As. J. Food Ag-Ind. **2009**, *Special Issue*, S2-S9

Asian Journal of Food and Agro-Industry
ISSN 1906-3040
Available online at www.ajofai.info

Research Article

Antifungal activity of essential oils from basil (*Ocimum basilicum* Linn.) and sweet fennel (*Ocimum gratissimum* Linn.): Alternative strategies to control pathogenic fungi in organic rice

Apinya Piyo, Julara Udomsilp, Peerayot Khang-Khun and Pitipong Thobunluepop*

Department of Agricultural Technology, Faculty of Technology, Mahasarakham University, Mahasarakham 44000 Thailand.

*Author to whom correspondence should be addressed, email: pitipongtho@yahoo.com

This paper was originally presented at the International Symposium "Go Organic", Bangkok, Thailand, August 2009.

Abstract

This in vitro study was aimed to evaluate the mycelium growth and spore germination inhibition properties of Thai medicinal essential oils. The oil from two species of Thai medicinal plant; Basil (*Ocimum basilicum* Linn) and Sweet Fennel (*Ocimum gratissimum* linn.), were applied against 7 species of 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 while un-used amounts of these oils were used as the control. The experiment was conducted *in vitro*, using potato dextrose agar (PDA) under Complete Randomized Design (CRD) with 3 replications. The data of mycelium growth inhibition recorded at 7 days after inoculation at 25±2^{o}C showed that Basil Oil at a concentration of 0.6%v/v showed the strongest mycelium growth inhibition of *F.moniliform* (100%), *F. proliferratum* (49.6%) and *P. grisea* (100%). At 2.0%v/v, *B. oryzae*, *A. brassicicola* and *A. flavus* was inhibited by 97.40, 94.62 and 59.25%, respectively. However, basil essential oil was not effective in controlling *R. solani*. Sweet Fennel Oil could inhibit mycelium growth of all pathogenic fungi when the concentration applied was over 0.8 %v/v. The data of spore germination inhibition recorded at 24 hr after inoculation at 25±2^{o}C showed that, at 0.8%v/v; basil essential oil could control *F. monoliform* (91.31%) and *A. brassicicola* (99.74%). On the other hand, at 2.0%, *F. proliferratum*, *P. grisea*, *B. oryzae*, *R. solani*, and *A. flavus* were inhibited. Sweet Fennel Oil could completely inhibit (100%) spore

germination of all pathogenic fungi when the concentration applied was over 0.8 %v/v. Thus, the experiment suggested that essential oils from Thai medicinal plants demonstrated antifungal properties, in both mycelium growth and spore germination, on the rice pathogenic fungi. These properties were dependent on plant spiciness, concentration and the testing conditions.

Keywords: biological control, medicinal plants, *Alternaria brassicicola*, *Aspergillus flavus, Bipolaris oryzae*, *Fusarium moniliforme, Fusarium proliferatum, Pyricularia grisea*, *Rhizoctonia solani*

Introduction

Rice (*Oryza sativa* L.) is a major dietary staple food for much of the world's population, particularly in Asia [1]. Rice, at all stages of production is known to be susceptible to attack from many pathogenic fungi, particularly *Alternaria brassicicola*, *Aspergillus flavus, Bipolaris oryzae*, *Fusarium moniliforme, Fusarium proliferatum, Pyricularia grisea* and *Rhizoctonia solani*. However, the use of chemical treatment to control these fungi has raised many concerns due to their impact on human health and the environment [2]. They have also been shown to have negative effects on the food chain and increase fungicide resistance problems [3].

Many medicinal plants contain active compounds which are able to inhibit microbial growth [4]. Researchers have been able to achieve similar control results using this natural method when compared with chemical fungicide [5]. Thus, the aim of this study was to screen for the best *in vitro* antifungal activity of Basil Oil (*Ocimum basilicum* Linn) and Sweet Fennel Oil (*Ocimum gratissimum* Linn.) against rice pathogenic fungi as a possible alternative to synthetic chemical antifungal compounds.

