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ANTIFUNGAL ACTIVITY OF *Allamanda cathartica* STEM AND LEAF CRUDE EXTRACTS AGAINST *Pyricularia oryzae* PATHOGEN OF RICE BLAST DISEASE

KHAIRUN NUR BINTI ALI

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ABSTRACT

This study presents the potential use of natural product from *Allamanda cathartica* leaves and stems extract, as biofungicides and their antifungal activity against *Pyricularia oryzae* pathogen of rice blast disease *in vitro* and *in vivo*. The objectives of the study are to observe and evaluate the mechanisms of antifungal activity of *A. cathartica* leave and stem crude extracts against P. oryzae, pathogen of rice blast disease microscopically and to determine the phytochemical constituent in the *A. cathartica* leave and stem crude extracts that act as an antifungal activity agent. The results from the experiments revealed that chloroform and methanol stem extracts of *A. cathartica* possessed fungistatic or fungicidal properties on the growth of *P. oryzae*. However, chloroform stem crude extract of *Allamanda cathartica* shows greater inhibition zone (60% inhibition) against *P. oryzae* compared to methanol stem crude extracts (57.5% inhibition) with the radial growth of 1.6 cm and 1.7 cm respectively. The others crude extracts showed slightly antifungal activity against fungal pathogen. Furthermore, the chloroform and methanol stem crude extracts showed stronger inhibitory action than the positive control, Mancozeb® (41% Inhibition). The effective inhibitory concentration of active crude extract was at 7500 ppm. Microscopic observations of antifungal activity of active extracts resulted in hyphal degradation where hyphae were retarded and lysis. The screening of antifungal activity of fractions of active crude extract showed the good antifungal activity was at fraction 4 (70% hexane: 30% ethanol) with the radial growth inhibition 1.8 cm (55% inhibition). The effectiveness of active crude extract *in vivo* assays were conducted and found that the curative controls were more effective than preventive control. Antifungal effects of the most effective extracts were supported by the detection of major bioactive compound identified by GC-MS as hexadecanoic acid, nonadecane, pentadecane, tert-hexadecanethiol, heptacosane, and octadecanoic acid,

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TABLE OF CONTENTS

CHAPTER ONE
INTRODUCTION

1.1 Background

Rice is a major crop that has been planted in Malaysia for a long time besides oil palm and rubber. It is a staple food for many Asian countries. Rice is planted worldwide to support the human population in areas such Thailand, India and Malaysia. Rice is an economically important crop in Malaysia for profit and income generation for the country. Unfortunately, rice can be infected by various types of diseases mainly affected by the bacteria, viruses and fungi. Rice disease is a major constraint in rice production that can cause losses. The most common diseases in rice plantation are rice blast, bacteria leaf blight, sheath blight and stem rot. One of the important and significant diseases of rice is rice blast. Generally, rice blast can cause losses up to 90% of the rice production depending on the part of plant infected (Shahijahan, Sajad, Nabi Joo, and Masood, 2010). Rice blast mostly happen in all major rice developing areas of the world and accounts for devastating epidemics and a considerable decrease each year in rice production especially in Malaysia.

Rice blast is cause by Ascomycete fungus, *Magnaporthe grisea* Barr (anamorph *Pyricularia grisea* Sacc., synonym *Pyricularia oryzae* C a v.) which is the most destructive and aggressive fungus under favorable condition in rice world (Rossman, Howard and Valent, 1990). The attacks come both in terms of its distribution and the damage caused. Rice blast disease has long been recognized as the most destructive rice disease especially in Malaysia which contributed to major economic loss from their infection and cause the losses up to 15% (Yoon, Kim, Ryu, et al. 2011). Usually, the infection of rice blast disease occurs from soil borne conidia and the symptoms appear as lesion and spots (Guochang and Shuyuan, 2001). Rice blasts are the most serious disease that happens due the excessive amount of nitrogen fertilizers and less water per ton (Zeigler, Leong, and Teng, 1994). When the excessive amount of nitrogen in the field, it will encourage sporulation of spore of *P. oryzae* and increase blast intensity (Greer and Webster, 2001; Groth, 2006). Unfortunately, it is challenging to reduce rice blast disease since *P. oryzae* can persist in soil and remains for years.