In vitro antifungal activity evaluation of five plant extracts against five plant pathogenic fungi causing rice and economic crop diseases

Arom Jantasorn*, Boontida Moungsrimuangdee and Tida Dethoup

ABSTRACT

Plant diseases caused by fungi are one of significant destructive pathogens to economic crops of Thailand and worldwide. The most common plant pathogenic fungi infected crops in Thailand are *Pyricularia oryzae* Cavara., *Rhizoctonia solani* (J.G Kuhn), *Phytophthora palmivora* Butler., *Sclerotium rolfsii* Sacc., and *Colletotrichum gloeosporioides* Penz. and Sacc. Control of these pathogens is by using synthetic fungicides which are expensive and harmful to environment. This study aims at investigating the efficacy of the extract of five plants - *Hydnocarpus anthelminthicus* Pierre ex Laness., *Crateva magna* (Lour.) DC., *Caesalpinia sappan* L., *Xanthophyllum lanceatum* J. J. Sm., and *Carallia brachiata* (Lour.) Merr. to inhibit the growth of five plant pathogenic fungi in *in vitro* conditions at various concentrations. At the 10,000 ppm concentration *H. anthelminthicus* fruit extracts exhibited reduction in antifungal potential to growth inhibition, and recorded 100 % growth inhibition against *P. oryzae*, *P. palmivora* and *R. solani* followed by *S. rolfsii* at 96.33 % when compared with water control. *X. lanceatum* fruit extract that logged excellent inhibitory activity against *P. oryzae*. Antifungal potential was observed with the extract of *C. sappan*, which recorded the best inhibitory activity against *P. palmivora* and *S. rolfsii* at 88.89 and 78.89 % respectively. Results from this study demonstrated that the ability of some plant extracts *viz., H. anthelminthicus, X. lancelatum* and *C. sappan* could be used to control the growth of plant pathogenic fungi and may be applied as an alternative method to reduce fungicide.

MS History: 22.12.2015 (Received)-14.03.2016 (Revised)- 14.03.2016 (Accepted)

Key words: Antifungal, plant extracts, plant disease control, plant pathogenic fungi.

Citation: Jantasorn, A., Moungsrimuangdee, B. and Dethoup, T. 2016. *In vitro* antifungal activity evaluation of five plant extracts against five plant pathogenic fungi causing rice and economic crop diseases. *Journal of Biopesticides*, 9 (1): 1-7.

INTRODUCTION

The diseases produced from fungiform cause a significant loss of many economic crops worldwide. The fungi generate the greatest impact in terms of reduction in crop productivity or post harvest losses and leads to a huge loss to mankind (Tapwal *et al*., 2011). Crop losses from fungal infection are estimated to be about 14 per cent (Agrios, 2005). Among the plant pathogenic fungi, *Phytophthora palmivora* Butler. causing root and stem rot, *Colletotrichum gloeosporioides* Penz. and Sacc. responsible for anthracnose and *Sclerotium rolfsii* Sacc. are reported as the most destructive pathogens and cause extensive damage and yield losses (Abdel-Fattah *et al*., 2011; Al-Askar, 2012; Alwathnani and Perveen, 2012).

Rice blast disease is one of the most devastating diseases in many paddy fields around the world (Ou, 1985; Couch *et al*., 2002), especially in north, northeast and south Thailand. The causal agent, *Pyricularia oryzae* Cavara. is a ubiquitous air born pathogen which can damage all stages of rice. Sheath blight caused by *Rhizoctonia solani* (J.G Kuhn) is also widespread among the rice crops in Thailand. It produces yield losses of 20 - 30 % and can be found in all stages of rice.

Management of these pathogens is required, as at least 10 per cent of food is lost due to plant diseases (Strange and Scott, 2005). Presently, many researchers are trying to identify effective natural products for controlling diseases thereby replacing synthetic pesticides (Kim *et al*., 2005). The antifungal activity from the plant extracts have been shown to be effective against plant pathogen and eco-friendly to the environment (Latha *et al*., 2009; Duru and Onyedineke, 2010). Previously, the various plant extracts that have been reported as a source of bio-pesticide because of the substances of plant extract inhibited the growth of plant pathogens and reduced the hazard to human health and environment. The presence of antifungal compounds in higher plants has long been recognized as important factors for controlling some plant diseases (Tapwal *et al*., 2011). Recently, many researchers in the world show interest in the application of plant product as bio- pesticide (Singh and Srivastava, 2013). Through the use of bio-pesticides, there can be a decrease in the use of chemical pesticides which have an undesirable effect on other organisms present in the environment and humans through the food chain. Some medicinal plants that have antifungal properties can also be a source of plant bio-pesticide (Aslam *et al*., 2010).

