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Research article

Efficacy of Thai plant extracts against grain discoloration disease in rice (*Oryza sativa* L.)

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Abstract

The antifungal effect was examined of ethanolic crude extracts of four plants on the mycelial growth and conidial germination of *Curvularia lunata* and *Fusarium sacchari*, which are the principal causal agents of grain discoloration disease in rice. The four plants studied were: *Cassia spectabilis* (leaves and flowers); *Syzygium aromaticum* (buds); *Alpinia galanga;* and *Curcuma zedoaria* (aerial part and rhizome). The extract of *S. aromaticum* showed 100% inhibition on the mycelial growth and conidial germination of both *C. lunata* and *F. sacchari* at all concentrations. The extract of *A. galanga* rhizome at 10,000 parts per million (ppm) also completely inhibited both mycelial growth and conidial germination of *F. sacchari*. The extracts of leaves and flowers from *C. spectabilis* displayed 100% inhibition against conidial germination at both 5,000 ppm and 10,000 ppm. The ethanolic extracts of *S. aromaticum* and *A. galanga* rhizome were separated based on column chromatography and used for bioassay. The active compounds were characterized as eugenol from *S. aromaticum* and 1′-acetoxychavical acetate (ACA) from *A. galanga*. The minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of the active compounds were determined using 96-well microplates against the fungal pathogens. The ACA fraction showed the highest antifungal activity against *C. lunata* (MIC = 23.43 ppm and MFC = 23.43 ppm) and *F. sacchari* (MIC = 46.87 ppm and MFC = 93.75 ppm). The results indicated the tested plant extracts had the potential to be used as eco-friendly pesticides to control grain dislocation disease in rice.

Introduction

Rice (*Oryza sativa*) is Thailand's most important staple food crop and principal agricultural export (Boonreung and Boonlertnirun, 2013). However, rice production is subject to a variety of diseases, with grain discoloration (GD) being amongst the most pernicious. GD (also known as dirty panicle of rice [*O. sativa*]) is a complex disease caused by the fungal pathogens such as *Sarocladium* sp., *Bipolaris* sp., *Alternaria* sp., *Fusarium* sp., *Phoma* sp., *Curvularia* sp. and *Trichoconiella* sp. (Ou, 1985; IRRI, 2002). GD has also been reported in Brazil (Prabhu et al., 2012), Iran (Amini et al., 2016a), Pakistan (Arshad et al., 2009) and Korea (Jeong et al., 2003), indicating its widespread presence. In Thailand, GD is emerging as one of the principal threats to rice cultivation, with *A. padwickii*, *C. lunata*, *F. semitectum* and *Helminthosporium oryzae* having been identified as principal causal agents (Prathuangwong et al., 2013).

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In managing GD, farmers have relied on the application of synthetic fungicides (Bhajbhuje, 2015). This was largely because of the dearth of GD-resistant rice varieties, and a lack of information on alternative agricultural practices to inhibit GD. The latter comprise methods such as the application of biological control agents and plant extracts eliminating the need for chemical fungicides (Swami and Alane, 2013), which are not only expensive, but also hazardous to health and the environment (Bhajbhuje, 2015).

Liquid plant extracts serve as an effective, organic inhibitory to various microorganisms. Extract of *Cymbopogon citratus* and *Ocimum basilicum* has been reported to exhibit antifungal properties under laboratory, greenhouse and field conditions (Amini et al., 2016b). Extracts of galangal, either with water or ethanol, have been reported to completely inhibit the conidial germination of *Curvularia* sp., *Fusarium* sp., *C. gloesporioides*, *Pestalotiopsis* sp. and *C. orbiculare* (Yulia, 2005).

Sawatdikarn (2016) studied the antifungal effects of crude extracts of some medicinal plants against *Fusarium* sp., the pathogen of dirty panicle disease in rice. These extracts included: *Boesenbergia pandurata*, *Curcuma longa*, *C. aromatica*, *C. xanthorrhiza*, *Zingiber officinale*, *Z. cassumunar*, *A. galanga*, *Amonum xanthioides*, *A. krervanh*, *Kaempferia galanga*, *K. parviflora*, *Syzygium aromaticum*, *Allium sativum*, *A. ascolonicum*, *Cymbopogon citratus*, *C. nardus*, *Leptochloa chinensis*, *Eupatorium odoratum*, *Piper betle*, *Synedrella nodiflora*, *Cassia siamea*, *Sorghum bicolor*, *Rosmarinus officinalis* and *Origanum vulgare.* It was found that crude extracts of *S. aromaticum* and *O. vulgare* showed 100% inhibition on mycelial growth of the pathogen at all concentrations. The crude extract of *C. siamea* has also been shown to have an antifungal effect against *Curvularia* sp. at 10,000 parts per million (ppm) (Sawatdikarn, 2011). The extracts of *A. galanga* and *S. aromaticum* were reported to have high antifungal activity against *Curvularia* sp. and *Fusarium* sp. (Yulia, 2005; Mongkol et al., 2015; Sawatdikarn, 2016). Therefore, the extracts of these plants, and that of *C. zedoaria,* constitute promising candidates to test for controlling GD in rice.

