

Full Length Research Paper

Antifungal activity of selected medicinal plants against *Alternaria* species: The pathogen of dirty panicle disease in rice

Sawatdikarn Sanit

Department of Applied Science, Faculty of Science and Technology, Pranakhon Si Ayutthaya Rajabhat University, Thailand.

Received 12 June, 2013; Accepted 8 March, 2016

Antifungal activity of the ethanolic crude extracts from ten plants, namely: *Curcuma xanthorrhiza*, *Curcuma aromatica*, *Kaempferia parviflora*, *Syzygium aromaticum*, *Origanum vulgare*, *Synedrella nodiflora*, *Sorghum bicolor*, *Rosmarinus officinalis*, *Piper longum* and *Eupatorium odoratum* were tested against *Alternaria* species (the pathogen of dirty panicle disease in rice) by poisoned food technique at 0, 1,000, 2,500, 5,000, 7,500 and 10,000 ppm. The inhibition of mycelial growth and spore germination were evaluated. The results showed *C. aromatica*, *S. aromaticum* and *O. vulgare* crude extracts at 1,000 ppm, the *R. officinalis* crude extracts at 5,000 ppm, and the *C. xanthorrhiza* crude extracts at 10,000 ppm showed the highest inhibition of mycelial growth at 100%, whereas *S. nodiflora* and *S. bicolor* crude extracts at 10,000 ppm had the inhibition at 56 and 58%, respectively. For inhibition of spore germination, the three plant crude extracts; *C. aromatica*, *S. aromaticum* and *O. vulgare* at 1,000 ppm and *C. xanthorrhiza*, *S. nodiflora*, *S. bicolor*, *R. officinalis* and *P. longum* crude extracts at 2,500 ppm reached the highest inhibition of spore germination at 100%, whereas *E. odoratum* and *K. parviflora* crude extracts at 10,000 ppm had the inhibition at 20 and 55%, respectively.

Key words: Antifungal activity, dirty panicle disease, *Alternaria* species, medicinal crude extract, rice.

INTRODUCTION

Dirty panicle disease is one of the most serious diseases of rice, affecting rice production in the world and rice production grown in Thailand and tropical locations (Ou, 1985). The causal organisms are several pathogens, *Fusarium* species, *Alternaria* species, *Cercospora* species and *Curvularia* species (Abdelmonem, 2000). The panicle dirty control had several methods, mechanical,

cultural, biological and chemical method. Chemical control is the best method for dirty panicle disease, whereas this method is harmful for environmental condition, consumer and user. Nowadays, the farmers use the biological control for dirty panicle control. The medicinal herb crude extracts for the seed borne pathogen control have attracted wide interest, the

E-mail: SSanit33@hotmail.com

Author(s) agree that this article remain permanently open access under the terms of the [Creative Commons Attribution License 4.0 International License](https://creativecommons.org/licenses/by/4.0/)

medicinal herbs comprises a rich phytochemicals and biochemicals substances to be used as biopesticide which are more friendly environment than synthetic chemicals.

There are reports that phytochemicals and biochemicals substances of some plants showed fungicidal activity against pathogenic fungi; *Syzygium aromaticum* crude extract at 1,000 ppm showed 100% inhibition on mycelial growth of *F. oxysporum* and *F. equiseti* (Kritzinger et al., 2002) and the *S. aromaticum* crude extract can be used for *Staphylococcus aureus* control (Briozzo et al., 1989). Rosemary (*Rosmarinus officinalis*) crude extracts showed the growth inhibition of three pathogens, namely *Leuconostoc mesenteroides*, *Listeria monocytogenes* and *S. aureus* (Campo et al., 2000). For the some medicinal crude extracts on mycelial growth of some pathogens control including the oregano (*Origanum vulgare*) crude extract for *Bacillus cereus* control (Paster et al., 1995); *Curcuma xanthorrhiza* for three pathogen control, namely *B. cereus*, *S. aureus* and *Escherichia coli* (Husein et al., 2009); *Curcuma aromatica* for *S. aureus* and *E. coli* control (Saleem et al., 2011) and *Kaempferia parviflora* crude extracts for *B. cereus* and *S. aureus* control (Butkhup and Samappito, 2011).

No information of ten medicinal herb crude extracts on inhibition of mycelial growth and spore germination of *Alternaria* spp. (pathogen of dirty panicle disease in rice). The objective of this research was to evaluate ten medicinal herb crude extracts on the mycelial growth and spore germination of *Alternaria* spp. in Central area, Thailand.