Plant bio-pesticides are cheap, locally available, non toxic, and are easily degradable. There is a great demand for them as alternative agents to control plant pathogenic fungi (Hadizadeh *et al*., 2009). Because of development of resistance towards the synthetic fungicide and accumulation of residues, the use of natural products for fungal disease management is considered one of the better alternatives (Gujar *et al*., 2012). This study was undertaken to screen the crude extracts of five *viz.*, *Hydnocarpus anthelminthicus* Pierre ex Laness., *Crateva magna* (Lour.) DC., *Caesalpinia sappan* L., *Xanthophyllum lanceatum* J. J. Sm., and *Carallia brachiata* (Lour.) Merr. for their inhibition activity in laboratory conditions against *Pyricularia oryzae* Cavara., *Rhizoctonia solani* (J.G Kuhn), *Phytophthora palmivora* Butler., *Sclerotium rolfsii* Sacc., and *Colletotrichum gloeosporioides* Penz. and Sacc.

MATERIALS AND METHODS

Preparation of the plant extracts

H. anthelminthicus (fruits), *C. magna* (fruits), *C. sappan* (barks), *X. lanceatum* (fruits) and *C. brachiata* (barks) were collected from the riparian forest at Bodhivijjalaya College, Srinakharinwirot University, Sakaeo campus. 100 g of each dry plant was macerated with 900 ml of 70% ethanol for 7 days at room temperature and then filtrated with Whatman No.1 filter paper to give the organic solutions and then evaporated under reduced pressure to furnish the crude ethanol extracts of each plant.

In vitro antifungal activity evaluation

Dilution plate method was used for the evaluation of the *in vitro* antimycelial growth of plant pathogenic fungi. The concentrations of 1, 10, 100, 1,000 and 10,000 ppm of each extract were tested according to Boonsang *et al*. (2014). Each treatment was performed with five replications in a complete randomized design. The effectiveness of the antifungal activity was assessed based on the percentage of mycelial growth inhibition which was calculated using the formula: G1-G2 / G1 × 100, where G1 is the colony radius of the fungi in control, and G2 the colony radius of fungi in treatments.

Data analyses

Data obtained were submitted to the analysis of variance (ANOVA) and means were compared by Duncan's multiple range test (P<0.05) using the statistical program SPSS version 16 (IBM Corporation, Somers, NY).

RESULTS AND DISCUSSIONS

The data indicating the reduction in growth of five plant pathogenic fungi in response to the tested plant extracts is shown in table 1. Seven days after inoculation, the growth of all the five pathogenic fungi was found to have inverse relationship with the concentration of plant extracts used. The highest antifungal activity against *P. oryzae* was recorded for *H. anthelminthicus* and *X. lanceatum* fruit

Table 1. *In vitro* inhibitory effect of plant crude extracts at various concentrations on the mycelia growth of five plant pathogenic fungi