The present research aimed to test the efficacy of certain plant extracts namely, *C. spectabilis* (leaves and flowers), *S. aromaticum* (buds), *A. galanga*, and *C. zedoaria* (aerial part and rhizome) in suppressing fungi associated with GD symptom in rice.

Materials and Methods

Collection and preparation of plants materials

In Thailand in June 2017, four plant species were collected: *C. spectabilis* (leaves and flowers), *A. galanga*, *C. zedoaria* (aerial part and rhizome) and *S. aromaticum* (bud) as shown in Table 1. Samples were collected from various sources: a garden in Silpakorn University, Phetchaburi IT campus, Cha-Am, Phetchaburi; a garden in Nongplub, Hua-Hin, Prachubkhirikhan; and from a medicinal plant shop in Bangkok (Table 1). The collected plants were cut into small pieces and dried at 60°C for 3 d. Then, the plant pieces were separately soaked for maceration in a solution of 95% ethanol. After maceration, the plant parts were filtered and dried using a rotary evaporator to obtain crude extracts and left in a laminar flow cabinet to remove the solvent until the samples were completely dried. The latter were then refrigerated at 4°C to produce the stock solutions further analyses.

Preparation of rice seeds and isolation of pathogens

Two hundred rice panicles (var. RD-61) with severe GD symptoms were collected from rice fields at two locations in Phetchaburi province: Muang and Cha-Am districts, in 2017. *Curvularia lunata* and *Fusarium sacchari* from the rice seeds with GD symptom were isolated and identified using DNA sequencing of the internal transcriber spacer region of the rRNA gene. These fungi were maintained on Petri dishes containing potato dextrose agar (PDA) and incubated at room temperature (26–32°C) for 7 d before the experiments (Madi et al., 2017).

Antifungal activity of plant extracts

The antifungal activity of each plant extract against *C. lunata* and *F. sacchari* was tested using a food poisoning technique (Kumar et al., 2008). The crude extract of each sample, prepared as detailed above, was used to create stock solutions for each sample. This was carried out using the crude extract from each sample at 0.1 g/mL, 0.5 g/mL and 1 g/mL to produce concentrations of 100,000 ppm, 500,000 ppm and 1,000,000 ppm, respectively. The stock solution from each sample (at 0.1 mL) of the plant extracts was individually added to 9.9 mL of sterile PDA medium. Then, this mixture was poured into Petri dishes to obtain final concentrations of 1,000 ppm, 5,000 ppm and 10,000 ppm. A PDA medium consisting of pure ethanol served as a nil control. A small disc (6 mm in diameter) of the growing mycelia from the fungal culture, which had been grown on PDA for 7 d, was cut and transferred aseptically onto the center of the PDA dish, which had been prepared with each concentration of the crude extracts. The PDA dishes amended with acetone were used as a negative control and those amended with difenoconazole + propiconazole (225 ppm) (Armure® 300 EC, Syngenta Crop Protection Monthey AG, Switzerland) were used as a positive control (Takham et al., 2017).

Table 1 List of collected plant species

Family	Scientific name	Plant parts	Source
Caesalpiniaceae	*Cassia spectabilis*	Flower & leaf	Garden, Cha-Am district, Phetchaburi
Myrtaceae	*Syzygium aromaticum*	Dried buds	Medicinal Plant Shop, Bangkok
Zingiberaceae	*Alpinia galanga*	Rhizome & aerial part	Garden, Hua-Hin, Prachubkhirikhan
Zingiberaceae	*Curcuma zedoaria*	Rhizome & aerial part	Garden, Hua-Hin, Prachubkhirikhan

These plates were incubated at 27°C for 7 d, after which the growth of the fungus was assessed. Fungal colony diameter of the three crude extract compounds and nil control were measured, and the percentage of mycelial growth inhibition was calculated using the Equation 1 proposed by Yang et al. (2005):

Mycelial growth inhibition (%) = ((A – B)/ A) × 100 (1)

where A is the colony diameter of the control treatment and B is the colony diameter of the treated crude extracts. Each treatment was replicated three times.

Inhibition of spore germination by plant extracts

Inhibition of spore germination was tested by mixing 0.5mL of spore suspension (1 ×10^5 spores/mL) of *C. lunata* and *F. sacchari* with 0.5 mL of different concentrations (1,000 ppm, 5,000 ppm and 10,000 ppm) of each plant extracts on glass slides. The nil control treatment was carried out by mixing spore suspension with distilled water. Then, the slides were incubated at 25°C for 16 hr on a sterilized U-shaped glass rod in Petri dishes containing sterilized water.

After 16 hr, the slides were removed from the plates and used for assessing spore germination. A drop of lacto phenol cotton blue was placed on the spore suspension and inspected under a compound microscope (×40). Each treatment consisted of three replications. Spore germination was measured, and the inhibition percentage was calculated using the Equation 2 proposed by Kumari et al. (2013):

Inhibition (%) = ((C-T)/ T) × 100 (2)

where C is the germination percentage of spores in the negative control and T is the germination percentage of spores in the treatment.

