UNIVERSITI PUTRA MALAYSIA

EFFECT OF ANTIMICROBIAL EXTRACTS OF *PIPER BETLE AND CLINACANTHUS NUTANS* ON *IN VITRO* GROWTH OF SELECTED FUNGAL PATHOGENS

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By

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A project report submitted to Faculty of Agriculture, Universiti Putra Malaysia in fulfilment of the requirement of PRT4999 (Final Year Project) for the award of the degree in Bachelor of Agricultural Science

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CERTIFICATION

This project report entitled '**Effect of Antimicrobial Extracts of *Piper betle* and *Clinacanthus nutans* on *in vitro* growth of selected fungal pathogens'** is prepared by Nur Maliya Bt Rahim and submitted to the Faculty of Agriculture in fulfilment of the requirement of PRT4999(Final Year Project) for the award of the degree in Bachelor of Agricultural Science.

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LIST OF ABBREVIATIONS

YHRC	:	Yellow Head Rhibdovirus
DPPH	:	2,2-diphenyl-1-picrylhydrazyl
NMR	:	Nuclear Magnetic Resonance
IR	:	Infrared spectroscopy
TBARS	:	Thiobarbituric acid reactive substances
MIC	:	Minimal Inhibitory Concentration
MFC	:	Minimal Fungicidal Concentration
cm	:	centimetres
mm	:	millimetre
%	:	Percentage
PIDG	:	Percentage of Inhibition Diameter Growth
ml	:	millilitres
L	:	Litre
>	:	more than
<	:	less than
°C	:	degree Celsius
rpm	:	rate per minute
h	:	Hour
g	:	Gram
kg	:	kilogram
:	:	ratio of

ABSTRACT

Plant pathogenic fungi have been identified as the causal agent of various types of plant diseases in agricultural crops in Malaysia. Due to this problem, many methods of control had been introduced. The most common method is by using chemical control. However, application of chemical fungicides causes a major destruction to the environment because of high synthetic toxicity elements. The use of *Piper betle* and *Clinacanthus nutans* extracts as biopesticides is a good application towards sustainable agriculture. In view of this, this research was conducted 1) to extract crude antimicrobial compounds from *P.betle* and *C. nutans* and 2) to investigate the effectiveness of different concentrations of crude antimicrobial extracts on the *in vitro* growth of selected plant fungal pathogens. The selected fungal pathogens were *Rhizoctonia solani, Ganoderma boninense, Fusarium oxysporum* f sp *cubense* and *Exserohilum rostratum.* The leaves were washed, air dried at room temperature and ground using a blender. The leaf powder were soaked in methanol and loaded in orbital shaker. The mixture was filtered and the filtrate was concentrated using rotary evaporator to obtain dried extract. There were four concentrations (5, 10, 15 and 20%) used and compared with control (0%). Poisoned agar plates with *P. betle* extract showed inhibition on the mycelial growth and number of spores. The percent inhibition of diameter growth (PIDG) of *F.o.c* was 94.04% at 12^{th} day, *G. boninense* 89.42% at 12^{th} day, *R. solani* 82.84% at 4^{th} day and *E. rostratum* 43.74% at 8^{th} day. The number of spores produced ($4x10^6$) in 20% by *F.o.c* in *P.betle* extract is 969 while for *R.solani* is 178. Poisoned agar plates with *C. nutans* extract showed PIDG of *E.rostratum* was 64.42% at 8^{th} day *R. solani* 58.86% at 4^{th} day, *G. boninense* 27.48% at 12^{th} day and *F.o.c* 10.36% at 12^{th} day. The

number of spore produced ($4x10^6$) in 20% by *F.o.c* in *C.nutans* extract is 1723 while for *R.solani* is 38. Thus, this showed that the extracts of *P. betle* and *C. nutans* have the potential to be used as bio-fungicides as a safe alternative to synthetic fungicides.