Pathogens	Conc. of plant crude extracts (ppm)	% mycelial growth inhibition at concentrations				
		H. anthelminthicus	*X. lanceatum*	*C. magna*	*C. sappan*	*C. brachiata*
Pyricularia oryzae	1	0^{ze}	0^{ze}	0^{ze}	0^{ze}	0^{ze}
	10	0^{ze}	0^{ze}	0^{ze}	0^{ze}	0^{ze}
	100	0^{ze}	0^{ze}	0^{ze}	0^{ze}	0^{ze}
	1,000	17.78^{tu}	55.56^{h}	0^{ze}	0^{ze}	5.56^{zc}
	10,000	100^{a}	100^{a}	21.48^{r}	5.56^{zc}	19.63^{s}
Rhizoctonia solani	1	0^{ze}	0^{ze}	0^{ze}	0^{ze}	0^{ze}
	10	0^{ze}	0^{ze}	0^{ze}	0^{ze}	0^{ze}
	100	0^{ze}	0^{ze}	0^{ze}	0^{ze}	0^{ze}
	1,000	53.3^{i}	45.50^{k}	0^{ze}	0^{ze}	0^{ze}
	10,000	100^{a}	57.41^{f}	17.41^{uv}	0^{ze}	4.81^{zc}
Phytophthora palmivora	1	0^{ze}	0^{ze}	0^{ze}	0^{ze}	0^{ze}
	10	0^{ze}	0^{ze}	0^{ze}	0^{ze}	0^{ze}
	100	0^{ze}	16.89^{v}	0^{ze}	0^{ze}	0^{ze}
	1,000	0^{ze}	53.33^{i}	22.78^{q}	23.68^{f}	0^{ze}
	10,000	100^{a}	53.33^{i}	56.33^{g}	88.89^{c}	21.14^{r}
Colletotrichum gloeosporioides	1	0^{ze}	0^{ze}	0^{ze}	0^{ze}	0^{ze}
	10	0^{ze}	0^{ze}	0^{ze}	0^{ze}	0^{ze}
	100	0^{ze}	0^{ze}	0^{ze}	0^{ze}	0^{ze}
	1,000	13.33^{y}	14.78^{w}	11.11^{za}	6.67^{zb}	5.89^{zc}
	10,000	51.11^{k}	20.00^{s}	52.22^{j}	34.78^{o}	18.11^{t}
Sclerotium rolfsii	1	0^{ze}	0^{ze}	0^{ze}	0^{ze}	0^{ze}
	10	0^{ze}	0^{ze}	0^{ze}	0^{ze}	0^{ze}
	100	0^{ze}	11.89^{z}	0^{ze}	0^{ze}	0^{ze}
	1,000	38.33^{n}	40.78^{m}	14.11^{x}	43.00^{l}	0^{ze}
	10,000	96.33^{b}	52.22^{j}	63.00e	78.89^{d}	0^{ze}

Means followed by the same letter do not significantly different at $P<0.05$, when analysed using Duncan's multiple range test of One-Way ANOVA.

extracts at a concentration of 10,000 ppm when compared with all other plant extract treatments. The growth of *P. oryzae* continued to decrease seven days after inoculation at different concentrations of *H. anthelminthicus* fruits extract. At concentrations of 10,000 ppm of *H. anthelminthicus* extract showed 100 % growth inhibition against *R. solani* and *P. palmivora* when compared with the control. However, the same extract at a concentration of 10,000 ppm was found to inhibit the growth of *S. rolfsii* and *C. gloeosporioides* on it at 96.33% and 51.11% and at low concentrations (1, 10, and 100 ppm), it failed to inhibit the mycelia growth. *X. lanceatum* fruit extract also recorded 100% growth inhibition against *P. oryzae* at a concentration of 10,000 ppm followed by *R. solani*, *P. palmivora*, *S. rolfsii*, and *C. gloeosporioides*. The extracts from *C. magna* and *C. sappan* at 10,000 ppm were found most effective in inhibiting the growth of *P. palmivora* and *S. rolfsii* at 56.33%, 63.00%, 88.89% and 78.89% respectively. The plates treated with *C. brachiata* extract recorded minimal growth inhibition against the five pathogenic fungi. Fruit extracts from *H. anthelminthicus* was the most effective in reducing the mycelial growth of *R. solani*, *P. oryzae*, *P. palmivora*, *S. rolfsii* and *C.gloeosporioides* with increasing concentration. *X. lanceatum* fruit extraction recorded excellent inhibitory activity against *P. oryzae*. However, the extract from *C. sappan* was more efficient in the growth inhibition of *P. palmivora* and *S. rolfsii* as compared with *C. magna* extract (Fig. 1).

