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Research Article

Antifungal properties of essential oils from Thai medical plants against rice pathogenic fungi

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Abstract

This *in vitro* study was aimed to evaluate the mycelium growth and spore germination inhibition properties of essential oils. Two Thai medicinal plants; Frankincense oil (*Boswellia carteri* Bird.) and Cassia oil (*Acacia farnesiana* Linn) were applied against 7 species of economically important rice pathogenic fungi; *Alternaria brassicicola, Aspergillus flavus, Bipolaris oryzae, Fusarium moniliforme, Fusarium proliferatum, Pyricularia arisea* and *Rhizoctonia solani*. The mycelium growth and spore germination inhibition techniques were applied to record the efficiency of these essential oils at 0.4, 0.6, 0.8, 1.0 and 2.0 %(v/v) and an un-used amount of essential oils were subjected as control. The experiment was *in vitro* studied on potato dextrose agar (PDA) under complete randomised design (CRD) with 3 replications. The data of mycelium growth inhibition was recorded at 7 days after inoculation at 25±20^{0}C showed that, Frankincense oil at concentration 2.0%v/v showed the strongest mycelium growth inhibition of *F.monoliform* (61.11%), *F. proliferratum* (16.66%), *P.grisea* (33.33%), *B. oryzae* (33.33%), *R. solani* (44.44%), *A. brassicicola* (71.29%) and *A. flavus* (11.11%). Cassia oil could inhibit mycelium growth of all pathogenic fungi when applied at a concentration over 1.0 %v/v. The data of spore germination inhibition recorded at 24 hr after inoculation at 25±20^{0}C showed that, at 2.0%v/v; Frankincense oil could control *F. monoliform* (84.78%), *B. oryzae* (76.27%), *R. solani,* (94.69%), *A. flavus* (88.80%) and *F. proliferratum*, *P. grisea* and *A. brassicicola* for 100%. Cassia oil at 1.0%v/v could completely inhibit *F. monoliform*, *F. proliferratum*, *P. grisea*, *B. oryzae, R. solani* and *A. brassicicola* (100%) *and A. flavus* for 76.00%. The experiment suggested that the selected essential oils from

Thai medicinal plants showed antifungal properties, on both mycelium growth and spore germination, for rice pathogenic fungi. These properties were dependent on plant and fungal species, concentration and the testing conditions.

Keywords: Frankincense oil, Cassia oil, mycelium, Thai medicinal plant, biocontrol

Introduction

Rice is a staple food for much of the world's population, particularly in Asia [1](Paranagama *et al*., 2003). However, specific conditions; temperature, relative humidity and moisture content of rice seed [2](Yasmin *et al*., 2008), may contribute to the rapid deterioration of stored rice by promoting fungal growth [3](Christensen and Saucer 1992). Fungal infections may discolour grains, change their chemical and nutritional characteristics and reduce germination [4](Paster *et al*., 1993). Pathogenic fungi may grow rapidly via the production of sexual parts such as spores, which may consist of one or more cells [5](Ramezani *et al*., 2002), which then produce asexually to form mycelium. The fungal rice diseases observed were; blast, brown leaf spot, sheath blight, bakanea and root rot, which are caused by seven major pathogenic fungi; *Pyricularia oryzae*, *Bipolaris oryzae*, *Fusarium moniliforme, Fusarium proliferatum*, *Alternaria brassicicola, Aspergillus flavu*s and *Rhizoctonia solani* respectively. These fungi may spread rapidly via the production of spores and mycelium respectively, which cause reductions in crop yield and degradation of grain quality [6](Rice, 1995). Thus, the aim of this study was to screen for the best *in vitro* mycelium growth and spore germination inhibition using frankincense oil (*Boswellia carteri* Birdw) and cassia oil (*Acacia farnesiana* Linn) against rice pathogenic fungi as a possible organic alternatives for synthetic chemical antifungal compounds.