ABSTRAK

Kulat patogen tumbuhan telah dikenalpasti sebagai salah satu ajen yang menjadi penyebab kepada kepelbagaian penyakit bagi tumbuhan dalam sektor pertanian di Malaysia. Disebabkan permasalahan ini, terdapat banyak cara pengawalan penyakit yang telah diperkenalkan. Antara cara pengawalan yang selalu digunakan adalah dengan menggunakan bahan kimia .Walaubagaimanapun, teknik menggunakan racun kulat ke atas tanaman mengakibatkan kemusnahan kepada alam sekitar disebabkan oleh kandungan toksik yang sangat tinggi. Oleh itu, penggunaan ekstrak Sireh dan Belalai Gajah sebagai salah satu biopesticides adalah salah satu langkah yang terbaik dalam usaha kearah melestarikan sektor pertanian. Untuk itu, kajian ini dibuat adalalah untuk 1) mengekstrak komponen antimikrob daripada Sireh dan Belalai Gajah dan 2) mengkaji keberkesanan kepekatan ekstrak yang berlainan terhadap pertumbuhan kulat. Kulat yang dipilih bagi kajian ini ialah *Rhizoctonia solani, Ganoderma boninense*, *Fusarium oxysporum* f.sp *cubense* dan juga *Exserohilum rostratum*. Daun yang diambil telah dibersihkan dan dikeringkan pada suhu bilik dan dikisar menggunakan pengisar. Serbuk daun tersebut direndam didalam methanol dan diletakkan di atas *orbital shaker***.** Larutan itu kemudiannya ditapis dan diletakkan pada *rotary evaporator* untuk mendapatkan ekstrak. Terdapat 4 kepekatan yang digunakan iaitu 5,10.15 dan 20% dan dibandingkan dengan 0% kepekatan (tanpa ekstrak). Agar yang telah diletakkan ekstrak Sireh menunjukkan pengurangan dalam pertumbuhan mycelia dan bilangan spora. Pengurangan dalam pertumbuhan mycelia *F.o.c* mencapai 94.04% pada hari ke-12, *G. boninense* 89.42% pada hari ke-12, *R. solani* 82.84% pada hari ke-4 and *E. rostratum* 43.74% pada hari ke-8. Bilangan spora ($4x10^6$) yang dihasilkan pada kepekatan 20% pula ialah 969 bagi *F.o.c* dan

178 bagi *R.solani.* Begitu juga dengan ekstrak Belalai Gajah. Pengurangan dalam pertumbuhan mycelia *E.rostratum* mencapai 64.42% pada hari ke-8, *R. solani* 58.86% pada hari ke-4 *F.o.c* 10.36% pada hari ke-12 dan *G. boninense* 27.48% pada hari ke-12. Bilangan spora ($4x10^6$) yang dihasilkan pada kepekatan 20% pula ialah 1723 bagi *F.o.c* dan 38 bagi *R.solani.* Ini menunjukkan bahawa ekstrak Sireh dan Belalai Gajah boleh dijadikan sebagai bio-fungicide sebagai salah satu alternatif kepada racun kulat kimia sintetik

CHAPTER ONE

INTRODUCTION

1.1 Sustainable agriculture

The term 'sustainability' is currently widely used in many aspects of our lives, and especially in agriculture because of the effect that certain crop production methods have on the environment (Hanson *et al*., 2007). Sustainable agriculture is the management and the utilization of the agricultural ecosystem in a way that maintains its biological diversity, productivity, regeneration capacity, vitality and ability to function, so that it can fulfill today and in the future significant ecological, economic and social functions at the local, national and also global levels in a way that it does not give harm to the ecosystem (Lewandowski *et al.*, 1999).

The sustainability in agriculture has faced some of the most important challenges in recent years (Hanson *et al.,* 2007). The first major challenge is the increasing of human population. When this happen, increased demand for agricultural land and resources will occur. Next, over dependence on fossil energy and the increased monetary and also environmental costs of nonrenewable resources. Lastly, global climate change (Brown 2006; Diamond 2005), and globalization (Hanson *et al.,* 2007). These issues are challenging agriculturists to develop more sustainable management systems like no other time in history. To meet the food and nutritional needs of a growing population, agriculture sector will need to move beyond the past emphasis on productivity to improved public health, social well-being and a good environment (Hanson *et al.*, 2007). Also, it is crucial to find alternative way to control plant diseases which do not harm the environment and at the same time increase yield and also improve product quality (Batish *et al.*, 2006).

In recent years, the importance of sustainable agriculture has risen to become one of the most important issues in agriculture. With the occurrence of plant disease in agriculture sector, it limits the agricultural production. Controlling plant disease using classical pesticide will increase serious concern about the quality of environment, level of toxicity in products and also pesticide resistance in which becoming a factor for the need of alternative pest management techniques.

