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BIOLOGICAL CONTROL OF FUNGI ASSOCIATES IN SOURSOP (*Annona muricata*) LEAF SPOT DISEASE BY USING *Trichoderma harzianum*

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ABSTRACT

Soursop (*Annona muricata*) is known for its ethnomedicinal uses, leading to the increasing demand for the fruits. In Malaysia, leaf spot disease is one of the major constraints in the soursop plantation. If unattended, the possibility for the disease to cause huge economic losses in the soursop industry are highly possible. Thus, this study aimed to determine the morphological characteristic of the isolated fungi associated with leaf spot disease of soursop, to assess molecular identification of the isolated fungi by amplification of ITS regions, to analyse the antagonistic activity of *Trichoderma harzianum* against identified fungal isolates by using an application of dual culture test *in vitro*, to determine the severity of infection of identified pathogenic fungi on the soursop saplings *in vivo,* and to determine the capability of *T. harzianum* as plant growth-promoting fungi (PGPF) on soursop saplings *in vivo*. The leaves and soil samples from the infected soursop trees with leaf spot disease symptoms were collected from the farms located in Seri Menanti, Negeri Sembilan and Bukit Kurau, Perak, Malaysia. Cultivation was done on Potato Dextrose Agar (PDA) medium. The morphology of the fungal isolates was identified using fungal slide culture method Then, further identification for the fungal species was done molecularly by amplification of ITS region. Antagonistic activity of *T. harzianum* against fungal isolates was identified using dual culture method and its capability as plant growth – promoting fungus was done by application of *T. harzianum* suspension on soursop saplings. From the results, twenty-six morphologically distinct fungi isolates were identified and only 12 fungal isolates were suspected to be pathogenic. The analysis discovered *Fusarium oxysporum, Aspergillus sydowii,* and *Diaporthe phaseolorum* demonstrated high severity of infection with a range of 15.59 - 49.94% severity value where the highest was recorded by *F. oxysporum*. Meanwhile, other isolates displayed mild severity infection with a range of values of 0-7.97%. The growth rate of the infected soursop was affected negatively for most of the saplings, but some also demonstrated a high growth rate which might be due to their defence system response towards the infection. In the meantime, *T. harzianum* managed to exhibit antagonistic activity towards all those twelve isolated fungi with the highest inhibition values recorded at 64.3% for *A. sydowii* and 52.5% for *F. oxysporum*, however, it failed to suppress the growth of *D. phaseolorum in vitro*. Other than that, it was found that *T. harzianum* also managed to increase the soursop plant growth in terms of the height, texture, colour, and size of the leaves as well as its dry weight. The soursop saplings treated by *T. harzianum* appeared higher, sturdy, greenish in colour, has bigger and healthier leaves compared to the control saplings. In addition, the soil analysis was done giving a few hints regarding proper soil conditions required for soursop plantation which include the optimum temperature, pH, and nutrient content level. In conclusion, demonstrating various levels of effectiveness, *T. harzianum* was revealed to have the capability and potential as an excellent biological control agent against the possible pathogenic fungi associated with the leaf spot disease of the soursop plant. Thus, aside from soil nutrient awareness, the application of *T. harzianum* could be considered as an alternative method for soursop leaf spot disease control in agriculture practices. Despite the experimental work limitations, it is believed that the data presented in this study offer valuable insights and new information to the existing knowledge. Nevertheless, an extensive study should be done to discover further understanding on the potential fungal pathogens and endophytes that were identified in this study.

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CHAPTER ONE
INTRODUCTION

1.1 Research Background

Annona muricata, a member of the Annonaceae family, the custard apple family, known as "Graviola" or "soursop" is a small tropical tree widely cultivated throughout tropical countries. Soursop is categorized under underutilised fruit due to its limited and scattered information on market value, medicinal uses, and toxicology. It just recently, the fruit is highlighted by researchers particularly in Malaysia despites the study on their medical benefits and chemical composition has been reported more than a decade ago where soursop fruit and tree has been utilised as traditional medicine for human disease treatment such as fevers, asthma, pain, coughs, wounds, and skin remedies (Adewole & Ojewole, 2009; Moghadamtousi et al., 2015; Martin del Campo-Rayas et al., 2022). This plant was described as "the cancer killer" due to its remarkable cytotoxicity against various cancer cell lines. The seeds and leaves of *A. muricata* have been widely used by the natives in South America for the treatment of cancer and tumours (Moghadamtousi et al., 2014; Peña et al., 2022). In tropical Africa, including Nigeria, *A. muricata* is also generally used in folk medicine as an anticancer agent (Adewole & Ojewole, 2009). The stem, barks, and leaves of *A. muricata* revealed noteworthy antiproliferative effects against cancer cells (Mishra et al., 2013; Asare et al., 2015). Apart from the medicinal benefits, the soursop also been utilized in food and beverages, making them in high demand (Forero & Waliszewski, 2022).

Like all plant trees, soursop is also susceptible to pests and diseases. Many cases of soursop plant disease had been reported around the world. Morton, 1966 reported that in Mexico, *Colletotrichium gloeosporoides* and *Rhizopus stolonifer* were among the early fungi that were identified associated with the pre-harvesting deterioration of soursop fruit in Colombian plantations. Escobar and Sanchez (1993) pointed out that *Phytophthora* species and *Rhizoctonia* species were among the fungal pathogens that attack the roots of *A. muricata* causing root rot diseases. In another research by Morton (2000), four fungal isolates: *Botryodiplodia theobromae, Fusarium spp, Rhizopus stolonifer and Aspergillus niger* were associated with the pre-harvest deterioration of soursop. The pre-harvest rot of immature fruits was associated with *Lasiodiplodia*