Materials and Methods

The *in vitro* study was aimed to evaluate the mycelium growth and spore germination inhibition properties of two essential oils. Basil and Sweet Fennel oils were applied against 7 species of economically-damaging rice pathogenic fungi; *Alternaria brassicicola, Aspergillus flavus, Bipolaris oryzae, Fusarium moniliforme, Fusarium proliferatum, Pyricularia grisea* and *Rhizoctonia solani*. The experiment was carried out at the Department of Agricultural Technology, Faculty of Technology, Mahasarakham University (MSU). The effectiveness of essential oil from Basil and Sweet Fennel against mycelium and spore germination inhibition at different concentrations was studied. The experiment was conducted using CRD with 3 replications. Essential oils at concentrations; 0, 0.4, 0.6, 0.8, 1.0 and 2.0 %v/v, on PDA medium, were *in vitro* tested. The agar diffusion method was applied to evaluate their effectiveness. The data was displayed in means after analysis of the last significant difference at 95% (LSD≤0.05) by Statistix Program (version 8.0).

Mycelium growth inhibition analysis

The experiments were conducted by using the agar overlay technique [6]. The essential oil from Basil and Sweet Fennel at different concentrations (0, 0.4, 0.6, 0.8, 1.0, and 2.0 %v/v) on PDA medium was *in vitro* tested against the fungi mycelium growth. The colony diameter was measured and the mycelium inhibition percentage was calculated by following the method of Deans and Svoboda [7] (Eq. 1). Three replications of each treatment were tested and the average was calculated. Control sets were simultaneously run without using the essential oil.

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

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 inhibition analysis

Spores of *Alternaria brassicicola, Aspergillus flavus, Bipolaris oryzae, Fusarium moniliforme, Fusarium proliferatum, Pyricularia arisea* and *Rhizoctonia solani* were used as test fungi. Spore concentration of these fungi was adjusted to approximately 10^6 cfu ml^{-1} by using a 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 5 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, then incubated for 24 hrs at 25±2^{o}C [6] (Pitipong,2008) and spore inhibition was calculated by following Eq. 1.

Results

The data of mycelium growth inhibition recorded at 7 days after inoculation at 25±20^{o}C showed that both Basil oil and Sweet Fennel essential oil treatment could inhibit fungal mycelium growth (Figure 1). Basil oil at a concentration of 0.6%v/v showed the strongest mycelium growth inhibition of *F.monoliform* (100%), *F. proliferratum* (49.6%) and *P. grisea* (100%). At 2.0%v/v, *B. oryzae*, *A. brassicicola* and *A. flavus* were inhibited by 97.40, 94.62 and 59.25%, respectively. However, basil essential oil was not effective in controlling *R. solani*. Sweet Fennel could inhibit mycelium growth of all pathogenic fungi when the concentration applied was over 0.8 %v/v (Figure 2).