Materials and Methods

The pathogenic fungi; *Alternaria brassicicola, Aspergillus flavus, Bipolaris oryzae, Fusarium moniliforme, Fusarium proliferatum, Pyricularia arisea* and *Rhizoctonia solani* were obtained from the Department of Agriculture, Thailand. The experiments were conducted at the Department of Agricultural Technology, Faculty of Technology, Maha Sarakham University (MSU), Thailand in 2009. The experiment was laid out in a Completely Randomized Design (CRD) at various concentrations; 0.4, 0.6, 0.8, 1.0 and 2.0 %(v/v) and un-used portions of essential oils were subjected as control, of two essential oils; frankincense oil (*Boswellia carteri* Birdw) and cassia oil (*Acacia farnesiana* Linn), following 3 replications. The *in vitro* study of mycelium growth and spore germination inhibition techniques were applied to record the efficiency of these essential oils.

Mycelium growth inhibition

The application of the two essential oils at different concentrations, 0.4, 0.6, 0.8, 1.0 and 2.0 %(v/v) on PDA medium was *in vitro* tested against the fungal mycelium growth. PDA medium (20 ml) was dispensed into Petri dish and 5 mm diameter of the tested fungi cut from the middle of 7-day-old cultures were incubated upside down separately to each assay plant and incubated for 96 hrs at 25±2^{o}C. The inhibition percentage was calculated by following the equation of Pitipong [7](2008);

$$\text{Inhibition (\%)} = [(C-T)/C] \times 100 \qquad \text{(Eq. 1)}$$

When: C was the colony diameter of the mycelium on the control plate (mm), and T was the colony diameter of the mycelium on the treatment plate (mm).

Spore germination inhibition

Spore concentration of *Alternaria brassicicola, Aspergillus flavus, Bipolaris oryzae, Fusarium moniliforme, Fusarium proliferatum, Pyricularia arisea* and *Rhizoctonia solani* was adjusted to approximately to 10^6 cfu mL^{-1} by the hemacytometer. Sterile microscope slides were dropped with 10 µL of PDA aqueous medium to obtain a thin agar layer on the slide, then 10 µL of spore suspension sample was gently spread on each slide. An uncovered watch glass containing either 10 µL of sterile water as control, or 5 µL of plant essential oils at concentrations 0.4, 0.6, 0.8, 1.0 and 2.0 %(v/v) were dropped onto the slide, incubated for 24 hrs at 25±2°C and spore germination inhibition was calculated following Eq. 1.

Results

The data of mycelium growth inhibition recorded at 7 days after inoculation at 25±20°C showed that frankincense and cassia essential oil treatment could inhibit fungal mycelium growth (Figure 1).