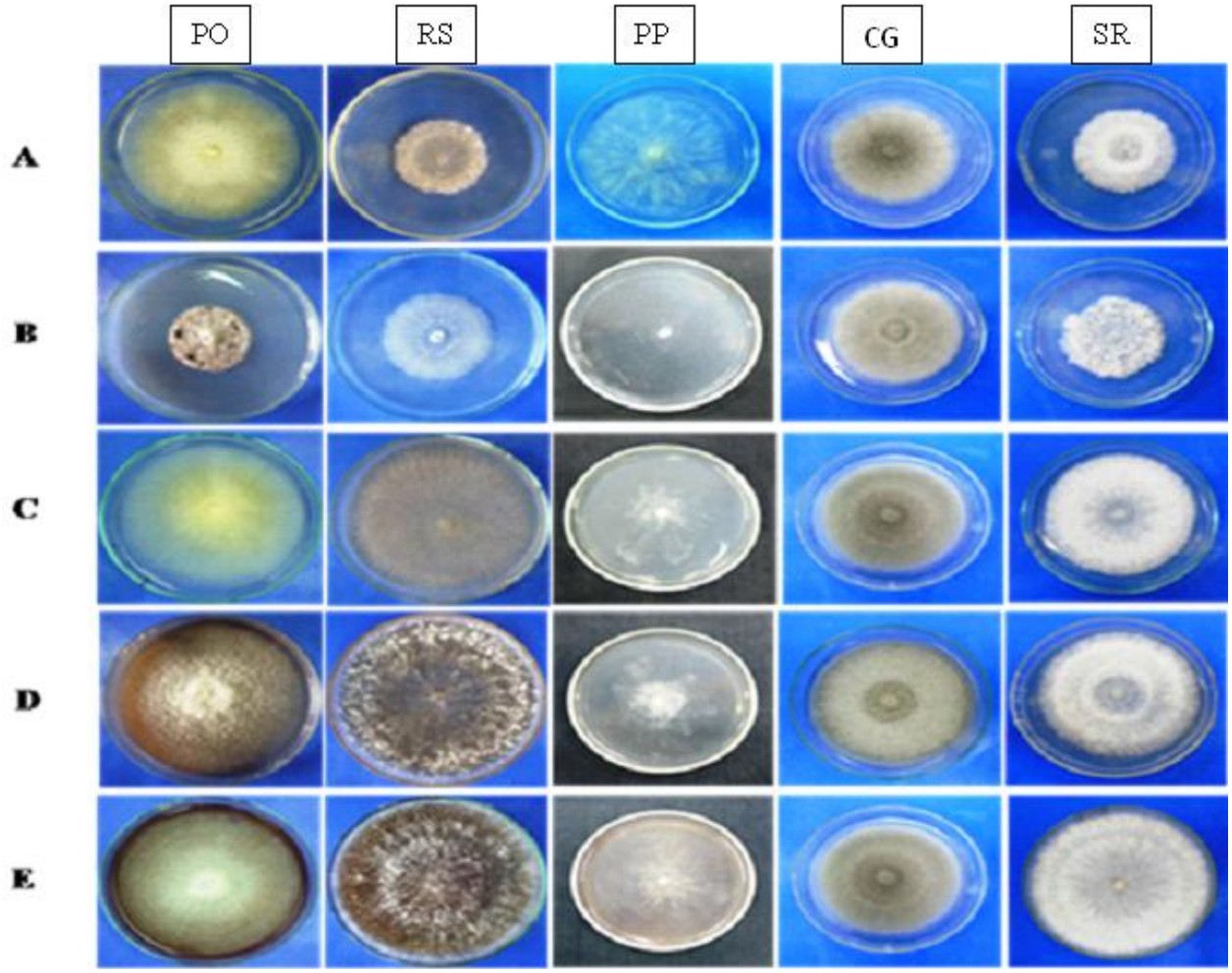

Figure 1 Antifungal effect of plant crude extracts at concentration of 1,000 ppm against *Pyricularia oryzae* (PO), *Rhizoctonia solani* (RS), *Phytophthora palmivora* (PP), *Colletotrichum gloeosporioides*(CG) and *Sclerotium rolfsii* (SR) on PDA at 28◦C for 7 days; A) = *Hydnocarpus anthelminthicus,* B) = *Xanthophyllum lanceatum,* C) = *Crateva magna* , D) =*Caesalpinia sappan* , E) = *Carallia brachiata*

The results indicated that all plant extracts had different levels of antifungal activity against the tested pathogenic fungi. The most effective fruit extract was found to be *H. anthelminthicus* which recorded growth inhibition against *P. oryzae*, *R. solani*, *P. palmivora* and *S. rolfsii* when compared with the control. On the preliminary tests in the laboratory, the fruit extract from *H. anthelminthicus* was tested on two species of pathogenic bacteria, *Xanthomonas citri* subsp. *citri* and *Xanthomanas oryzae*. The extract inhibited the growth of bacteria (data not shown). In another study, the antifungal activity from pomegranate, camel thorn, and caper was tested on different pathogens and has been reported by various researchers (Dahham *et al*., 2010; Abd-Ellatif *et al*., 2011).