MATERIALS AND METHODS

This work was conducted at the Department of Applied Science, Faculty of Science and Technology, Phranakhon Si Ayutthaya Rajabhat University, Phranakhon Si Ayutthaya for 2010 to 2011 to test the antifungal activity of *C. xanthorrhiza*, *C. aromatica*, *K. parviflora*, *S. aromaticum*, *O. vulgare*, *Synedrella nodiflora*, *Sorghum bicolor*, *R. officinalis*, *Piper longum* and *Eupatorium odoratum* against *Alternaria* spp. (pathogen of dirty panicle disease in rice) in distilled water and ethanol by using food poisoning technique (Prasad et al., 2010).

Preparation of rice seeds and isolation of pathogen

Rice seeds were obtained from three location in Central area, Phranakhon Si Ayutthaya, Aungthong and Prathumthani province. *Alternaria* spp. from the rice seeds were isolated and maintained on petri dishes containing in Potato dextrose agar (PDA) and incubated at 25°C for 3 days before the tests. The preparation of rice seeds and the isolation of pathogen followed by the methods of Sawatdikarn (2011).

Collection of and preparation of plants samples

Ten medicinal crude extracts used in this study were obtained from three location in Phranakhon Si Ayutthaya province, Bangban, Wangnoi and Bangpa-in area. Fresh rhizomes of *C. xanthorrhiza*, *C.*

aromatica and *K. parviflora* were collected from Bangban, fresh leaves of *O. vulgare*, *S. nodiflora*, *S. bicolor*, *R. officinalis* and *E. odoratum* were collected from Wangnoi and fresh bud and fruit parts of *S. aromaticum* and *P. longum*, respectively were collected from Bangpa-in. They were washed with tap water and air dried for three days to eliminate surface moisture. Then each part of the medicinal plants was packed into envelop and kept in oven at 105°C temperature until it is dried. Dried parts were ground separately in an electric grinder to obtain powder which was than kept in plastic bags before the tests.

Preparation of crude extracts

100 grams of the dried powdered plant were soaked in 1,000 ml of 90% ethanol. These mixtures were refluxed followed by agitation at 200 rpm for 1 h. The ethanolic extracts were squeezed and filtered by muslin cloth. The crude extracts were placed into a wide tray to evaporate ethanol and added with water plant extracts (Prasad et al., 2010).

Antifungal activity test

Mycelial growth test

Food poisoning technique: Diffusates were added in PDA and poured into petri dishes. PDA medium added only with ethanol and water served as control treatment. Each petri dishes was inoculated with 5 mm plug of pure isolate taken from margins of actively growing culture of pathogen. All petri dishes were incubated at 25°C. The screening of crude extracts for antifungal activity was conducted using the agar dilution method. Different crude extracts were tested using food poisoning technique. Each tested crude extracts was used at different concentrations; 0 (control treatment), 1,000, 2,500, 5,000, 7,500 and 10,000 ppm. The petri dishes were incubated in room temperature for 7 days. The efficacy of treatment was assessed from all the four plate by measuring fungal colony development (cm). The mycelial growth inhibition (M) with respect to the control treatment was calculated from the formula (Sheng-Yang et al., 2005).

$$M = [(A-B) / A] \times 100$$

where A is the colony diameter of the control treatment and B is the colony diameter of the treated crude extracts.

Spore germination test

Effect of ten medicinal crude extracts on spore germination of *Alternaria* spp. was carried out by the spore germination method (Ting-Ting et al., 2011). A spore suspension of *Alternaria* spp. was prepared by adding 10 ml of sterile water to 15 days old petri plate culture of *Alternaria* spp. grown at 22°C on PDA. The surface of agar was washed through a double layer of gauze with sterile water. The inoculum was adjusted to a concentration of 1×10^6 ml with haemocytometer. One milliliter of ten crude extract in 20 and 40 mg/ml was mixed with the same amount of *Alternaria* spp. spore suspension. The mixture was pipetted onto clean concave slips and incubated at room temperature for 24 h. In the control, the sterile water was mixed with spore suspension. Germination rate of spores was observed with microscope after incubation of 12 h.

Each tested crude extracts was used at different concentrations; 0 (control treatment), 1,000, 2,500, 5,000, 7,500 and 10,000 ppm. The efficacy of treatment was assessed from four slide after treatment by measuring number of spore germination. The spore

Table 1. Efficacy of different concentration of some medicinal plant crude extracts on mycelial growth inhibition of *Alternaria* spp.