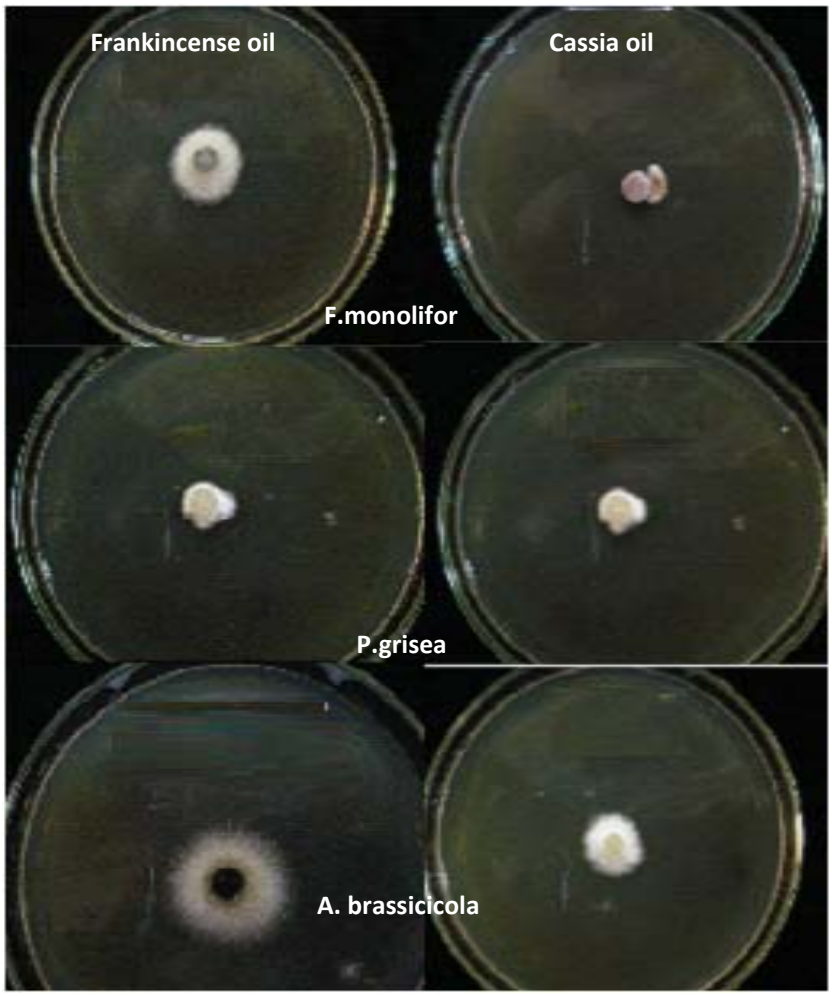

Figure 1. The effect of cassia oil and frankincense oil treatment at 2.0 %v/v on mycelium growth inhibition.

Frankincense oil at concentration 2.0%v/v showed the strongest mycelium growth inhibition of *F.monoliform* (61.11%), *F. proliferratum* (16.66%), *P.grisea* (33.33%), *B. oryzae* (33.33%), *R. solani* (44.44%), *A. brassicicola* (71.29%) and *A. flavus* (11.11%). Cassia oil could completely inhibit mycelium growth of all pathogenic fungi when applied at a concentration over 1.0 %v/v (Figure 2).

The data of spore germination inhibition was recorded at 24 hr after inoculation at 25±20^0 C showed that, at 2.0%v/v; frankincense oil could control *F. monoliform* (84.78%), *B. oryzae* (76.27%), *R. solani,* (94.69%), *A. flavus* (88.80%). and *F. proliferratum*, *P. grisea*, and *A. brassicicola* for 100%, while cassia oil at 1.0%v/v could completely inhibit *F. monoliform*, *F. proliferratum*, *P. grisea*, *B. oryzae, R. solani,* and *A. brassicicola* (100%) *and A. flavus* for 76.00% v/v (Figure 3).