Characterization of effective plant extract and bioassay

The ethanolic crude extracts of *S. aromaticum* (50 g) and *A. galanga* (20 g) were fractionated using column chromatography with silica gel (70–230 mesh). Step elution was conducted using 100% hexane and the polarity was increased with CH_2Cl_2 and EtOAc and finally with 20% MeOH in EtOAc. The collected fractions were combined using thin layer chromatography which was observed under an ultraviolet lamp (254–365 nm). The fractions were evaporated using a rotary evaporator to obtain nine and eight fractions (S1-S9 and A1-A8), respectively. All fractions were evaluated for bioassay against mycelial growth of *C. lunata* and *F. sacchari* at 1,000 ppm using the food poisoning technique described above with three replications. The active fractions were subjected to further characterization using silica gel column chromatography with a gradual increase in the polarity. The active compounds were isolated and compared with the proton nuclear magnetic resonance (^{1}H-NMR) data (Kumar, 2010).

Determination of minimum inhibitory concentration and minimum fungicidal concentration of isolated compounds against fungal pathogens

The MIC testing of isolated compounds against *F. sacchari* and *C. lunata* was conducted using the microwells dilution method with 96-well microplates containing sterilized potato dextrose broth (PDB) with three replicates (Mitscher et al., 1972). The stock solutions of either 1´-acetoxychavicol acetate (ACA) or eugenol were dissolved in acetone to obtain 3,000 ppm. A sample (100 µL) of each stock solution was mixed with 100 µL of the sterilized PDB in the first well of the 96-microwell plate, giving each mixture a concentration of 1,500 ppm. Then, 100 µL of the mixture from this first well was transferred into the second well, which already contained 100 µL of PDB. These serial dilutions were carried out until the final well reached a concentration of 11.71 ppm. The 100 µL of spore suspensions (1 ×10^6 spores/mL of each pathogen; either *C. lunata* or *F. sacchari*) was added into these eight wells, resulting in final concentrations in each well ranging from 750 ppm (in the first well) to 5.85 ppm (in the eighth well). The control treatment consisted of a mixture of acetone (100 µL) and PDB (100 µL) in which 100 µL of this mixture was transferred into the adjacent well. The spore suspension (at 100 µL) was dispensed into this well.

The microplate was incubated at 27°C for 48 hr after which the samples in each well showing complete inhibition (100% or an optically clear well) were individually transferred onto a PDA plate to determine the MFC. The plates were incubated at 27°C for 48 hr. The MIC endpoints represented the lowest concentrations that showed absence of growth or complete growth inhibition (100% inhibition or an optically clear well). The MFC was the lowest concentration with an absence of mycelial growth (Espinel-Ingroff, 2001). The treatments were replicated three times.

Statistical analysis

All experiments were conducted in triplicate with a completely randomized design. The data represented the mean ± SD of the triplicate values. Significant differences were determined based on one-way analysis of variance using R program (computer software package version 3.1.0) and Duncan's multiple range test was used to compare the means, with significance tested at $p < 0.05$.

Results

Antifungal activity of plant extracts

The antifungal activity of the ethanolic extracts of *C. spectabilis* (leaves and flowers), *S. aromaticum* (bud), *A. galanga*, and *C. zedoaria* (aerial part and rhizome) was tested against *C. lunata* and *F. sacchari* at different concentrations. Mycelial growth inhibition varied by plant and concentration.

The bud extract from *S. aromaticum* completely inhibited the mycelial growth of *C. lunata* and *F. sacchari* (Table 2 and Fig.1) at all concentrations. The rhizome extract from *A. galanga*, at a concentration of 10,000 ppm inhibited the mycelial growth of *F. sacchari* completely (100%) and inhibited that of *C. lunata* at 90.17% (Table 2 and Fig. 1). Difenoconazole + propiconazole (225 ppm) (Armure®), as a positive control, completely inhibited the mycelial growth of both *C. lunata* and *F. sacchari*.

Table 2 Effect of plant extracts against mycelial growth of phytopathogenic fungi *Curvularia lunata* and *Fusarium sacchari*

Plant species	Plant parts	*C. lunata*			*F. sacchari*		
		1,000 ppm	5,000 ppm	10,000 ppm	1,000 ppm	5,000 ppm	10,000 ppm
A. galanga	Aerial	6.55±1.04^{e}	20.23±0.51^{f}	39.69±2.02^{d}	13.20±1.44^{f}	14.34±2.93^{e}	24.15±2.18^{f}
	Rhizome	26.59±2.18^{d}	84.97±2.00^{b}	90.17±0.50^{b}	6.22±1.00^{e}	61.13±1.26^{b}	100.00±0.00^{a}
C. zedoaria	Aerial	36.99±2.29^{c}	37.57±2.00^{e}	38.92±2.02^{d}	0.94±1.50^{c}	37.36±1.26^{d}	39.24±1.15^{e}
	Rhizome	62.81±2.36^{b}	68.98±1.53^{c}	71.29±0.76^{c}	4.72±1.00^{b}	56.04±0.76^{c}	60.00±2.02^{b}
C. spectabilis	Leaf	35.07±2.47^{c}	62.24±2.84^{d}	72.06±0.58^{c}	5.85±2.85^{ef}	40.75±2.52^{d}	47.17±2.08^{d}
	Flower	37.19±2.57^{c}	59.92±1.26^{d}	71.87±2.84^{c}	20.75±1.00^{d}	41.13±2.29^{d}	50.56±1.53^{c}
S. aromaticum	Bud	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}

Mean ± SD values within a column superscripted with different lowercase letters are significantly different ($p < 0.05$).