Medicinal plant crude extracts	Mycelial growth inhibition degree (%)				
	1,000 ppm	2,500 ppm	5,000 ppm	7,500 ppm	10,000 ppm
<i>Curcuma xanthorrhiza</i>	84 ^b	87 ^b	89 ^b	92 ^b	100 ^a
<i>Curcuma aromatica</i>	100 ^a	100 ^a	100 ^a	100 ^a	100 ^a
<i>Kaempferia parviflora</i>	49 ^c	57 ^c	70 ^c	72 ^c	75 ^b
<i>Sygygium aromaticum</i>	100 ^a	100 ^a	100 ^a	100 ^a	100 ^a
<i>Origanum vulgare</i>	100 ^a	100 ^a	100 ^a	100 ^a	100 ^a
<i>Synedrella nodiflora</i>	25 ^d	42 ^d	53 ^d	55 ^d	56 ^c
<i>Sorghum bicolor</i>	40 ^c	43 ^d	50 ^d	53 ^d	58 ^c
<i>Rosmarinus officinalis</i>	88 ^b	90 ^b	100 ^a	100 ^a	100 ^a
<i>Piper longum</i>	47 ^c	52 ^c	68 ^c	70 ^c	75 ^b
<i>Eupatirium odoratum</i>	40 ^c	46 ^d	60 ^c	69 ^c	71 ^b
CV (%)	10.28	11.26	9.86	12.64	8.54

In each column, mean followed by a common letter are not significantly different at the 5% level by DMRT.

germination inhibition (S) with respect to the control treatment was calculated from the formula (Sheng-Yang et al., 2005).

$$S = [(A-B) / A] \times 100$$

where A is the spore germination number of the control treatment and B is the spore germination number of the treated crude extracts.

Statistical analysis

For statistical analysis, Duncan Multiple Range Test was used to compare the average.

RESULTS AND DISCUSSION

The ten medicinal herb crude extracts showed inhibition on mycelial growth of *Alternaria* spp. different concentrations (Table 1). The crude extracts of *C. aromatica* (Figure 1), *S. aromaticum* and *O. vulgare* at all concentrations, the *R. officinalis* crude extracts at 5,000 ppm and the *Curcuma xanthorrhiza* crude extracts at 10,000 ppm (Figure 2) showed 100% inhibition on mycelial growth, whereas the *S. nodiflora* (Figure 3) and *S. bicolor* (Figure 4) crude extracts at 10,000 ppm gave the highest inhibition of 56 and 58%, respectively.

C. aromatica, *S. aromaticum* and *O. vulgare* crude extracts showed that 100% inhibition on spore germination (Table 2) at all concentrations can use crude extracts of these species for *Alternaria* spp. control (the pathogen of dirty panicle disease in rice) at all concentration (1,000 to 10,000 ppm).

The *C. aromatica* crude extracts showed 100% inhibition on mycelial growth and spore germination at all concentrations (1,000-10,000 ppm); this result agrees with *C. aromatica* for *S. aureus* and *E. coli* control (Saleem et al., 2011). For *S. aromaticum*, the crude

extracts showed 100% inhibition on mycelial growth and spore germination at all concentration (1,000-10,000 ppm); this result agrees with the clove oils for antimicrobial activity on some pathogen; *S. aureus* (Briozzo et al., 1989; Tranter et al., 1993), *Fusarium oxysporum* and *F. equiseti* (Kritzing et al., 2002) and *Phomopsis azadirachtae* (Prasad et al., 2010) showed the mycelial growth inhibition.

O. vulgare crude extracts showed 100% inhibition on mycelial growth and spore germination of *Alternaria* spp. at all concentration (1,000 to 10,000 ppm); this result agrees with the *O. vulgare* crude extracts inhibited the growth of *Bacillus cereus* (Paster et al., 1995). For the three crude extracts showed the inhibited mycelial growth in *Alternaria* spp., because phytochemical effects for antimicrobial activity, curcumin from the rhizome of *C. aromatica* (Saleem et al., 2011), phenolic compound and eugenol from the bud or fruit of *S. aromaticum* (Briozzo et al., 1989; Maarse, 1991) and carvacrol from the leaves of *O. vulgare* (Ultee et al., 2000; Chaudhry et al., 2007).

The two crude extracts, *R. officinalis* (5,000 to 10,000 ppm) and *C. xanthorrhiza* (10,000 ppm) showed 100% of inhibition on mycelial growth of *Alternaria* spp. (Table 1). This result agrees with the *R. officinalis* crude extracts showed the growth inhibition of *Leuconostoc mesenteroides*, *Listeria monocytogenes* and *S. aureus* (Campo et al., 2000) and the result indicated that the *C. xanthorrhiza* crude extracts showed the inhibition for three pathogen control, namely *B. cereus*, *S. aureus* and *E. coli* (Husein et al., 2009).