Our results revealed that the highest antifungal activity against *P. oryzae* was reported with *H. anthelminthicus* and *X. lanceatum* fruit extracts at a concentration of 10,000 ppm and the extract from *H. anthelminthicus* at the same concentration (Fig. 2). 100% growth inhibition was recorded against *R. solani*, *P. palmivora* and *S. rolfsii* when compared with the control. The efficacies of natural products from plants in inhibiting the growth of

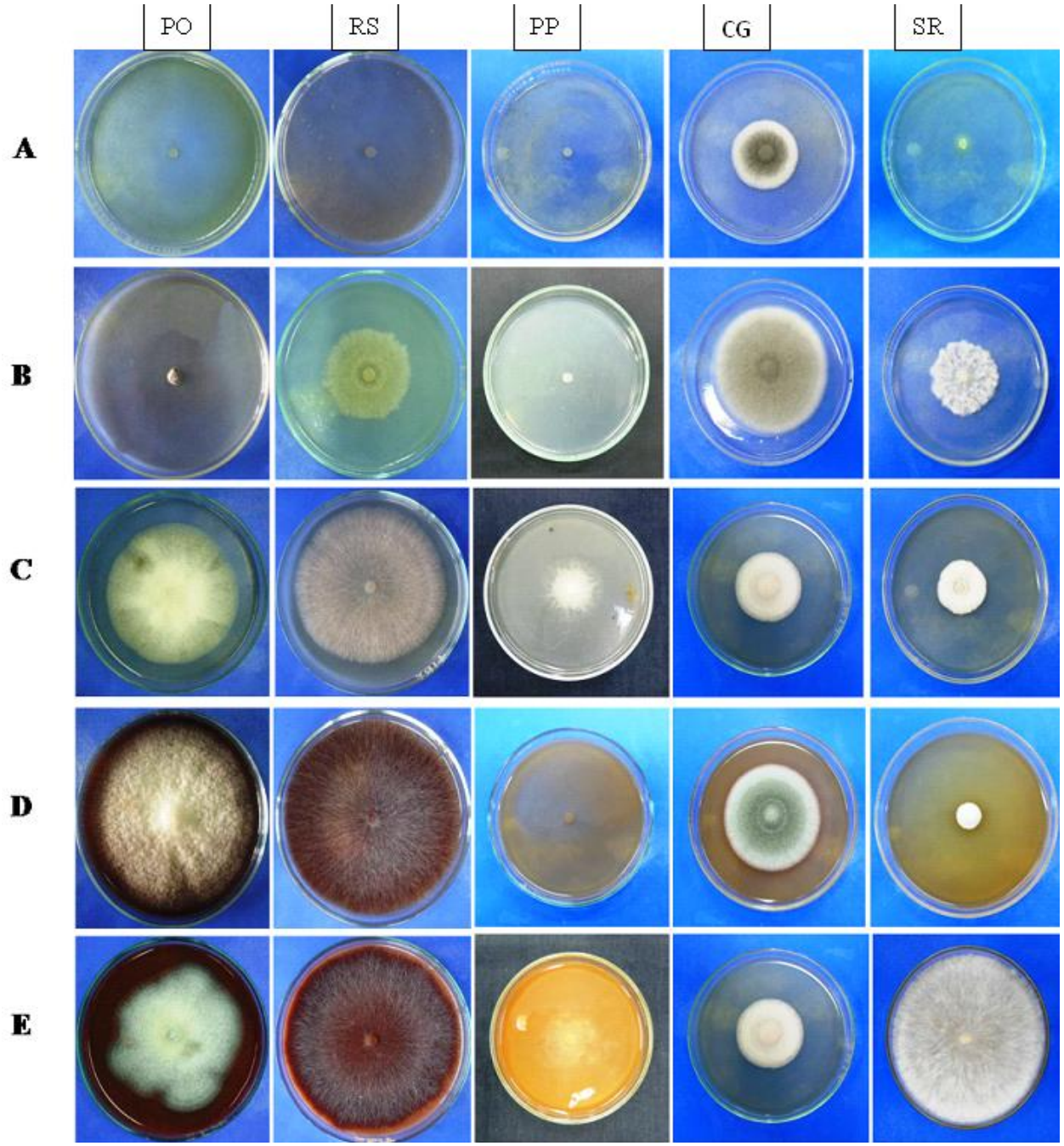

Figure 2 Antifungal effect of plant crude extracts at concentration of 10,000 ppm against *Pyricularia oryzae* (PO), *Rhizoctonia solani* (RS), *Phytophthora palmivora* (PP), *Colletotrichum gloeosporioides* (CG) and *Sclerotium rolfsii* (SR) on PDA at 28◦C for 7 days: A) = *Hydnocarpus anthelminthicus*, B) = *Xanthophyllum lanceatum*, C) = *Crateva magna*, D) =*Caesalpinia sappan*, E) = *Carallia brachiata*