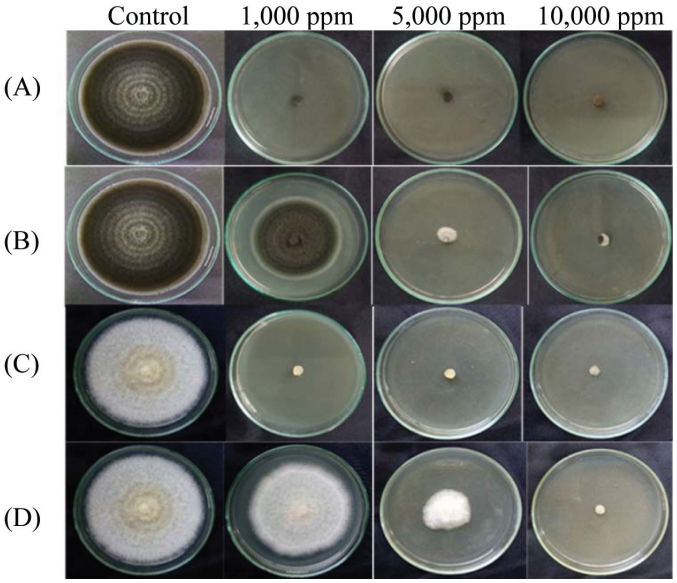

Fig. 1 Effect of crude extracts of (A, C) *Syzygium aromaticum*; (B, D) *Alpinia galanga* rhizome on mycelial growth of (A, B) *Curvularia lunata*; (C, D) *Fusarium sacchari* at different concentrations

Effect of plant extracts on spore germination

The bud extract from *S. aromaticum* also completely inhibited spore germination of both *C. lunata* and *F. sacchari* at all concentrations. The leaf extract from *C. spectabilis,* at 5,000 and 10,000 ppm, also completely inhibited spore germination of both pathogens. The flower extract of C. *spectabilis* at 10,000 ppm completely inhibited spore germination of *C. lunata* and *F. sacchari* (Table 3).

Interestingly, at 10,000 ppm, the rhizome extract of *A. galanga* completely inhibited spore germination of both tested fungi. The rhizome extract of *C. zedoarya* achieved 94.26% inhibition to *F. sacchari*, *while* the aerial parts of *C. zedoarya* and *A. galanga* achieved 83.31 and 80.67 percent inhibition to *C. lunata*, respectively (Table 3).

Antifungal activity of S. aromaticum and A. galanga extracts

The ethanol extracts from the *S. aromaticum* bud and *A. galanga* rhizome had high antifungal activity against *C. lunata* and *F. sacchari.* The column chromatography technique produced nine fractions from the bud of *S. aromaticum* and eight fractions from the rhizome of *A. galanga*.

Subsequent bioassay tests showed that fractions S3 and S4 **(**from the bud of ***S. aromaticum*****)** completely inhibited the mycelial growth of both *F. sacchari* and *C. lunata*. By contrast, fractions S6, S7 and S8 had the lowest capacity to inhibit the mycelial growth

Table 3 Percentage inhibition of plant extracts on spore germination of *Curvularia lunata* and *Fusarium sacchari* at different concentrations

Plant species	Plant parts	*C. lunata*			*F. sacchari*		
		1,000 ppm	5,000 ppm	10,000 ppm	1,000 ppm	5,000 ppm	10,000 ppm
A. galanga	Aerial	25.85±2.59^{d}	40.23±2.35^{f}	80.67±0.95^{c}	1.23±2.14^{f}	39.63±0.64^{f}	42.11±1.21^{d}
	Rhizome	90.00±2.08^{b}	93.69±1.28^{b}	100.00±0.00^{a}	39.86±2.05^{e}	60.66±0.58^{d}	100^{a}
C. zedoaria	Aerial	13.39±2.96^{e}	46.20±2.50^{e}	83.31±2.00^{b}	43.73±1.08^{d}	52.65±0.28^{e}	66.69±1.71^{c}
	Rhizome	35.31±2.87^{c}	54.12±1.41^{d}	68.04±1.78^{d}	44.90±0.80^{d}	76.01±1.07^{c}	94.26±1.70^{b}
C. spectabilis	Leaf	86.85±1.66^{b}	100^{a}	100^{a}	57.08±2.60^{c}	100^{a}	100^{a}
	Flower	9.71±1.57^{e}	86.00±1.51^{c}	100^{a}	66.29±0.66^{b}	87.35±1.84^{b}	100^{a}
S. aromaticum	Bud	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}

Mean ± SD values within a column superscripted with different lowercase letters are significantly different ($p < 0.05$).

of both pathogens (Table 4). As such, the highest active fractions (S3 and S4 from the bud of *S. aromaticum*) were selected for further characterization.