The three crude extracts, namely *C. xanthorrhiza*, *R. officinalis* and *P. longum* at 2,500 ppm reached the highest inhibition of spore germination at 100% (Table 2). For the *R. officinalis* and *C. xanthorrhiza* crude extracts showed the inhibition of mycelial growth and spore germination of *Alternaria* spp., because of phytochemical potential for antimicrobial activity, carnosic acid and

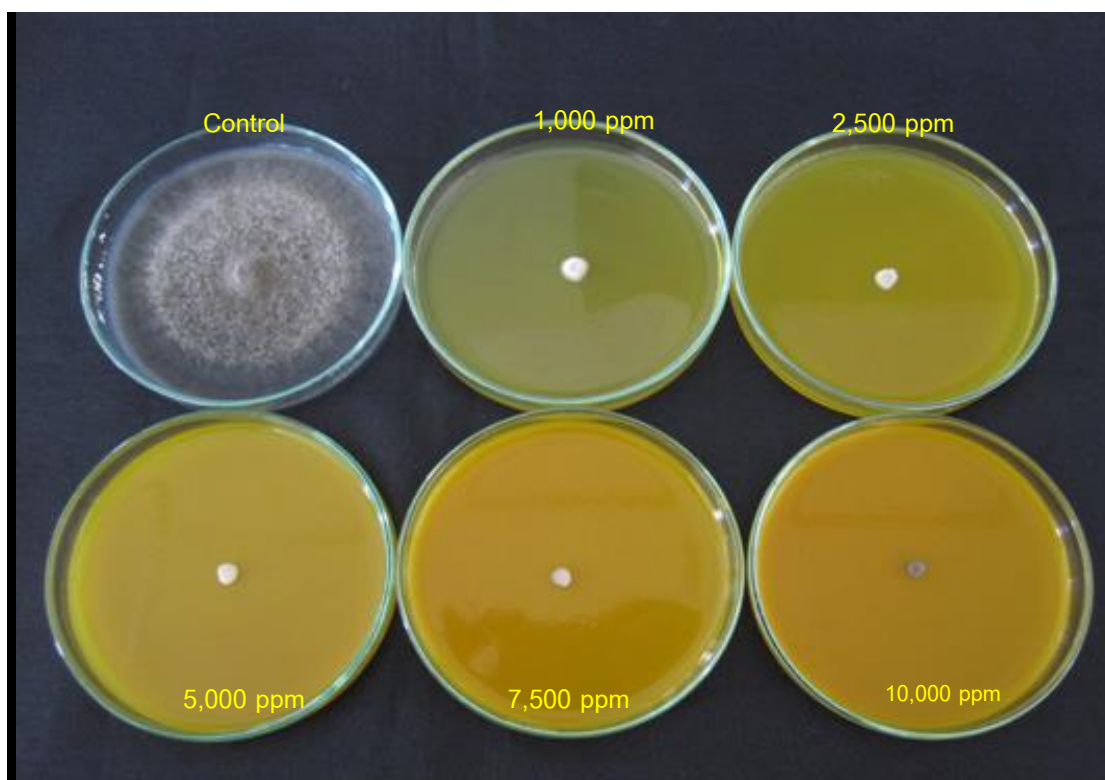


Figure 1. Effect of *Curcuma aromatica* crude extract on the mycelial growth of *Alternaria* sp. at different concentrations (Control treatment, 1,000 2,500 5,000 7,500 and 10,000 ppm).