pathogenic fungi have been reported earlier. Mariappan *et al.* (1995) also reported that the leaf extracts from *Z. jujube* inhibited the growth of rice blast disease caused by *P.oryzae*. The antifungal activity from the leaf extracts of *D. stramonium* against the growth of *R. solani* was recorded as the maximum reduction in the growth of disease and four other soil borne fungi (Yossry *et al.*, 1998).

The antifungal activities of tested plant extracts are attributed to chemical compounds, belonging mainly to six groups, *viz.*,

coumarins, pyrones, steroids, phenolics and phenol acid, flavonoids, isoflavonoids, alkaloids and other compounds. However, only a few chemical compound products from plants are being used in agriculture for plant protection (Mitra *et al*., 1984). The plant extracts are a bio-pesticide control agent that is crucial for two particular reasons. The first reason is its safe usage for people and environmental accumulation. The second is its ability to control the pathogens and to prevent the pathogens from developing resistance to fungicide (Derbalah *et al*., 2011). The use of natural products from plants could be a major step towards the application of bio-pesticides as a measure for the prevention and control of plant diseases.

The antifungal activities of the five plants extracts in this study indicated that some plant species have an effect in causing the highest reduction of growth inhibition on plant pathogenic fungi. The results were confirmed in all of the tested plant extracts which inhibited the growth of the five pathogenic fungi at different concentrations of extraction. Although some plant extracts only slightly reduced the growth inhibition**,** plant species collected in this study will determine the extraction that could provide antifungal activity against all five plant pathogenic fungi, *P. oryzae*, *R. solani*, *P. palmivora*, *C. gloeosporioides* and *S. rolfsii*. For the natural management of plant diseases, some plant extracts, *H. anthelminthicus*, *X. lanceatum*, *C. magna* and *C. sappan* could be applied to control plant pathogenic fungi and be employed as the alternative method in reducing the fungicide. Subsequently, through the usage of bio-pesticides, there will be a reduction of hazards to human health and the environment.

ACKNOWLEDGEMENTS

This work was financially supported by the Strategic Wisdom and Research Institute, Srinakharinwirot University, under the project number 043/2558. We also thank Mr. Thanaprosong Oiuphisittraiwat for technical support.

REFERENCES

Abdel-Fattah, G.M., El-Haddad S.A., Hafez E.E. and Rashad Y.M. 2011. Induction of defense responses in common bean plants by *Arbuscular mycorrhizal* fungi. *Microbiological Research*, **166**(4): 268–281

Abd-Ellatif, S., Abdel Rahman, S. M. and Deraz, S. F. 2011. Promising antifungal effect of some folkloric medicinal plants collected from El- Hammam habitat, Egypt against dangerous pathogenic and toxinogenic fungi. *Journal of Agricultural and Biological Science*, **6(9)**: 25–32.

Agrios, G.M. 2005. Plant Pathology. 5th ed. AP, New York, NY, 922 **PP**.

Aslam, A., Naz, F., Arshad, M., Qureshi, R. and Rauf, C. A. 2010. *In Vitro* antifungal activity of selected medicinal plant diffusates against *Alternaria solani, Rhizoctonia solani* and *Macrophomina phaseolina. Pakistan Journal of Botany,* **42**: 2911-2919.

Al-Askar, A. A. 2012. In Vitro antifungal activity of three Saudi plant extracts against some phytopathogenic fungi. *Journal of Plant Protection Research*, **52(4)**: 458-462.

Alwathnani H.A. and Perveen K. 2012. Biological control of fusarium wilt of tomato by antagonist fungi and cyanobacteria. *African Journal of Biotechnology*, **11(5)**: 1100–1105.