After the separation, fractions A3 and A4 from the rhizome of *A. galanga* exhibited complete inhibition of mycelial growth of *F. sacchari*. *C. lunata* A2 was the second most effective fraction of mycial growth of *C. lunata* with a inhibition factor of 72.09% (Table 4).

Further isolation of the S3 and S4 fractions (from the bud of *S. aromaticum*) using silica gel column chromatography (70–230 mesh) yielded S3-5, an active compound (12.39 g), brownish-yellow-colored liquid, which was identified based on ^{1}H-NMR and the comparison of the ^{1}H-NMR spectral data of eugenol (Kumar, 2010). The NMR data active compound was identified as eugenol (Table 5). The unknown active fractions A3 and A4 (from the rhizome of *A. galanga*) were further isolated using silica gel column chromatography (70–230 mesh). The isolation yielded A3-1 (1.18 g), a colorless, liquid active compound. Subsequently, this A3-1 compound was identified using ^{1}H-NMR. The data from this analysis were compared with that of Mongkol et al. (2015) to identify the unknown chemical agent as 1′-acetoxychavical acetate (ACA), as shown in Table 6. The structures of both eugenol and ACA are shown in Fig. 2.

Table 4 Effect of fraction isolated from *Alpinia galanga* rhizome (A1–A8) and *Syzygium aromaticum* buds (S1–S9) on mycelial growth of *Curvularia lunata* and *Fusarium sacchari* at 1,000 ppm

Fractions	Percentage inhibition		Fractions	Percentage inhibition	
	C. lunata	*F. sacchari*		*C. lunata*	*F. sacchari*
A1	50.29±1.53^e	27.41±2.84^e	S1	61.51±2.00^b	65.20±1.80^b
A2	72.08±1.53^b	59.63±2.36^b	S2	19.24±2.52^e	34.60±2.29^d
A3	100^a	100^a	S3	100^a	100^a
A4	100^a	100^a	S4	100^a	100^a
A5	55.07±1.89^d	48.33±2.02^c	S5	36.22±2.52^d	40.00±1.10^c
A6	63.67±2.47^c	40.93±2.02^d	S6	6.41±2.52^g	10.40±1.15^f
A7	41.88±2.84^f	6.30±2.08^f	S7	5.66±2.89^g	16.80±2.08^e
A8	11.86±2.75^g	0^g	S8	15.09±1.73^f	12.80±2.52^f
			S9	54.34±0.58^c	33.80±2.25^d

Mean values within a column superscripted with different lowercase letters are significantly different ($p < 0.05$).

Table 5 Comparison of ^{1}H-NMR spectral data of eugenol and isolated compounds from buds of *Syzygium aromaticum*

Chemical shift (ppm)	
Eugenol (Kumar, 2010) *	Isolated compound**
3.38 (d, 2H)	3.34 (d, 2H)
3.86 (s, 3H)	3.88 (s, 3H)
5.12 (d, 1H)	5.07 (d, 1H)
5.19 (d, 1H)	5.11 (d, 1H)
5.96 (m, 1H)	5.94 (m, 1H)
6.74 (s, 1H)	6.69 (s, 1H)
6.76 (d, 1H)	6.71 (d, 1H)
6.92 (d, 1H)	6.87 (d, 1H)

*^{1}H- NMR spectra were measured in $CDCl_3$ at 300 MHz.
** ^{1}H- NMR spectra were measured in $CDCl_3$ at 400 MHz.

Table 6 Comparison of ^{1}H-NMR spectral data of 1′-acetoxychavical acetate and isolated compound from the bud of *Alpinia galanga*

Chemical shift (ppm)	
ACA (Mongkol, 2014)*	Isolated compound**
2.10 (s, 3H)	2.10 (s, 3H)
2.29 (s, 3H)	2.29 (s, 3H)
5.27 (d, 1H)	5.26 (d, 1H)
5.98 (m, 1H)	5.97 (m, 1H)
6.26 (d, 1H)	6.26 (d, 1H)
7.07 (d, 2H)	7.08 (d, 2H)
7.36 (d, 2H)	7.37 (d, 2H)

ACA = 1′-acetoxychavical acetate**.**
* ^{1}H-NMR were measured in $CDCl_3$ at 400 MHz.
** ^{1}H-NMR were measured in $CDCl_3$ at 500 MHz.

