirmed after microscopic observation especially shapes of conidia and other criteria such as conidiophore, phialides and chlamydospore (Figure 3).

We observed that the *Fusarium* sp. rapidly grow on PDA medium and produced robust woolly mycelium than others (Figure 2a). Microconidia was more common on hyphae growing on the agar. The mode of formation of microconidia, i.e. monophialides or polyphialides as well as the presence of microconidia chains and chlamydospores were observed for *Fusarium* morphology characteristics identification (Leslie and Summerell, 2006) (Figure 3a). Most of the macroconidia showed a distinct basal foot cell and whereas the microconidia were formed on simple chain with or without branches (Figure 3a). However, some species were observed showing some differences of macroconidia shapes such as *F. oxysporum* showed short with a thin walled and *F. semitectum* showed slender with a curved dorsal surface. *Fusarium* species are known to cause *Fusarium* wilt disease of black pepper and in China, it had caused a major decline in pepper production (Xiong *et al.*, 2015). In addition, Shahnazi *et al.* (2012) had reported that *Fusarium* species also giving yellowing disease that impact in economic losses at pepper plantations in Malaysia.

The genus *Curvularia* consists of more than 40 species and its taxonomy changed many times to accommodate species formerly classified as *Bipolaris* spp. (Zhang *et al.*, 2004; Kusai *et al.*, 2015). The cottony mycelial produced a border shape (regular or irregular) with pigmentation of colony ranges from black, moss green and sometime grey (Figure 2b). Conidia are ellipsoidal (Zhang *et al.*, 2004) (Figure 3b) that could easily be distinguished from the other fungi species such as *Fusarium* sp., *Penicillium* sp. and *Trichoderma* sp. The *Curvularia* species are well known to cause leaf spot and leaf blight symptoms in maize, rice, beans and cowpea (Liu *et al*., 2010; Al-Jaradi *et al*., 2018). Although, little is known about it effects on black pepper nevertheless, there is a potential when conditions are favourable for foliar diseases to develop.

Both *Trichoderma* sp. and *Penicillium* sp. were easily distinguished morphologically from their pigmentation and formation on the PDA. The pure culture visually classified as *Trichoderma* sp.

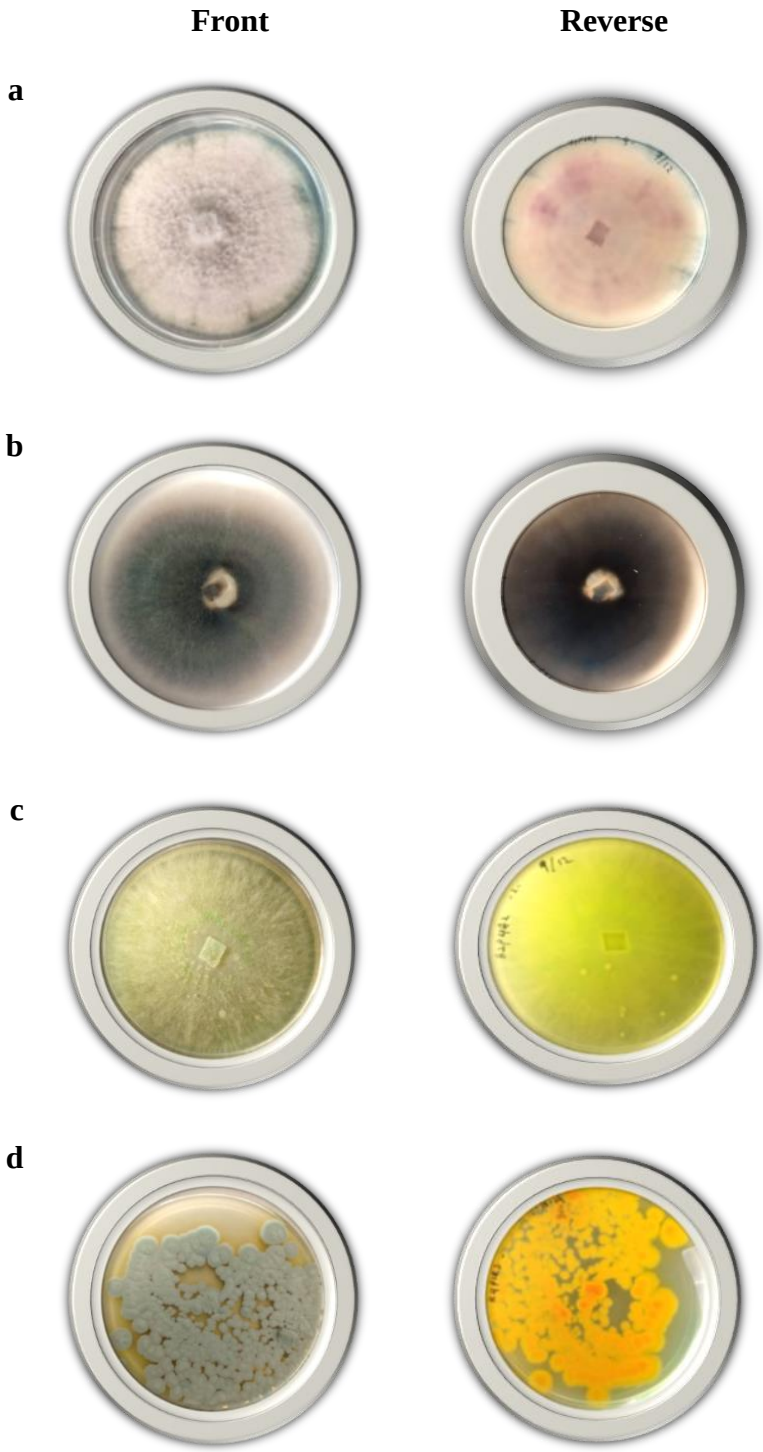

Figure 2. The variation of pigmentation and morphology of the fungal colonies on Potato Dextrose Agar (PDA) medium a) *Fusarium* sp., b) *Curvularia* sp., c) *Trichoderma* sp. and d) *Penicillium* sp.

