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CHARACTERIZATION OF ENDOPHYTIC FUNGI FROM *Garcinia atroviridis* WITH ANTAGONISTIC EFFECT AGAINST C*olletotrichum gloeosporioides*

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ABSTRACT

Fungal endophytes produce a broad variety of bioactive compounds with potential to address some of the unmet human needs. Medicinal plants have an important role to play in the search for new strains of endophytes fungi, as it is possible that their beneficial characteristics are as a result of the metabolites produced by their endophytic community. However, inspite of this potential as repositories of bioactive compounds, the fungal endophytes of Malaysian medicinal plants remain largely underexplored. This thesis reports on studies that were conducted to bioprospect for endophytic fungi with antagonistic activity against *Collectrichum gloeosporioides* hosted by the plants *Garcinia atroviridis* used in Malaysian traditional medicine. The endophytic fungus isolates were isolated from different parts of the host plant (fruits, leaves and branches), where the samples were collected from two different geographical location in Nasuha Herbs Farm, Muar. A total of 243 endophytic fungi were isolated from 600 *G. atrovidis* segments. Overall, fungal isolation rate in *G. atrovidis* segments was 0.41 and colonization rate was 71.5% and the highest being found in the higher location. The colonization rate was higher in the leaf (50.2%) than the branch (33.3%) and fruit (16.5%). The fungal richness was also higher in leaf as compared to in other parts. Morphological analysis grouped these isolates into 21 distinct groups. Further, ITS gene sequencing analysis identified these isolates as *Annulohypoxylon* sp, *Colletotrichum gloeosporioides*, *Pestalotiopsis* sp., *Pestalotiopsis* sp., *Diaporthe* sp., *Lasiodiplodi theobromae*, *Pestalotiopsis neglecta* , *Bjerkandera adusta*, *Nigrospora sphaerica* and *Colletotrichum sp.* The effect of different cultivation medium on fungal growth revealed that the growth requirements of each endophyte fungi isolate significantly ($p<0.05$) differs from each other. Potato Dextrose Agar (PDA) exhibited the most suitable medium to growth endophyte fungus with highest radial growth compared to Malt Extract Agar (MAE) and Water Extract Agar (WA). Spearman-rho analysis revealed a significant ($p<0.05$) correlation between type of medium and growth rate among endophyte isolates. Among all the endophytes tested, 12 isolates showed strong inhibition towards *C. gloeosporioides*, with the highest inhibition percentage possessed *N. sphaerica* (87.94%). The *in vitro* antagonistic activities as two types of activities in this study; mycoparasitism and competition were found. Our findings suggest that endophytes *Annulohypoxylon* sp., *Pestalotiopsis* sp., *Lasiodiplodia theobromae*, *Bjerkandera adusta*, *Nigrospora sphaerica* and *Diaporthe sp.* are promising candidates for their use in biological control due to their antagonistic activity against the mycelia growth of anthracnose diseases-associated fungi.

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CHAPTER ONE
INTRODUCTION

1.1 Research background

Plant disease, caused by pathogenic microorganisms such as fungi, bacteria and virus have often posed a serious threat to the agricultural sector as it extremely affects the quality and quantity of crop yields. Colletotrichum is Ascomycota fungi genus that are symbionts to plants as endophyte fungus or phytopathogen. This genus represents many successful phytopathogens which collectively cause anthracnose disease on a wide range of economically important plants in subtropical and tropical countries (de Silva *et al.*, 2019; Dowling *et al.*, 2020). Anthracnose, the most prominent postharvest disease in tropical fruits and latent infections are typically in growing fruits before harvested (Gutiérrez-Martínez *et al.*, 2016). One of the most pathogenic species of this genus is *Colletotrichum gloeosporioides (C. gloeosporioides)*, which responsible for low yield and poor quality of cash crops such as mango, banana, papaya, dragon fruits and grapefruits due to the disease (Cruz-Lagunas *et al.*, 2020; Zakaria, 2021).

Currently, chemical fungicides are the most effective method to manage plant diseases. However, the long-term affects of extensive fungicidal usage and its toxicity towards not only targeted pathogenic organisms, farmers and consumers, also to the environment, has raised public concerns. Recently, there has been a rising attempt to replace the synthetically produced fungicides with more eco-friendly compounds. Plants have always been viewed as bio-factories of potentially secondary metabolites. However, this approach seems infeasible due to plant slow growth rates and harvesting rare and endangered species pose a risk in biodiversity. Hence, it is warranted to search for new potential producers of novel bioactive compounds, especially endophyte microorganisms that are symbionts with various plant hosts.

Endophytic symbionts, often a bacterium or fungus, present asymptomatically in its plant host. These microorganisms may inhabit tissues of leaves, stems, roots, seeds, barks and flowers with different rate of colonization and species richness