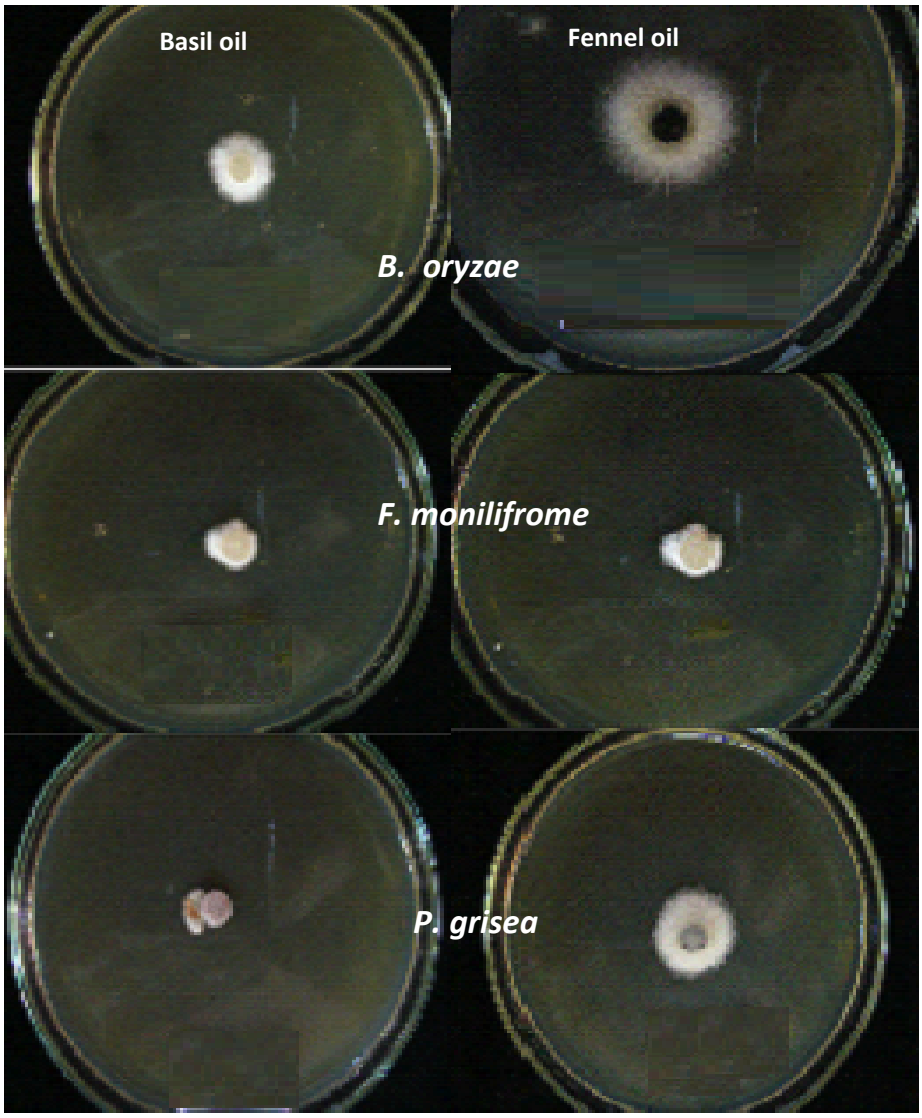

Figure 1. Effect of Basil and Sweet Fennel oil treatment at 2.0 %v/v on mycelium growth inhibition of *B. oryzae, F. moniliforme* and *P. grisea.*

