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Morphological and molecular characterization of *Fusarium* spp. associated with *Fusarium* wilt disease of *Piper nigrum* L. in Northwestern region of Sarawak

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ABSTRACT

Aims: *Piper nigrum* L. (black pepper) is an economically important commodity plant in Malaysia, which generated RM 200.95 million from pepper export in the year of 2018. However, the increase in pepper production is restricted by diseases. *Fusarium* wilt is one of the major diseases of *P. nigrum* L. The objectives for this study were to isolate *Fusarium* spp. associated with *Fusarium* wilt of *P. nigrum* L. from selected pepper farms in the northwestern region of Sarawak and to characterize the *Fusarium* spp. isolated morphologically and molecularly.

Methodology and results: *Fusarium* spp. were isolated from diseased root samples. The pathogen was grown on potato dextrose agar (PDA) under dark condition at circa (ca.) 25 °C for morphological characterisation. Molecular characterisation was done by using internal transcribed spacer (ITS). Phylogenetic tree was constructed to study the genetic relationship of the isolates. *Fusarium solani, F. oxysporum, F. proliferatum* were the three *Fusarium* species identified. There were variations in morphological characters observed between and among the species, including the colony form, margin, elevation, surface appearance and pigmentation. No distinctive morphological characteristic was specific to a location. In addition, growth rate, macroconidia sporulation rate, and microconidia sporulation rate of the isolates were not correlated. In molecular phylogeny, the three *Fusarium* species were separated into three distinct clades representing the three identified species. The genetic relatedness between isolates within each species was depicted in the tree.

Conclusion, significance and impact of study: Variations were observed among isolates in this study based on morphological and molecular characterization. This study would contribute information on the variations of *Fusarium* spp. associated with *Fusarium* wilt of *P. nigrum* L. from the northwestern region of Sarawak.

Keywords: Fusarium, Fusarium wilt, internal transcribed spacer (ITS), Piper nigrum L., black pepper

INTRODUCTION

Black pepper (*Piper nigrum* L.) is a perennial woody vine which produces berries that are commonly known as peppercorns. As this spice is being widely used in traditional medicine and culinary purposes, *P. nigrum* L. is the most economically important species among the *Piperaceae* family. The plants are commercially cultivated in countries such as Malaysia, Brazil, Vietnam, India and Indonesia (Tiing *et al.*, 2012). According to Malaysian Pepper Board (2018), the pepper production of the nation reached 31,073 tonnes with the total export of 11,714 tonnes that worth RM 200.95 million in 2018. Among the total coverage area of 17,437 hectares of pepper plantation, 98% of the cultivation is located in Sarawak and operated intensively by the local smallholders with an average 2000 vines per hectare (Adama *et al.*, 2018).

Crop loss due to diseases is one of the primary constraints in increasing the yield of pepper production in Malaysia which results in diminishing of 2% of the pepper cultivation area annually (Krishnamurthy *et al.*, 2010). One of the major diseases is *Fusarium* wilt, which is also known as slow wilt or yellowing disease (Gordo *et al.*, 2012; Tiing *et al.*, 2012). The disease may cause as high as 32% of annual loss in Indonesia (Mustika, 1990). Duarte and Albuquerque (1991) also reported that the pepper plantation economic lifespan will drop from 20 years to 6-8 years due to *Fusarium* infestation.

The most typical symptom of the disease is the brown to black lesion in the xylem tissue of the plant root (Eng, 2011). The infected plant will start yellowing and drooping of leaves occurs in whole canopy, then the plant appeared to be flaccid, foliar and foot rot due to the malfunction of the roots (Eng, 2011; da Luz *et al.*, 2017). Later, the

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