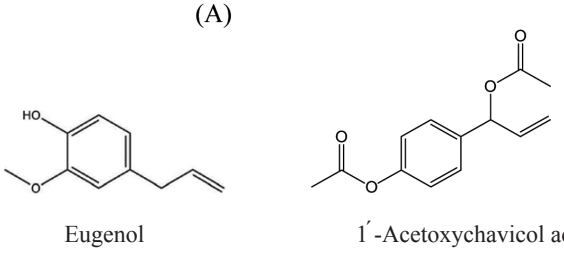

Fig. 2 Structures of active compouds isolated from (A): *Syzygium aromaticum* (eugenol) and *(*B*): Alpinia galanga* rhizome (1′-acetoxychavical acetate)

Minimum inhibitory concentration and minimum fungicidal concentration

The effectiveness of the MIC and MFC values of the isolated compounds from the bud of *S. aromaticum* and rhizome of *A. galanga* against two fungi is shown in Table 7. ACA had the highest effectiveness against *C. lunata* (MIC = 23.43 ppm and MFC = 23.43 ppm) and *F. sacchari* (MIC = 46.87 ppm and MFC = 93.75 ppm).

Eugenol exhibited moderate activity against both pathogens (MIC = 187.5 ppm). Even so, it possessed weak MFC inhibition against both *C. lunata* and *F. sacchari* (MFC = 750 ppm), as shown in Table 7.

Table 7 Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of isolated compounds against pathogenic fungi

Compound	MIC (ppm)		MFC (ppm)	
	C. lunata	*F. sacchari*	*C. lunata*	*F. sacchari*
1´-Acetoxychavical acetate	23.43	46.87	23.43	93.75
Eugenol	187.50	187.50	750.00	750.00

Discussion

Certain chemical extracts from plants having fungicidal properties may be used to control plant pathogenic fungi (Yulia, 2005). Extracts from several plants (*Curcuma* sp., *S. aromaticum, A. galanga* and *C. spectabilis*) have been reported to possess biological activity such as antimicrobial, antifungal, anti-inflammatory and antioxidant activities (Ayoola et al., 2008; Sangetha et al., 2008; Latha et al., 2009).

The bud extract of *S. aromaticum* at all concentrations in the present study showed complete inhibition to mycelial growth and spore germination of *C. lunata* and *F. sacchari*. Two reports have indicated the efficacy of the bud extract of *S. aromaticum* in inhibiting *Curvularia sp.* (Sawatdikarn, 2011) and *Fusarium sp.* (Sawatdikarn, 2016). The low concentration of the bud extract of *S. aromaticum* (1,000 ppm) was confirmed as effective against *F. sacchari* and *C. lunata*, making this plant material a potential candidate for use in controlling this important rice disease, particularly in agricultural regions where clove is abundant.

The present study showed the bud extract of *S. aromaticum* was also effective in inhibiting conidial germination. These results make clove a candidate for practical use by farmers because the spore is the primary inoculum initiating grain discoloration disease in the field. Spores of the *F. sacchari* and *C. lunata*, which are the main inoculum-causing diseases in the rice field, have been reported to be damaged by clove oil (Yulia, 2005). Sprayed extract of clove deposited on rice plant tissues such as panicles should damage the spores of the pathogens, preventing them from germinating and infecting the rice plant.

The rhizome extract of *A. galanga* showed inhibition on mycelial growth and conidial germination of both *C. lunata* and *F. sacchari* at 10,000 ppm. Both ethanol and water extracts of *A. galanga* were reported to be effective in inhibiting the conidial germination of *Curvularia* sp. and *Fusarium* sp. (Yulia, 2005). The high efficacy of water extract of *A. galanga* rhizome indicated that *A. galanga* was suitable for use by farmers owing to its ease of preparation and ready availability. That said, *C. spectabilis*, which is a tree commonly found throughout Thailand, should also be adopted for use to control GD because flower and leaf extracts of this plant are highly effective in inhibiting spore germination of *C. lunata* and *F. sacchari* (Table 3). Even so, further testing is required of the water extract of this plant against the fungal pathogens.

The rhizome extract of *C. zedoaria* showed moderate inhibition of mycelial growth of *C. lunata* and *F. sacchari* at all concentrations. However, the crude extract of this plant part displayed a wide range of inhibitory activity against the conidial germination of the pathogens at all concentrations. Sawatdikarn (2016) reported that three plants crude extracts in the Zingiberaceae species (*C. longa*, *C. aromatic*, and *C. xanthorrhiza)* showed at the highest inhibition on mycelial growth of *F. sacchari* at 5,000–10,000 ppm concentrations. Although these various compounds are inhibitory to fungi, they may not be suitable for use as, for example, some species are very rare, such as *C. zedoaria*, and thus are not readily available for mass application in Thailand.

Plants have the ability to synthesize aromatic secondary metabolites such as phenols, phenolic acids, flavonoids and tannins (Cowan, 1999). The compounds, such as carvacrol, eugenol, and thymol, are highly active against plant pathogens (Das et al., 2010). In the present study, *S. aromaticum* bud also contained eugenol, an essential oil which has been reported to be abundantly present in *S. aromaticum* at 90.6% (Barnes et al., 2007; Xie et al., 2015). The substance 1´- acetoxychavical acetate (ACA) was isolated from extract of *A. galanga* (Madi, 2017) and this substance was the major constituent of the organic extract of *A. galanga* (Janssen and Scheffer, 1985; Dadang, 1999). The phytotoxicity effect of *S. aromaticum* bud and *A. galanga* rhizome to rice is necessary to ensure farmers do not reject any recommendations on their use.