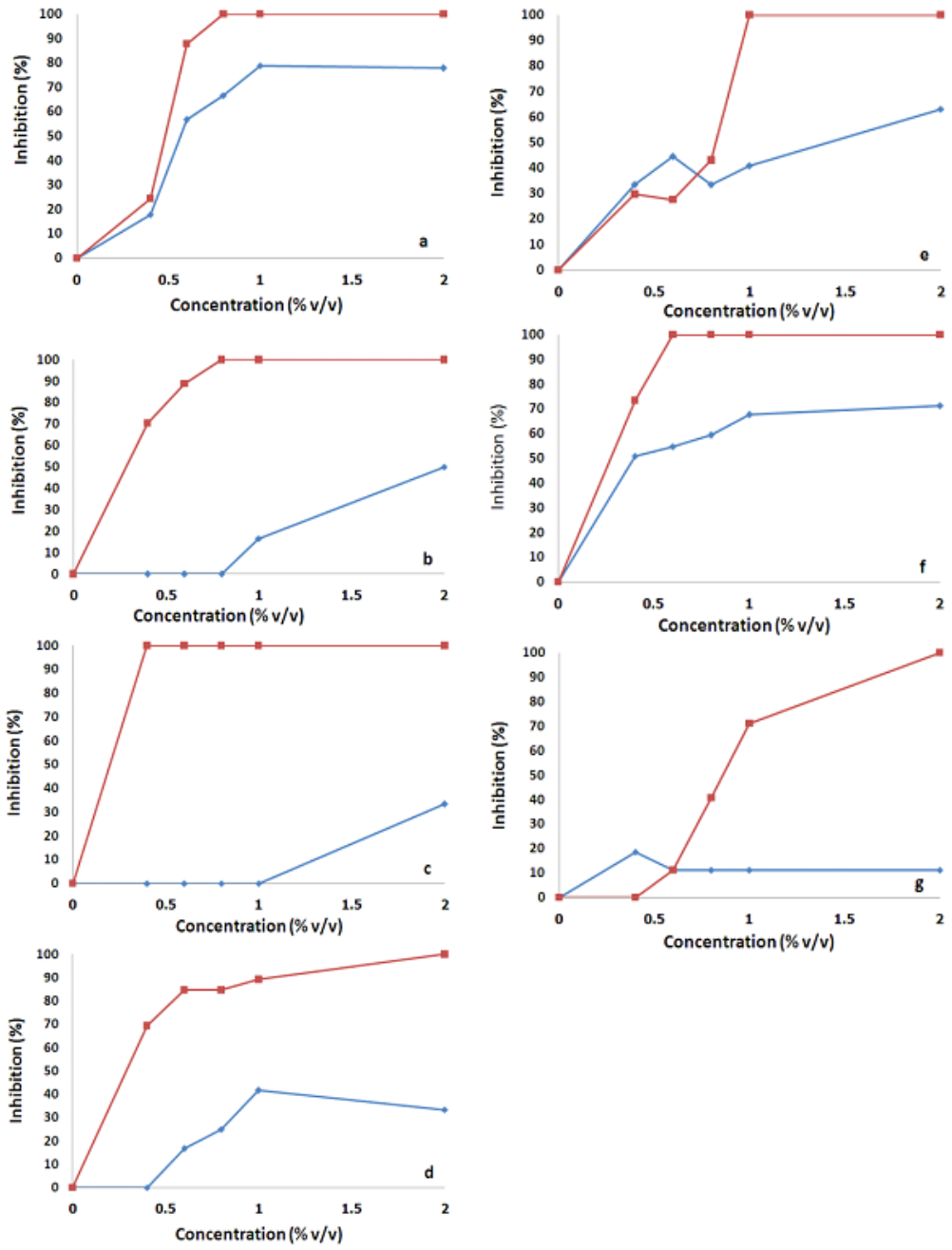

Figure 2. The mycelium growth inhibition effect at various concentrations of *Boswellia carteri* Birdw and *Acacia farnesiana* Linn essential oils on *F. Monoliform* (a), *F.Proliferratum* (b), *P. Grisea* (c), *B. Oryzae* (d), *R. Solanai* (e), *A. Brassicicola* (f), *A. Flavus* (g); □: *Boswellia carteri Birdw* (Cassia oil), ◊: *Cassia Flower Acacia farnesiana* Linn (Frankincense oil).