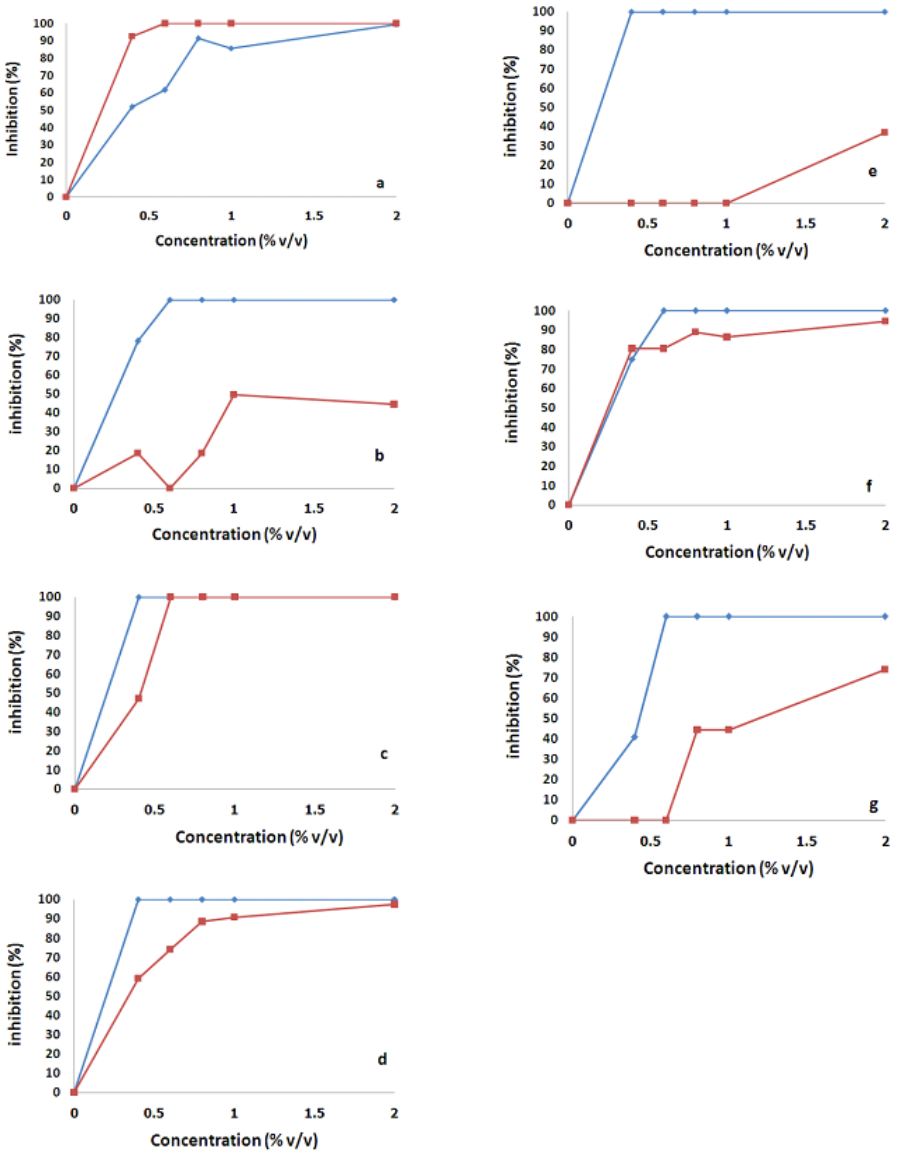

Figure 2. Mycelium growth inhibition effect at various concentrations of *Ocimum basilicum* and *Ocimum gratissimum* essential oils on *F. monoliform* (a), *F. proliferratum* (b), *P. grisea* (c), *B. oryzae* (d), *R. solanai* (e), *A. brassicicola* (f), *A. flavus* (g); □: *Ocimum basilicum* (Basil oil), ◊: *Ocimum gratissimum* (Sweet Fennel oil).

The data of spore germination inhibition recorded at 24 hrs after inoculation at 25±2 ^{o}C showed that, at 0.8%v/v; basil essential oil could control *F. moniliform* (91.31%) and *A. brassicicola* (99.74%). On the other hand, at 2.0%v/v, *F. proliferratum*, *P. grisea*, *B. oryzae*, *R. solani* and *A. flavus* were also inhibited. Sweet Fennel oil could completely inhibit (100%) spore germination of all tested pathogenic fungi when the concentration applied was over 0.8 %v/v (Figure 3).