In the present study, the MIC and MFC of eugenol (isolated from *S. aromaticum*) and *ACA* (isolated *from A. galanga)* were determined against *F. sacchari* and *C. lunata*. The ACA had significant MIC and MFC values against both *F. sacchari* and *C. lunata* (Table 7). These two values of ACA, isolated from *A. galanga*, provided evidence that this plant would be suitable for use in large-scale production to control GD disease in a farm setting. Eugenol had a moderate MIC value with its MFC at the highest concentrations after testing against both *F. sacchari* and *C. lunata*. That the values of MIC and MFC of *S. aromaticum* were higher than those of *A. galanga* indicated that a large quantity of this plant is required for practical applications. However, it should be noted that because *S. aromaticum* is highly valued as a medicinal plant and is sold for that purpose in Thailand, its adoption as an antifungal may be discouraged on economic grounds. Nevertheless, the findings provided significant evidence on the potential of these two plants to control *C. lunata* and *F. sacchari*, two main fungi which are causal agents of GD. The question of which plant may be more suitable for a given agricultural region will depend, in part, on the availability of each plant in a given country or region.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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References

Amini, M.M., Alizadeh, M.R., Padasht, F., Elahinia, S.A., Khodaparast, S.A. 2016a. Rice grain discoloration effect on physical properties and head rice yield in three rice cultivars. Qual. Assur. Saf. Crop. 8: 283–288. doi.org/10.3920/QAS2014.0582

Amini, J., Farhang, V., Javadi, T., Nazemi, J. 2016b. Antifungal effect of plant essential oils on controlling *Phytophthora* species. Plant. Pathol. J. 32: 16–24. doi.org/10.5423/PPJ.OA.05.2015.0091

Arshad, H.M.I., Khan, J.A., Naz, S., Khan, S.N., Akram, M. 2009. Grain discoloration disease complex: a new threat for rice crop and its management. Pakistan J. Phytopathol. 21: 31–36.

Ayoola, G.A., Lawore, F.M., Adelowotan, T., Aibinu, I.E., Adenipekun, E., Coker, H.A.B., Odugbemi, T.O. 2008. Chemical analysis and antimicrobial activity of the essential oil of *Syzigium aromaticum* (clove). Afr. J. Microbiol. Res. 2: 162–166.

Barnes, J., Anderson, L.A., Phillipson, J.D. 2007. Herbal Medicines, 3rd ed. Pharmaceutical Press. London, UK.

Bhajbhuje, M.N. 2015. Potential of some botanicals against *Curvularia* and *Fusarium* species. Int. J. Life Sci. 3: 399–402.

Boonreung, C., Boonlertnirun, S. 2013. Efficiency of chitosan for controlling dirty panicle disease in rice plants. ARPN J. Agric. Biol. Sci. 8: 1990–6145.

Cowan, M.M. 1999. Plant products as antimicrobial agents. Clin. Microbiol. Rev. 12: 564–82.

Dadang, 1999. Insect regulatory activity and active substances of Indonesian plants particularly to the diamondback moth. M.Sc. thesis, Graduate School of Agriculture, Tokyo University of Agriculture. Tokyo, Japan. doi.org/10.11501/3153606 [in Japanese]

Das, K., Tiwari, R.K., Shrivastava, D.K. 2010. Techniques for evaluation of medicinal plant products as antimicrobial agent: current methods and future trends. J. Med. Plants Res. 4: 104–111. doi.org/10.5897/JMPR09.030

Espinel-Ingroff, A. 2001. *In vitro* fungicidal activities of voriconazole, itraconazole, and amphotericin B against opportunistic moniliaceous and dematiaceous fungi. J. Clin. Microbiol. 39: 954–958. doi.org/10.1128/JCM.39.3.954-958.2001

IRRI. 2002. Standard evaluation system for rice (SES). International Rice Research Institute. Manila, the Philippines.

Janssen, A., Scheffer, J. 1985. Acetoxychavicol acetate, an antifungal component of *Alpinia galanga*. Plant. Med. 51: 507–511. doi.org/10.1055/s–2007–969577

Jeong, Y., Kim, J., Kim, S., Kang, Y., Nagamatsu T., Hwang, I. 2003. Toxoflavin produced by *Burkholderia glumae* causing rice grain rot is responsible for inducing bacterial wilt in many field crops. Plant. Dis. 87: 890–895. doi.org/10.1094/PDIS.2003.87.8.890

Kumari, A., Kumar, R., Maurya, S., Choudhary, J.S., Kumar, S. 2013. Antifungal efficacy of aqueous extracts of neem cake, karanj cake and vermicompost against some phytopathogenic fungi. Bioscan. 8: 671–674.