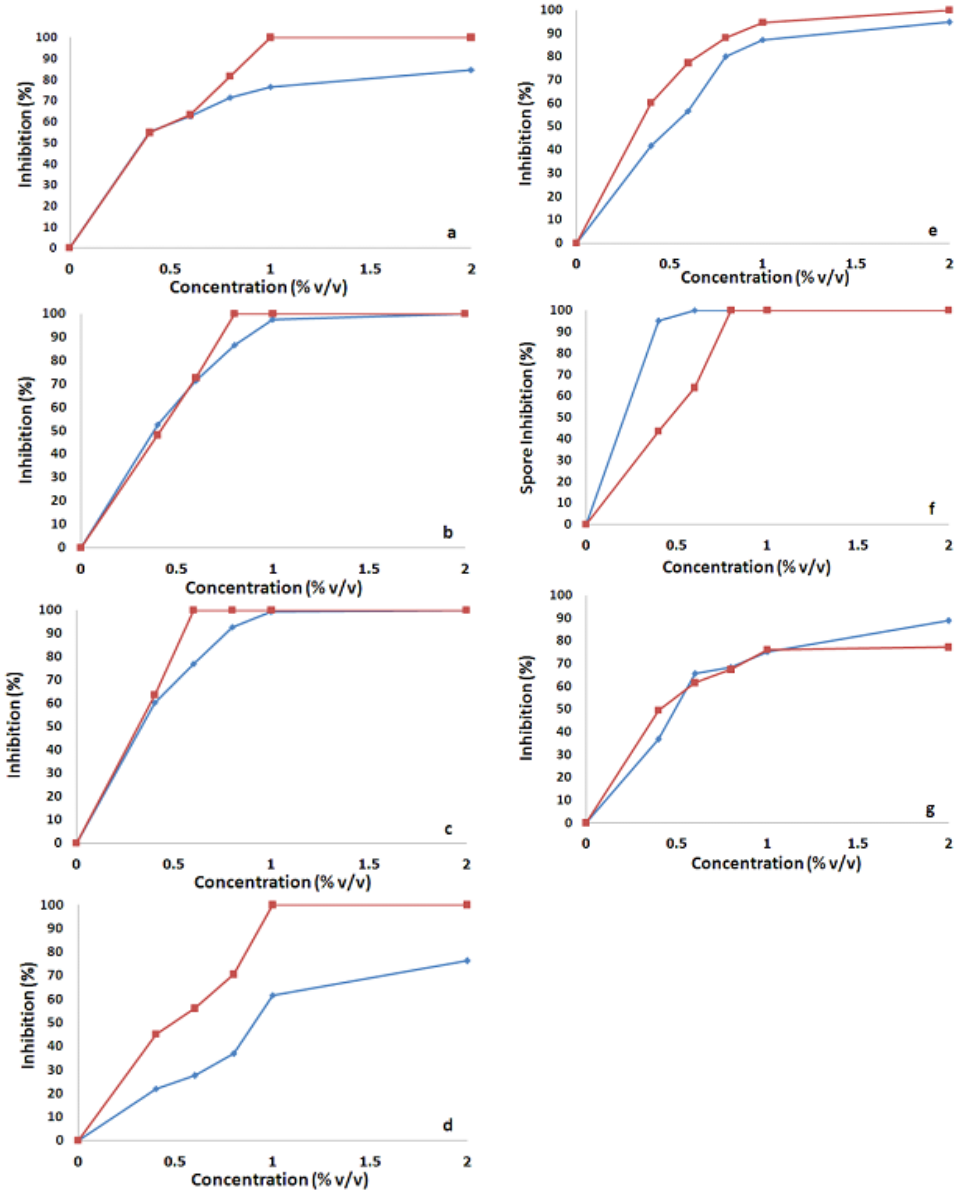

Figure 3. The spore germination effect at various concentrations of *Boswellia carteri Birdw* and *Cassia Flower Acacia farnesiana* Linn essential oils on *F. Monoliform* (a), *F.Proliferratum* (b), *P. Grisea* (c), *B. Oryzae* (d), *R. Solanai* (e), *A. Brassicicola* (f), *A. Flavus* (g); □: *Boswellia carteri Birdw* (Cassia oil), ◊: *Cassia Flower Acacia farnesiana* Linn (Frankincense oil).

The EC_{50} values indicated that frankincense oil showed strongest spore germination inhibition on *A. Brassicicola* at 0.12 %(v/v), while cassia oil showed spore germination inhibition on *P. Grisea* at 0.13 %(v/v). For mycelium growth inhibition, the EC_{50} values of frankincense oil on *A. Brassicicola* was 0.77 %(v/v), while cassia oil was 0.06 %(v/v) (Table 1).

Table 1. The EC_{50} of mycelium growth and spore germination inhibition of frankincense and cassia essential oils against rice pathogenic fungi.

Pathogenic fungi	Spore germination inhibition At 24 hrs		Mycelium growth inhibition At 7 days	
	Frankincense oil	Cassia oil	Frankincense oil	Cassia oil
F. monoliform	0.56	0.43	0.80	0.41
F. proliferratum	0.40	0.36	1.43	0.14
P. grisea	0.30	0.13	2.42	0.16
B. oryzae	1.13	0.55	2.37	0.27
R. solanai	0.57	0.33	1.33	0.80
A. brassicicola	0.12	0.42	0.77	0.06
A. flavus	0.66	0.64	16.26	0.73

Discussion and Conclusion

The efficacy tests of two essential oils were undertaken by mycelium growth and spore germination inhibition under *in vitro* conditions against rice pathogenic fungi. The experimental results showed that cassia oil at concentration 2.0%(v/v) completely inhibited mycelium growth and spore germination on *A. brassicicola, A. flavus, B. oryzae, F. moniliforme, F. proliferatum, P. arisea* and *R. solani*. On the other hand, frankincense oil showed susceptibility on mycelium growth and spore germination inhibition on all species pathogenic fungi. Farid *et al.* [7] and Fiori *et al*. [8], reported that the efficacy of medicinal plants against various species of pathogenic fungi was dependent on the origin of the medicinal plant and the sensitivity of the target fungi. The present results showed that essential oils of the medicinal plants; frankincense oil (*Boswellia carteri* Birdw) and cassia flower oil (*Acacia farnesiana* Linn) are able to inhibit the mycelium growth and spore germination of *Alternaria brassicicola, Aspergillus flavus, Bipolaris oryzae, Fusarium moniliforme, Fusarium proliferatum, Pyricularia arisea* and *Rhizoctonia solani*, which is a clear indication of their potential as an alternative source of synthetic fungicides to control these pathogenic fungi.

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