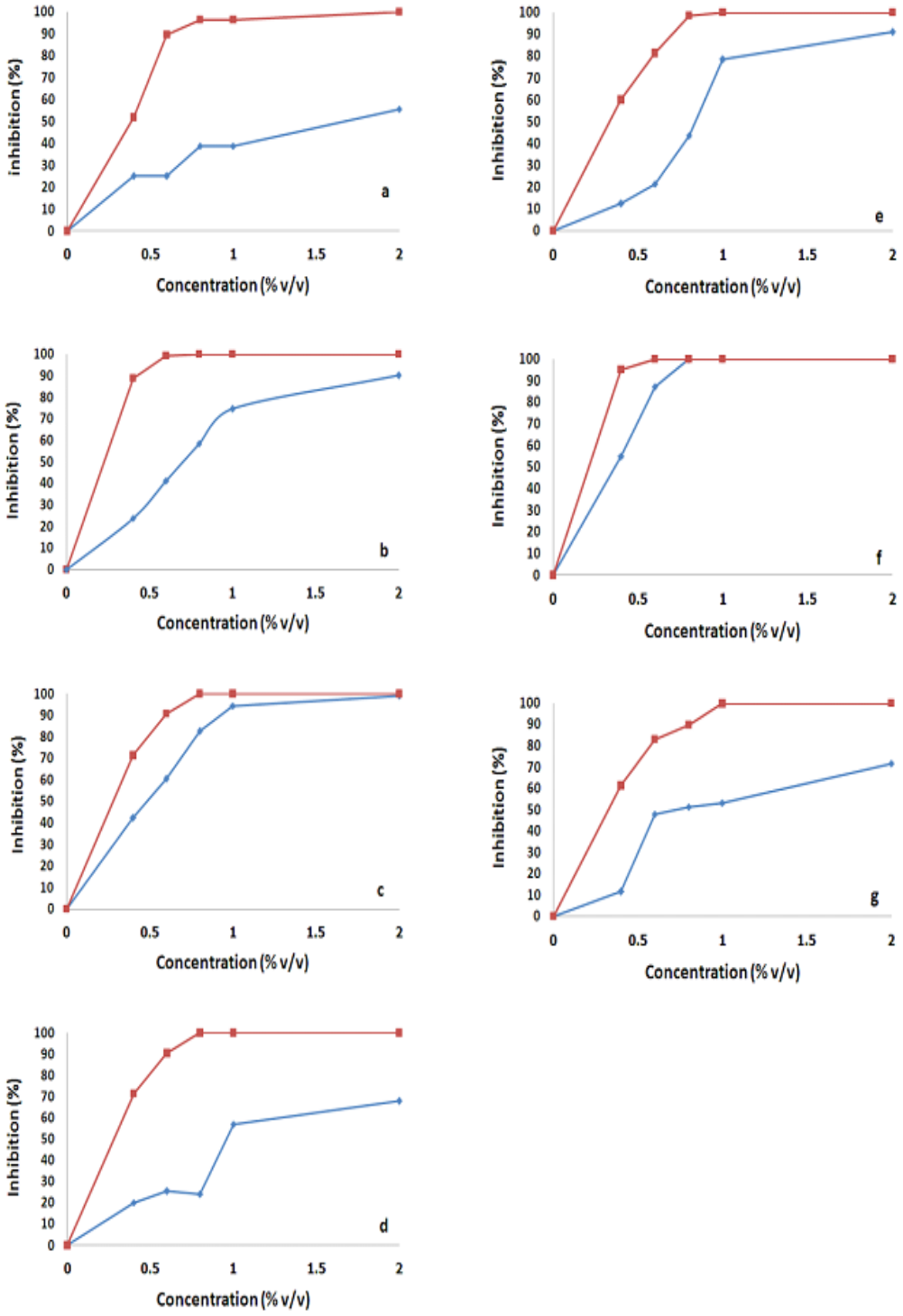

Figure 3. Spore germination inhibition effect at various concentrations of *Ocimum basilicum* and *Ocimum gratissimum* essential oils on *F. moniliform* (a), *F. proliferratum* (b), *P. grisea* (c), *B. oryzae* (d), *R. solanai* (e), *A. brassicicola* (f), *A. flavus* (g); □: (Basil oil), ◊: (Sweet Fennel oil).

The EC_{50} values indicated that Sweet Fennel oil showed the strongest spore germination inhibition on *A. Brassicicola* at 0.26%(v/v), while Basil oil showed spore germination inhibition on *F. Proliferratum* at 0.06%(v/v). For mycelium growth inhibition, the EC_{50} values of Sweet Fennel oil on *F. Moniliform* was 0.17%(v/v), while Basil oil showed mycelium growth inhibition on *F. Proliferratum* at 0.02%(v/v) (Table 1).

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

Pathogenic fungi	Spore germination inhibition at 24 hrs		Mycelium growth inhibition at 7 days	
	Sweet Fennel	Basil oil	Sweet Fennel	Basil oil
F. moniliform	0.46	0.10	0.17	1.55
F. proliferratum	0.84	0.06	10.03	0.02
P. grisea	0.52	0.13	0.23	0.16
B. oryzae	1.31	0.13	0.35	0.16
R. solanai	0.98	0.26	2.13	0.16
A. brassicicola	0.26	0.12	0.18	0.04
A. flavus	1.10	0.28	1.03	0.27

Results and Discussion

The present study found that essential oil from Basil and Sweet Fennel at a concentration of 2.0% v/v could inhibit fungal mycelium growth. These results are in accord with Soliman *et al*., [8], who reported that essential oils of basil and spearmint caused complete growth inhibition of four fungi at 3000 ppm, *A. ochraceus* was affected severely by spearmint and basil oils, while *A. parasiticus* and *F. moniliforme* were moderately affected, *A. flavus* was more resistant to basil and spearmint oils.

Basil oil for 24 hrs at concentration 2.0%v/v could completely inhibit spore germination of all the species of fungi tested, while Sweet Fennel oil showed susceptibility on *F. moniliforme*, *F. proliferratum*, *R. solanai*, *B. oryzae* and *A. flavus*, which was in accordance with the findings of Fiori *et al*. [9], who reported that essential oils of *C . citratus, A. conyzoides and E. citriodora* provided 100% inhibition of the mycelium growth and germination of spores of *D .bryoniae*.

The results of this experiment suggested that the two essential oils from Thai medicinal plants clearly showed antifungal properties, on both mycelium growth and spore

germination of rice pathogenic fungi. However, the strength of these properties depends on the plant and fungal species, concentration of the testing oil and the testing conditions, all of which will have some affect on the antifungal properties of essential oils.

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