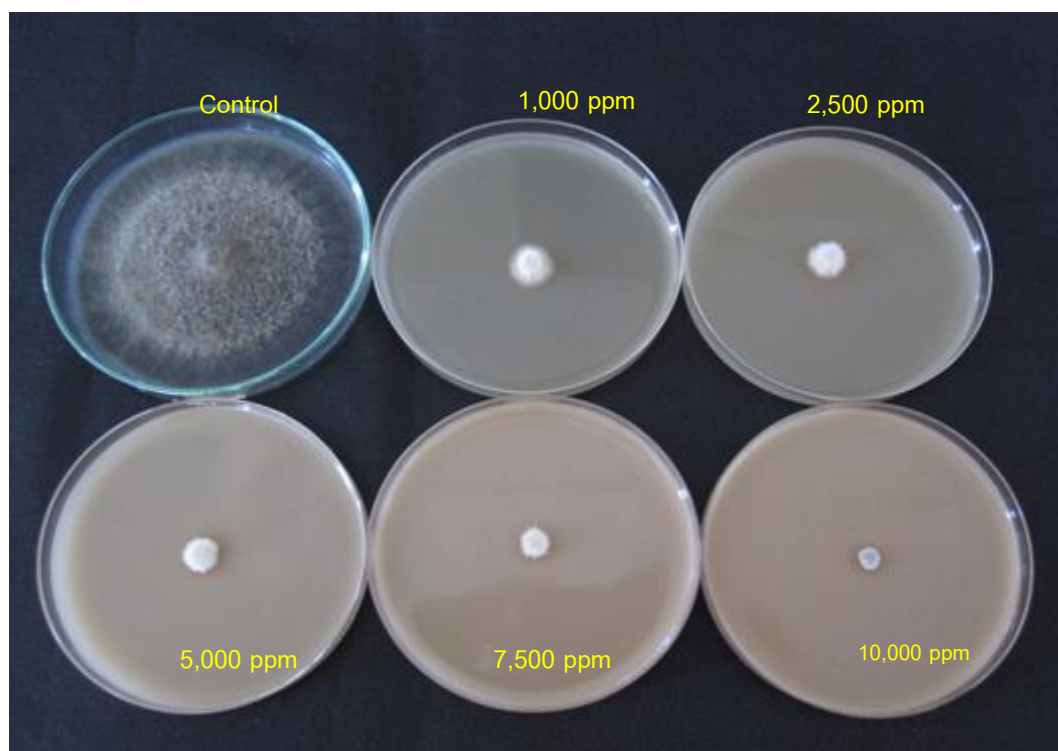


Figure 2. Effect of *Curcuma xanthorrhiza* crude extract on the mycelial growth of *Alternaria* sp. at different concentrations (Control treatment, 1,000 2,500 5,000 7,500 and 10,000 ppm).

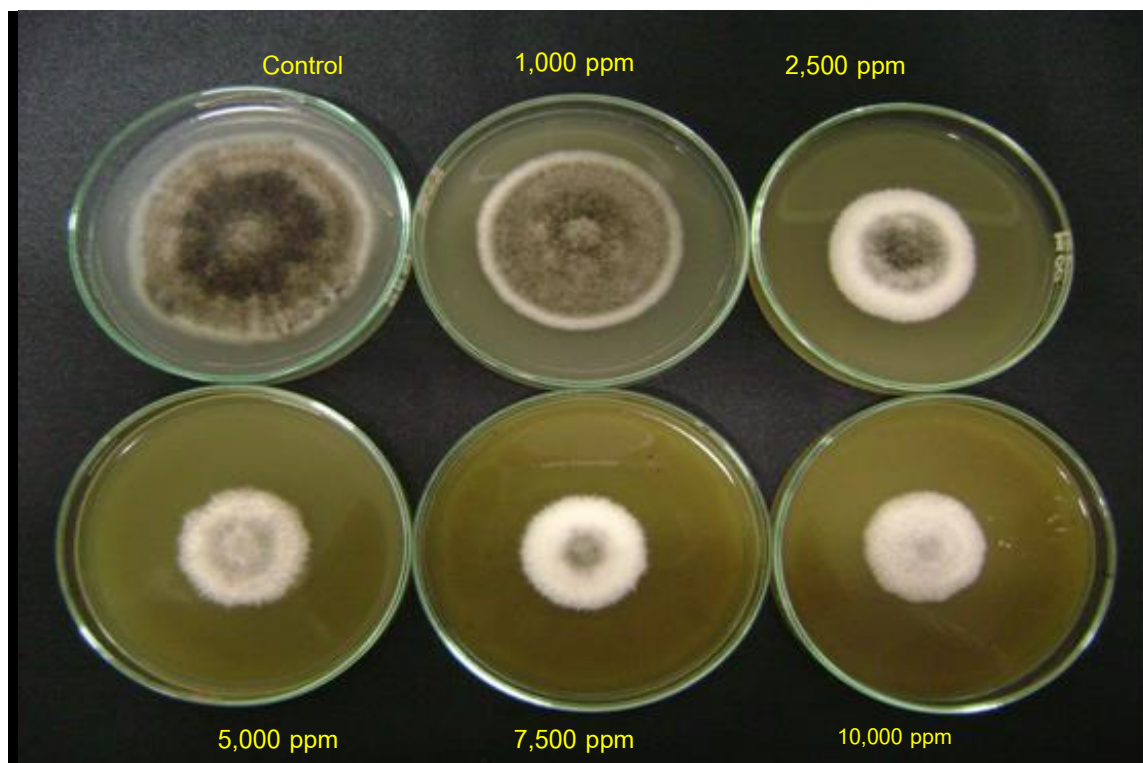


Figure 3. Effect of *Synedrella nodiflora* crude extract on the mycelial growth of *Alternaria* sp. at different concentrations (Control treatment, 1,000 2,500 5,000 7,500 and 10,000 ppm).