Kumar, A., Shukla, R., Singh, P., Prasad, C.S., Dubey, N.K. 2008. Assessment of *Thymus vulgaris* L. essential oil as a safe botanical preservative against post-harvest fungi infestation of food commodities. Innov. Food Sci. Emerg. Technol. 9:575–580. doi.org/10.1016/j.ifset.2007.12.005

Kumar, L. 2010. Antifungal, antibacterial and insect repellent chemical molecules of *Ocimum sanctum* L. Ph.D. thesis, Maharaj Singh college, Chaudhary Charan Singh University. Saharanpur, India.

Latha, C., Shriram, V.D., Jahagirdar, S.S., Dhakephalkar, P.K., Rojatkar, S.R. 2009. Antiplasmid activity of 1′-acetoxychavicol acetate from *Alpinia galanga* against multi-drug resistant bacteria**.** J**.** Ethnopharmacol**.** 123**:** 522–525. doi.org/10.1016/j.jep.2009.03.028

Madi, I.S.O. 2017. Fungi associated with grain discoloration (GD) of rice (*Oryza sativa* L.) (Var. RD61) and the efficacy of some plant extracts in suppressing GD causal agents. M.Sc. thesis, Faculty of Animal Science and Agricultural Technology, Silpakorn University. Phetchaburi, Thailand.

Madi, I**.**S**.**O**.**, Meetum, P**.**, Mongkol, R**.**, Kanjanamaneesathian, M**.** 2017**.** Analysis of the symptom of grain discoloration in rice (*Oryza sativa*) (var**.** RD-61**).** In**:** Proceedings of the 13rd Asian Congress on Biotechnology 2017 **(**ACB2017**) "**Bioinnovation and Bioeconomy**".** Khonkhaen, Thailand, pp**.** 114–1–114–10**.**

Mitscher, L.A., Leu, R.P., Bathala, M.S., Wu, W.N., Beal, J.L., White, R. 1972. Antimicrobial agents from higher plants. I: Introduction, rational and methodology. Lloydia 35: 157–166.

Mongkol, R., Chavasiri, W., Ishida, M., Matsuda, K., Morimoto, M. 2015. Phytotoxic and antiphytopathogenic compounds from Thai *Alpinia galanga* (L.) Willd. rhizomes. Weed Biol. Manag. 15: 87–93. doi.org/10.1111/wbm.12071

Ou, S.H. 1985. Rice Diseases, 2nd ed., Commonwealth Mycological Institute. Kew, UK.

Prabhu, A.S., Filho, M.P.B., Datnoff, L.E., Snyder, G.H., Berni, R.F., Rodrigues, F. A., Dallagnol, L.J. 2012. Silicon reduces brown spot severity and grain discoloration on several rice genotypes. Trop. Plant Pathol. 37: 409–414. doi.org/10.1590/S1982–56762012000600005

Prathuangwong, S., Athinuwat, D., Chuaboon, W., Chatnaparat, T., Buensanteai, N. 2013. Bioformulation *Pseudomonas fluorescens* SP007s against dirty panicle disease of rice. Afr. J. Microbiol. Res. 7: 5274–5283. doi.org/10.5897/AJMR2013.2503

Sangetha, S., Zuraini, Z., Sasidharan, S., Suryani, S. 2008. Fungicidal effect and oral acute toxicity of *Cassia spectabilis* leaf extract. Jpn. J. Med. Mycol. 49: 299–304. doi.org/10.3314/jjmm.49.299

Sawatdikarn, S. 2011. Antifungal activity of twenty-four medicinal crude extracts against *Curvularia* sp., the pathogen of dirty panicle disease in rice. In: Proceedings of 37th Congress on Science and Technology of Thailand. Bangkok, Thailand, pp. 1–8.

Sawatdikarn, S. 2016. Antifungal activity of crude extracts of some medicinal plants against *Fusarium* sp., the pathogen of dirty panicle disease in rice. J. Med. Plants Res. 10: 248–255. doi.org/10.5897/JMPR2013.4426

Swami, C.S., Alane, S.K. 2013. Efficacy of some botanicals against seed - borne fungi of green gram (*Phaseolus aureus* roxb.). Biosci. Discov. 4: 107–110.

Takham, A., Ketsuwan, K., Yajai, P., et al. 2017. Survey of rice seed discoloration disease, pathogen diversity and fungicides efficacy on the growth of pathogenic fungi in northern Thailand. In**:** Proceedings of Academic Seminar on Rice 2017. Bangkok, Thailand, pp. 196–210. [in Thai]

Xie, Y., Yang, Z., Cao, D., Rong, F., Ding, H., Zhang, D. 2015. Antitermitic and antifungal activities of eugenol and its congeners from the flower buds of *Syzgium aromaticum* (clove). Ind. Crops Prod. 77: 780–786. doi.org/10.1016/j.indcrop.2015.09.044

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: 813–818. doi.org/10.1016/j.biortech.2004.07.010

Yulia, E. 2005. Antifungal activity of plant extracts and oils against fungal pathogens of pepper (*Piper nigrum* L.), cinnamon (*Cinnamomun zeylanicum* Blume.), and turmeric (*Curcuma domestica* Val.). M.Sc. thesis, School of Tropical Biology and the School of Veterinary and Biomedical Sciences, James Cook University. Townsville, QLD, Australia.