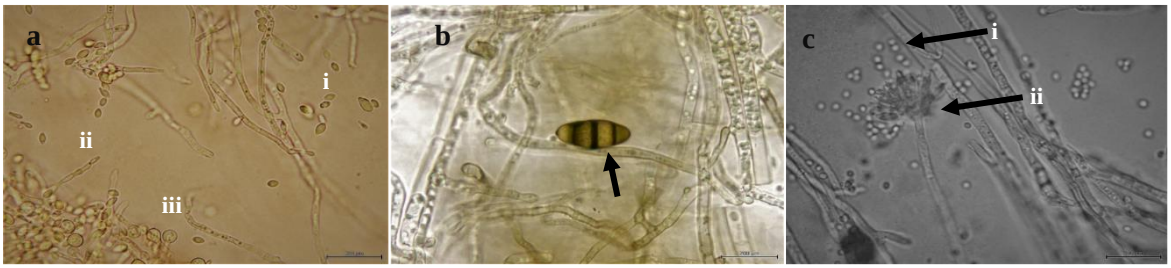

Figure 3. Microscopic characteristics of different fungi under 100 x 10 magnification. a. *Fusarium* sp. i: microconidia, ii: microconidia chain, iii: chlamydospore b. *Curvularia* sp. conidia (arrow) and c. *Penicillium* sp. i: conidia, ii: conidiophores

showed yellowish-light green pigmentation while the pure culture classified as *Penicillium* showed green pigmentation with white zone on PDA (Figure 2). For *Penicillium*, the colonies were rapidly growing, filamentous and cottony in texture (Figure 2d) that produced septate hyaline hyphae, branched conidiophores, phialides, and conidia (Figure 3c). *Penicillium* sp. is well known and also one of the most common fungi appearing in a diverse range of habitats, from soil to vegetation to air, indoor environments and various food product (Frisvad *et al.*, 2004). All the fungi identified in this study are well known plant pathogens whereas *Fusarium* and *Penicillium* are known to produce mycotoxins (Agrios, 2005; Perrone & Susca, 2017; Ji *et al.*, 2019). *Trichoderma* sp. may cause diseases in other plants but have also been reported to have the ability to reduce the foot rot pathogen *Phytophthora capsici* in pepper plants (Rajan *et al*., 2002).

Diversity of Fungi

The CFU was used to calculate the diversity of the six species that based on the Shannon-Weiner Index. Results showed that *Fusarium* sp. was the greatest (H' = 0.44) compared to other fungi species (Table 1). Thus, the black pepper plantation area is expected to be infected with *Fusarium* species when spores are abundance to invade the plant. Several factors may cause the spores abundance such as favourable conditions (weather and humidity) and the susceptible host (Agrios, 2005; Lacey & West, 2006).

Table 1. Diversity of fungi species at pepper plantation area isolated from air sampling

No.	Species (*i*)	Number in sample (CFU)	Species diversity (*H'*)
1	*Fusarium* sp.	2,071	0.44
2	*Fusarium oxysporum*	102	0.14
3	*Fusarium semitectum*	7	0.02
4	*Curvularia* sp.	3	0.00
5	*Trichoderma* sp.	106	0.14
6	*Penicillium* sp.	85	0.12

CONCLUSION

This study proved that *Fusarium* sp. was the dominant fungi species identified compared to other fungi pathogens at the black pepper Lembah Bidong, Terengganu. Several Fusarium species may appear at one area such as in this study, three *Fusarium* species were identified with distinct morphological characteristics (*Fusarium oxysporum* and *F. semitectum*). However, there is limitation in morphological identification when most of the *Fusarium* species produced similar banana-shaped macroconidia (Leslie & Summerell, 2006).

Although *Fusarium* species are well-known

soil-borne fungi, leaves infection will produce a massive microconidia and/or macroconidia. Consequently, could be dispersed throughout the area by air (Leslie & Summerell, 2006; West & Kimber, 2015; Lucas, 2020). All the species of *Fusarium* identified are known to cause diseases in pepper plants. *Fusarium oxysporium* causes *Fusarium* wilt in pepper plants and can cause great economic damage while *Fusarium semitectum* is reported to cause root rot. Some species of *Fusarium* are also known to cause leaf yellowing (Shahnazi *et al*., 2012).

The sampling date and season had favourable conditions for pathogen germination. This support the disease triangle concept which sates the importance of host, environment and pathogen for a disease to appear consequently resulting in disease epidemic (Agrios, 2005; Lucas, 2020). The mechanism of spores disperses such as tap and hail, will increase fungal pathogens infection at the field (Magyar *et al*, 2016). Therefore, by knowing the number of spores and species of fungi in the air will contribute to control measures instituted by the plantation. Moreover, it will help in decision making of the plantation especially in chemical control such as fungicides. A study by Siti Nordahliawate *et. al*, (2012) showed monitoring of air-borne spores and weather conditions can accurately predict when fungicide application maybe necessary.

At the field, air-borne spores contain several different fungi species that could be easily disseminated by wind blowing. Therefore, molecular approach could confirm the species when morphology identification shows a high degree of similarity and may cause misidentification. We believe that this study will benefit the pepper plantation to further monitor the air-borne fungi surrounding the field that may cause economically important diseases.

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