1.2 Problem statement of the study

Losses of agricultural products due to plant disease are increasing from day to day. It is recorded that in 2002, losses that were contributed by disease caused by pathogens and also other environmental factor was estimated about $220 billion (Agrios, 2004). Increasing of losses by agriculture product from time to time will eventually not going to feed all the population in this world that will increase up to 8,500 million in 2020 (Agrios, 2004).

There is various disease that lead to the crop damage every year and among them fungal diseases are very common (Naidu *et al.,* 1981). Although the use of synthetic fungicides in plant disease control has shown to reduce the amount of plant disease in agriculture, several of these have been found to display side-effects in the form of carcinogenicity, detrimental effects and other residual toxicities. It is believed that antimicrobial agents are present in higher plants. Some recent researches on the antifungal activity of extracts of several higher plants have shown the possibility of this extract as natural antifungal agents to control plant diseases (Qureshi SM *et al.,* 1997).

The use of systemic fungicides simplifies that not many systemic fungicide is practical to be used. Furthermore, there is also the rise of concerns for the problems of fungicide insensitivity, residues on edible product and for tree crops (Bailey and Jeger, 1992). One approach might be to test the plants that are traditionally used for their antifungal activities as potential sources in a drug development (Lucy *et al.,* 1999)

The plant disease that is caused by fungal pathogen is usually controlled by application of chemical based antifungal compounds. For example, *P. azadirachtae*

is sensitive to bavistin (Satesh MK, 1998). The chemical based pesticides are non-biodegradable and extremely toxic (Yadav SK, 2010; Lakshmeesha TR *et al.*, 2013). They have various drawbacks in terms of genotoxicity (Bolognesi C, 2003), hepatotoxicity (Cecchi A *et al.*, 2012), reproductive disorders (Richard S *et al.*, 2005) and immunosuppression (Agarwal *et al.*, 2012). Frequent use of fungicides has led to the emergence of resistant strains. Furthermore, environmental pollution caused by excessive use of agrochemicals, has increased the public pressure to reduce the use of synthetic fungicides in agriculture (Manasi K *et al.*, 2014). Hence, the need for effective and safe alternative has increased. Concerns have been raised about both the environmental impact, and the effect to human health when using these synthetic compounds (Abad MJ *et al.*, 2007; Lakshmeesha Tr *et al.*, 2013).

Synthetic fungicides have been used for almost 20 years for the control of post-harvest diseases of tropical and subtropical fruit. Many microbial pathogens, however, have begun to develop resistance to the most widely used chemicals, so there is a need to develop new fungicides with improved performance and less potential environmental impact (Suhaila *et al.*, 1996).

1.3 Introduction of herbal plants

To date, many researchers had been working on identifying antimicrobial compound in herbal plants as it is free from the side effect caused by the synthetic chemicals. The use of synthetic fungicide has been found to display side-effects in the form of carcinogenicity, detrimental effects and other residual toxicities. The alternative choice, therefore would be the use of botanical fungicides, which are advocated to be largely non-phytotoxic,systematic and easily biodegradable in nature (Fawcett CH *et al.,* 1970). Moreover, plant extracts could be an alternative to toxic fungicides for controlling plant pathogens since they are composed of various bioactive compounds such as alkaloids, flavonoids, glycosides, phenols, saponins, sterols and etc. (Lakshmeesha *et al.,* 2013). Huge efforts are being made to isolate bioactive products from medicinal plants for their possible utility in development of plant based biopesticides (Helton LR, 1996). The antimicrobial activity of different plant extracts has been reported by many studies. It is believed that antimicrobial agents are present in higher plants. Some recent researches on antifungal activity of extracts of several higher plants have indicated the possibility of their exploitation as natural antifungal agents for control of plant diseases. Many herbal plants are able to inhibit microbial growth since they contain active compounds (Begum J *et al.*, 2007). In the present study, we have evaluated the antimicrobial effects of the extract of two herbs, *Piper betle* and *Clinacanthus nutans* to control selected plant pathogen.

1.4 Objectives of the study

The general objective of this research was to investigate the effect of *P. betle* and *C. nutans* extract on the growth of selected plant pathogen *in vitro*

The specific objectives are:

1) To extract crude antimicrobial compounds from *P. betle* and *C. nutans.*

2) To investigate the effectiveness of different concentrations of crude antimicrobial extracts on the *in vitro* growth of selected plant fungal pathogens.

1.5 Hypothesis of the study

The hypothesis of this research was *P. betle* and *C. nutans* produce an antifungal compound which inhibits the growth of selected plant fungal pathogen.

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