Boonsang, N., Dethoup,T., Singburaudom, N., Gomes, N.G.M. and Kijjoa, A. 2014. *In vitro* antifungal activity screening of crude extracts of soil fungi against plant pathogenic fungi. *Journal of Biopesticides*, **7**: 156-166.

Couch, B.E. and Kohn, L.E. 2002. A multilocus gene genealogy concordant with host preference indicates segregation of a new species, *Magnaporthe oryzae*, from *M. grisea*. *Mycologia*, **94**: 683-693.

Dahham, S. S., Ali, M. N., Tabassum, H. and Khan, M. 2010. Studies on antibacterial and antifungal activity of pomegranate (*Punica granatum* L.). *American-Eurasian Journal of*

Agricultural and Environmental Sciences, **9(3)**: 273–281.

Daru, C. M. and Onyedeneke, N. E. 2010. *In vitro* study on the antimicrobial activity and phytochemical analysis of ethanolic extracts of the mesocarp of *Voacanga africana*. *American Journal of Plant Physiology*, **5**: 163-169.

Derbalah, A. S., El-Mahrouk, M. S. and El-Sayed, A. B. 2011. Efficacy and safety of some plant extracts against tomato early blight disease caused by *Alternaria solani*. *Plant Pathology Journal*, **10(3)**: 115-121.

Gujar, J. and Talwankar, D. 2012. Antifungal potential of crude plant extract on some pathogenic fungi. *World Journal of Science and Technology*, **2**: 58-62.

Hadizadeh, I., Peivastegan, B. and Kolahi, M. 2009. Antifungal activity of nettle (*Urticad ioica L.*), colocynth (*Citrullus colocynthis L. Schrad*), oleander (*Nerium oleander L.*) and konar (*Ziziphusspina-christi L.*) extracts on plants pathogenic fungi. *Pakistan Journal of Biological Sciences*, **12**: 58-63.

Kim, D. I., Park, J. D., Kim, S. G., Kuk, H., Jang, M. J. and Kim, S. S. 2005. Screening of some crude plant extracts for heir acaricidal and insecticidal efficacies. *Journal of Asia-Pacific Entomology*, **8**: 93–100.

Latha, P., Anand, T., Ragupathi, N., Prakasam, V. and Samiyappan. 2009. Antimicrobial activity of plant extracts and induction of systemic resistance in tomato plant by mixtures of PGPR strains and Zimmu leaf extract against *Alternaria solani*. *Biological Control*, **50**: 85-93.

Mariappan, V., Rajeswari, E. and Kamalakannan, A. 1995. Management of rice blast, Piricularia oryzae by using neem (*Azadirachta indica*) and other plant products. In*Neem for management of crop diseases* (Mariappan V. ed.). New Delhi, India: Associated publishing Co, 3-10 **PP**.

Mitra, S. R., Choudhuri, A. and Adityachaudhury, N. 1984. Production of antifungal compounds by higher plants-a review of recent researches. *Plant Physiology and Biochemistry*, **11**: 53-77.

Ou, S. H. 1985. Rice Diseases. 2nd ed. Commonwealth Mycological Institute, Kew, U.K. 235 **PP.**

Singh, P. and Srivastava, D. 2013. Phytochemical Screening and *In Vitro* Antifungal Investigation of *Parthenium Hysterophorus* extracts against *A. alternata*. *International Research Journal of Pharmacy*, **4**: 190-193.

Strange, R. N. and Scott, P. R. 2005. Plant diseases: a threat to global food security. *Annual Review of Phytopathology*, **43**: 83–116.

Tapwal, A., Nisha, Garg, S., Guatam, N. and Kumar, R. 2011. *In Vitro* antifungal potency of plant extracts against five phytopathogens. *Brazilian Archives Biology and Technology*, **54(6)**: 1093-1098.

Yossy, A. A., Ali S. M. A. and Imery, S. M.1998. Fungitoxic properties of some plant extracts against growth of soil borne disease fungi. *Annals of Agricultural Science*, **3**: 891-909.

Arom Jantasorn[1*], Boontida Moungsrimuangdee[1] and Tida Dethoup[2]

[1]Bodhivijjalaya College, Srinakharinwirot University, 114 Sukhumvit 23, Bangkok, 10110, Thailand.

[2]Department of Plant Pathology, Faculty of Agriculture, Kasetsart University, Bangkok, 10900, Thailand

[*]Corresponding author

E-mail: aromj@g.swu.ac.th