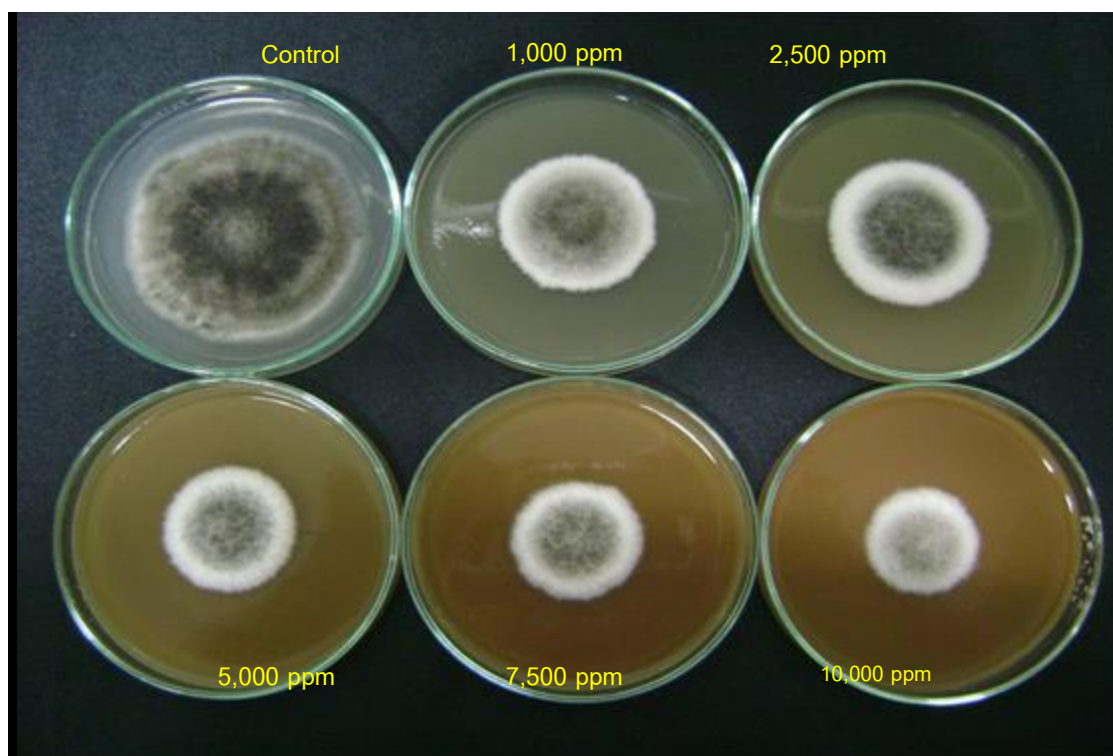


Figure 4. Effect of *Sorghum bicolor* crude extract on the mycelial growth of *Alternaria* sp. at different concentrations (Control treatment, 1,000 2,500 5,000 7,500 and 10,000 ppm).

Table 2. Efficacy of different concentration of some medicinal plants crude extracts on spore germination inhibition of *Alternaria* spp.

Medicinal plant crude extracts	Spore germination inhibition degree (%)				
	1,000 ppm	2,500 ppm	5,000 ppm	7,500 ppm	10,000 ppm
<i>Curcuma xanthorrhiza</i>	90 ^b	100 ^a	100 ^a	100 ^a	100 ^a
<i>Curcuma aromatica</i>	100 ^a	100 ^a	100 ^a	100 ^a	100 ^a
<i>Kaempferia parviflora</i>	0 ^d	0 ^b	25 ^b	30 ^b	55 ^b
<i>Synggium aromaticum</i>	100 ^a	100 ^a	100 ^a	100 ^a	100 ^a
<i>Origanum vulgare</i>	100 ^a	100 ^a	100 ^a	100 ^a	100 ^a
<i>Synedrella nodiflora</i>	90 ^b	100 ^a	100 ^a	100 ^a	100 ^a
<i>Sorghum bicolor</i>	80 ^c	100 ^a	100 ^a	100 ^a	100 ^a
<i>Rosmarinus officinalis</i>	85 ^b	100 ^a	100 ^a	100 ^a	100 ^a
<i>Piper longum</i>	85 ^b	100 ^a	100 ^a	100 ^a	100 ^a
<i>Eupatium odoratum</i>	0 ^d	0 ^b	0 ^c	10 ^c	20 ^c
C. V. (%)	9.24	3.65	6.54	8.78	6.85

In each column, mean followed by a common letter are not significantly different at the 5% level by DMRT.

rosmarinic acid from the leaves of *R. officinalis* (Frankel et al., 1996) and curcumin from the rhizome of *C. xanthorrhiza* (Husein et al., 2009).

