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Control of rice blast disease caused by *Magnaporthe oryzae* by application of antifungal nanomaterials from *Emericella nidulans*

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Abstract: Metabolites of *Emericella nidulans* (EN) were separated by chromatographic methods from crude hexane included emericellin and sterigmatocystin, while crude ethyl acetate found demethylsterigmatocystin. These metabolites proved to be antagonistic to *Magnaporthe oryzae,* the causal agent of rice blast. Crude extracts and nano-particles derived from EN inhibited *M. oryzae*. The ethyl acetate crude extract derived inhibited *M. oryzae* with an effective dose (ED_{50}) of 66 µg/mL. The nanoparticles showed better inhibition of *M. oryzae* than crude extracts at low concentrations. Nanoparticles, namely from crude ethyl acetate, crude methanol and crude hexane of EN were active against *M. oryzae* with ED_{50} of 4.2 µg/mL, 4.5 µg/mL, 8.9 µg/mL, respectively. It detected sakuranetin (rate of flow value is 0.09) in nano-EN treated rice leaves. These nanoparticles inhibited *M. oryzae* and acted as a new elicitor to induce immunity.

Keywords: antifungal effect; biocontrol; microbial elicitor; plant immunity; hypersensitive reaction

Emericella is a fungus with worldwide distribution and it is a common Ascomycete. Moosophon et al. (2009) found that *E. rugulosa* produces new bicyclo[3.3.1]nona-2,6-diene derivatives named isoemericellin, 14-methoxytajixanthone-25-acetate, rugulosone, shamixanthone, tajixanthone, tajixanthone hydrate and tajixanthone methanoate. The bicyclo[3.3.1]nona-2,6-diene derivatives actively inhibited *Plasmodium falciparum* (malaria disease) and *Mycobacterium tuberculosis* (tuberculosis) and was cytotoxic against cancer cell lines. A similar report by Sibounnavong and Soytong (2011) found that the tajixanthone compound from *E. rugulosa* ER01 acted as a control mechanism against the tomato wilt pathogen (*Fusarium oxysporum* f. sp. *lycopersici*). Furthermore, Moosophon et al. (2009) found that *E. rugulosa* ER01 produces tajixanthone, implying antibiosis against *M. oryzae*.

Magnaporthe oryzae causes rice blast and was recorded as rice fever disease in China as early as 1637. It infects the rice plant leading to yield loss of several hundred million tons annually, which has led to shortages in many countries (Wang & Valent 2009). Rice blast is considered as the principal disease of this crop and reaches a high incidence under favorable conditions. Control of the disease is commonly based on resistant varieties, cultural practices and chemical controls (Zeigler et al. 1994).

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Recently biological methods that use microorganisms to control disease have attracted more attention than other methods (Narayanasamy 2013).

The compound sakuranetin was discovered in rice (*Oryza sativa* L.) infected by *M. oryzae*. Sakuranetin is a flavonoid, and diterpenoids which are momilactones A and B, oryzalexins A–F, oryzalexin S, and phytocassanes A to E, (Kodama et al. 1992), oryzalexins A–F, S, and phytocassanes A–D (Dillon et al. 1997). Hasegawa et al. (2014) stated that the role of sakuranetin in blast immunity is that the young leaves of a resistant variety express a hypersensitive reaction (HR) in three days after inoculation with *M. oryzae*; while in a susceptible variety sakuranetin increases three days after inoculation without an HR.

Nanotechnology is a new scientific method to restructure materials at the molecular level. It involves building organic materials into defined structures (Soutter 2012). In recent years, nanotechnology in agriculture has received increasing attention (Li et al. 2011). The biological properties of organic nanomaterials have been studied to provide the efficient management of plant diseases (Salata 2004; Rai & Ingle 2012). The objectives of this research were to determine the control mechanisms of *E. nidulans* against the rice blast pathogen and to construct nanomaterials to control rice blast disease and induce disease immunity.

MATERIAL AND METHODS

Rice blast pathogen – *M. oryzae.* The pathogen was isolated from the symptomatic leaves of rice var. Pitsanulok 2 (PLS 2) using the tissue transplantation technique. Symptomatic leaves were cut into 0.5 × 0.5 cm square pieces from the advanced margins of lesions. These leaf pieces were soaked in 10% sodium hypochlorite for 10 min, cleaned three times with sterile distilled water, and left to dry on a sterilized tissue paper before being transferred to water agar. The hyphal tips from the advanced colony margins were transferred to rice flour agar until pure cultures were obtained. The pathogenicity test was performed according to Koch's postulates. The isolate was inoculated into wounded leaves of 20 rice seedlings planted in pots; wounds were created by puncturing in a diameter of 0.5 cm five times. The controls were wounded in the same manner but non-inoculated. The isolate was firstly identified by morphology, followed by molecular phylogeny (Pornsuriya et al. 2011). The morphology of the fungus was observed under binocular compound and scanning electron microscopes (PHILIPS XL 30 ESEM; Philips, Netherlands).

Antagonist – *E. nidulans.* The isolate used in the research was from the Plant Production Technology Department, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok. The isolate was firstly identified by morphological characters, followed by molecular phylogeny according to the method of Pornsuriya et al. (2011).

Molecular identification. Molecular phylogenetic analysis was done to confirm the species of *M. oryzae* and *E. nidulans.* The genomic DNA was separately extracted from each fungal isolate in potato dextrose broth for five days, filtered and freeze-dried. The DNA was extracted according to the methods of Altschul et al. (1997). Amplification was done by using the polymerase chain reaction for the internal transcribed spacer (ITS) region of ribosomal DNA with the universal primers, ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (Chuwa et al. 2015). The amplified products were processed on a 1% (w/v) agarose gel, sequenced and aligned in GenBank and BLAST analysis (Altschul et al. 1997). Sequences from closely related species were downloaded to produce the phylogenetic trees and aligned with CLUSTALW by MEGA software version 6.0 (Tamura et al. 2007). The phylogenetic trees were created by the neighbor-joining method.