The three crude extracts, *K. parviflora*, *P. longum* and *E. odoratum* at 10,000 ppm had the inhibition on mycelial growth at 75, 75 and 71%, respectively (Table 1). This result agrees with the *K. parviflora*, *P. longum* and *E. odoratum* crude extracts inhibited the growth of some pathogens; *K. parviflora* crude extracts for *B. cereus* and *S. aureus* control (Butkhup and Samappito, 2011) *P. longum* crude extracts for *Bacillus subtilis* control (Kumar et al., 2010) and *E. odoratum* showed the growth inhibition of two pathogen, namely *B. cereus* and *Aspergillus niger* (Owalabi et al., 2010). The phytochemical compounds in each species showed the inhibition on growth of pathogen; methoxyflavone from the rhizome of *K. parviflora* (Kummee et al., 2008), piperonaline from the fruit of *P. longum* (Lee et al., 2001) and alpha-pinene and beta-pinene from the leaves of *E. odoratum* (Owalabi et al., 2010).

S. nodiflora and *S. bicolor* crude extracts at 10,000 ppm gave the highest inhibition of mycelial growth at 56 and 58%, respectively (Table 1). *S. nodiflora* and *S. bicolor* crude extracts at 2,500 ppm showed the highest inhibition of spore germination at 100% (Table 2). This result agrees with the *S. nodiflora* crude extracts inhibited on growth in five pathogens, namely; *B. subtilis*, *S. aureus*, *E. coli*, *Candida albicans* and *Aspergillus flavus* (Bhogaonkar et al., 2010) and *S. bicolor* crude extracts inhibited on growth in three pathogens, namely; *Fusarium moniliforme*, *Curvularia lunata* and *A. flavus* (Seetharaman et al., 1997). The phytochemical compounds of *S. nodiflora* and *S. bicolor* crude extracts showed the inhibition on growth of some pathogens; alkaloids and flavanoids from the leaves of *S. nodiflora* (Bhogaonkar et al., 2010) and saponins and tannins from the leaves of *S. bicolor* (Soetan et al., 2006).

In total, this study indicated that the ten crude extracts can be used for *Alternaria* spp. control and three plant can be used for crude extracts of dirty panicle control. The crude extracts of three species, namely, *C. aromatica*, *S. aromaticum* and *O. vulgare* showed 100% inhibition on mycelial growth and spore germination of *Alternaria* spp. at all concentrations (1,000 to 10,000 ppm).

Conclusion

C. aromatica, *S. aromaticum* and *O. vulgare* crude extracts at 1,000 ppm, the *R. officinalis* crude extracts at 5,000 ppm and the *C. xanthorrhiza* crude extracts at 10,000 ppm showed the highest inhibition of mycelial growth at 100%, whereas the *S. nodiflora* and *S. bicolor* crude extracts at 10,000 ppm had the inhibition at 56 and 58%, respectively. For inhibition of spore germination, the three plant crude extracts, *C. aromatica*, *S. aromaticum* and *O. vulgare* at 1,000 ppm and the *C. xanthorrhiza*, *Synedrella nodiflora*, *S. bicolor*, *R. officinalis* and *P. longum* crude extracts at 2,500 ppm reached the highest inhibition of spore germination at 100%, whereas the *E. odoratum* and *K. parviflora* crude extracts at 10,000 ppm had the inhibition at 20 and 55%, respectively.

Conflict of Interests

The author has not declared any conflict of interests.

ACKNOWLEDGEMENTS

The author thanks the Faculty of Science and Technology, Phranakhon Si Ayutthaya Rajabhat University,

which supported this research and the Office of Higher Education Commission (OHEC) under Identity-Based Research Group (CHE-RES-IG) in 2010-2011, and Office of Higher Education Commission (OHEC) under Research Group of Good Agricultural Practices Procedures on Rice Production in 2011, which supported this research.

REFERENCES

- Abdelmonem AM (2000). Status of seed pathology and seed health testing in Egypt. *Seed Sci. Technol.* 28:533-547.
- Bhogaonkar P, Dagawal Y, Ghorpade DS (2010). Pharmacognostic studies and antimicrobial activity of *Synderella nodiflora* (L.) Gaertn. *Biosci. Disc.* 2:317-321.
- Briozzo J, Nunez L, Chirife J, Herszage L, Aquino MD (1989). Antimicrobial activity of clove oil dispersal in a concentrated sugar solution. *J. Appl. Bacteriol.* 66(1):69-75.
- Butkhup L, Samappito S (2011). Invitro free radical scavenging and antimicrobial activity of some selected Thai medicinal plants. *Res. J. Med. Plants* 5(3):254-265.
- Campo JD, Amiot M, Nguyen-Tae C (2000). Antimicrobial effects of rosemary extracts. *J. Food Prot.* 63(10):1359-1368.
- Chaudhry NMA, Saed S, Tarq P (2007). Antibacterial effects of oregano (*Origanum vulgare*) against gram negative bacilli. *Pak. J. Bot.* 39(2):609-613.
- Frankel EN, Hunag SW, Aeschbach R, Prior E (1996). Antioxidant activity of a rosemary extract and its constituents, carnosic acid, carnosol and rosmarinic acid, in bulk oil and oil-in water emulsion. *J. Agric. Food Chem.* 44(1):131-135.
- Husein S, Parhusip A, Ramasi EF (2009). Study on antibacterial activity from Temulawak (*Curcuma xanthorrhiza* Roxb.) rhizomes against pathogenic microbes cell destruction. *J. Appl. Ind. Biotechnol. Trop. Region* 2(1):1-4.
- Kritzinger Q, Aveling TAS, Marasas WFO (2002). Effect of essential plant oils on storage fungi, germination and emergence of cowpea seeds. *Seed Sci. Technol.* 30(3):609-619.
- Kumar BSA, Swamy VBN, Kumar PAA, Khan S (2010). Evaluation of antioxidant and antimicrobial activities of chitrakadi vati, A ayurvedic formulation. *J. Food Saf.* 12(2):158-164.
- Kummee S, Tewtrakul S, Subhadhirasakul S (2008). Antimicrobial activity of the ethanol extract and compounds from the rhizome of *Kaempferia parviflora*. *Songklanakarin J. Sci. Technol.* 30(4):463-466.
- Ou SH (1985). *Rice Disease*. Commonwealth Agricultural Bureaux 2nd Ed. Great Britain. 380 p.
- Owalabi MS, Ogundajo A, Yusuf KO, Lajide L, Villanueva HE, Tuten JA, Setzor WN (2010). Chemical composition and bioactivity of the essential oil of *Chromolaena odorata* from Nigeria. *Records Nat. Prod.* 4(1):72-78.
- Lee SE, Park BS, Kim MK, Choi WS, Kim HT, Cho KY, Lee SG, Lee HS (2001). Fungicidal activity of piperonaline. A piperidine alkaloid derived from long pepper, *Piper longum* L. against phytopathogenic fungi. *Crop Prot.* 20(6):523-528.
- Maarse H (1991). *Volatile Compounds in Food and Beverages*. Macel Dekker, INC., New York, USA. 764 p.
- Paster N, Menashero M, Ravid U, Juven B (1995). Antifungal activity of oregano and thyme essential oils applied as fumigants against fungi attacking stored grain. *J. Food Prot.* 58(1):81-85.
- Prasad MNN, Bhat S, Sreenivasa MY (2010). Antifungal activity of essential oils against *Phomopsis azadirachtae* - The causative of die-back disease of neem. *J. Agric. Technol.* 6(1):127-133.
- Saleem M, Daniel B, Murli K (2011). Antimicrobial activity of three different rhizomes of *Curcuma longa* and *Curcuma aomatica* on uropathogens of diabetic patients. *Int. J. Pharm. Pham. Sci.* 3(4):273-279.
- Sawatdikarn S (2011). Antifungal activity of twenty-four medicinal crude extracts against *Curvularia* sp., The pathogen of dirty panicle disease in rice. In 37th Congress on Science and Technology of Thailand. pp. 1-8.
- Seetharaman K, Whitehead E, Keller NP, Waniska RD, Rooney LW (1997). *In vitro* activity of sorghum seed antifungal proteins against grain mold pathogen. *J. Agric. Food Chem.* 45(9):3666-3671.
- Sheng-Yang W, Pin-Fun C, Shang-Tzen C (2005). Antifungal activities of essential oil and their constituents from indigenous cinnamon (*Cinnamomum osmophloeum*) leaves against wood decay fungi. *Bioresour. Technol.* 96(7):813-818.
- Soetan KO, Oyekunle MA, Aiyelaagbe OO, Rafunso MA (2006). Evaluation of the antimicrobial activity of saponin extract of *Sorghum bicolor* L. Moench. *Afr. J. Biotechnol.* 5(23):2405-2407.
- Ting-Ting W, Zhi-Hui C, Khan MA, Qing M, Ling AH (2011). The inhibitive effects of garlic bulb crude extract on *Fulvia fulva* of tomato. *Pak. J. Bot.* 43:2575-2580.
- Tranter H, Tassou SC, Nychas GJ (1993). The effect of the clove phenolic compound, oleuropien, on growth and enterotoxin B production by *Staphylococcus aureus*. *J. Appl. Bacteriol.* 74(3):253-9.
- Ultee A, Slump RA, Steging G, Smid EJ (2000). Antimicrobial activity of carvacrol toward *Bacillus cereus* on rice. *J. Food Prot.* 63(5):620-624.