Chemical elucidation. Dried fungal biomass of *E. nidulans* was extracted successively at room temperature with solvents. The filtrates were evaporated *in vacuo* to yield crude ethyl acetate, hexane and methanol extracts. The ethyl acetate and hexane extracts were separated by chromatographic methods and recrystallized to give pure compounds. The structure elucidation was based on IR, ^{1}H NMR, ^{13}C-NMR, 2D NMR and Mass Spectra.

Dual culture. Cultures of *M. oryzae* and *E. nidulans* were individually transferred on to potato dextrose agar (PDA) and incubated for 10 days at room temperature (28 °C). The fungal agar plugs of both tested fungi were separately subcultured on to PDA plates opposite to each other at the same distance. The experimental design was a completely randomized design repeated four

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times. The gathered data were spore production and colony diameter (cm). A haemacytometer was used to count the number of spores.

Bioassay test of metabolites from *E. nidulans* against *M. oryzae.* *E. nidulans* was cultured in PDB for 30 days at 30 °C, and the fungal biomass was harvested. The extraction used the solvents, ethyl acetate, hexane and methanol following the method of Thohinung et al. (2010) and yielded ethyl acetate, hexane and methanol crude extracts. The experimental design was performed using a factorial experiment and a CRD was repeated four times. Factor A represented the extracts of *E. nidulans* and factor B represented the concentrations of 0 µg/mL, 10 µg/mL, 50 µg/mL, 100 µg/mL, 500 µg/mL and 1 000 µg/mL. Data collected were spore production and colony growth. A statistical analysis of variance and means were computed. The spores were recorded by using a haemacytometer (Funakoshi 521-10; Funakoshi Co., Ltd., Japan). Effective dose (ED_{50}) was analysed using probit analysis. The Duncan's multiple range test compared the treatment means at $P \leq 0.05$ and 0.01.

Nano particles of *E. nidulans* testing for growth inhibition of *M. oryzae.* Nanoparticles were constructed from crude hexane, ethyl acetate and methanol extracts of *E. nidulans* based on electrospinning following the method of Dar and Soytong (2014) to yield nano-ENH, nano-ENE and nano-ENM, respectively. The characteristics of each of the nano-particles were observed under the scanning electron microscope (SEM) at the Faculty of Science, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand.

The experiment was conducted as a two-factor factorial using a CRD with four replications. Factor A was nano-particles and factor B was 0 µg/mL, 3 µg/mL, 5 µg/mL, 10 µg/mL and 15 µg/mL. Data recorded were as in the previous experiment.

Phytoalexin production *in vivo.* The experiment was carried out in rice var. PLS 2 in pots. Treatments were sakuranetin standard, nanoparticles of *E. nidulans* and chemical fungicide (tricyclazole). All treatments were inoculated with the rice blast pathogen. Phytoalexin was determined after treatment with nanoparticles constructed from crude extract mixtures from *E. nidulans* (EN) at a concentration of 15 µg/mL, chemical fungicide (tricyclazole) at 300 g/mL; and sterilized distilled water served as the negative control. Thirty-day-old seedlings of rice var. PLS 2 were inoculated with the rice blast pathogen at 1×10^5 spores/mL and fresh leaf samples were collected from the nano-EN and chemical fungicide treatments and control at three, five and seven days. The samples were crushed and soaked in solvent, filtered and passed through a rotary vacuum evaporator (VP50 Plus; LabTech, Inc., USA). Crude extract was analyzed by thin layer chromatography (TLC). Each 1.5 g fresh leaf sample was cleaned in methyl alcohol, cut into small pieces, ground, soaked in 10 mL methyl alcohol, put in water bath at 50 °C for 30 min, then passed through Whatman filter paper No. 4. Each filtrate was evaporated by a rotary vacuum evaporator to obtain crude extract. To the crude extract was added 3 mL methyl alcohol and 1 mL ethyl acetate. Phytoalexin was detected by TLC using the running solvent of chloroform and methyl alcohol at the ratio of 97 : 3. Five millilitres TLC solvent was added to the TLC tank. On the TLC plate was spotted each crude extract sample and the standard for comparison. The procedure was observed under UV light at 365 nm. The rate of flow value (Rf) was computed and compared with the standard sample of sakuranetin. The experiment was repeated three times. Rate of flow value was computed as Rf = distance spot travels/distance mobile phase travels.

RESULTS

Rice blast pathogen – *M. oryzae.* The causal agent was isolated from tissue symptomatic of rice blast and morphologically identified to be *M. oryzae*. The isolate produces an imperfect stage described as *Pyricularia oryzae*. When grown on PDA the culture is a grayish white colony, and conidia are borne on the apex of conidiophores. Colonies are circular with rough and smooth margins, grayish black in colour. The conidia are three-celled, pyriform with rounded base, and narrowed towards the tip (Figure 1). The phylogenic tree of taxa was confirmed to be related to *M. oryzae* inferred using a neighbor-joining method with the ITS rDNA sequence in which *Colletotrichum gloeosporioides* was the out group (Figure 2).

Antagonist – *E. nidulans.* *E. nidulans* was grown on PDA for 21 days. It produces abundant cleistothecia which contain asci, and each ascus has eight ascospores. Sterile hulle cells occur around the fruiting bodies (Figure 3). Molecular

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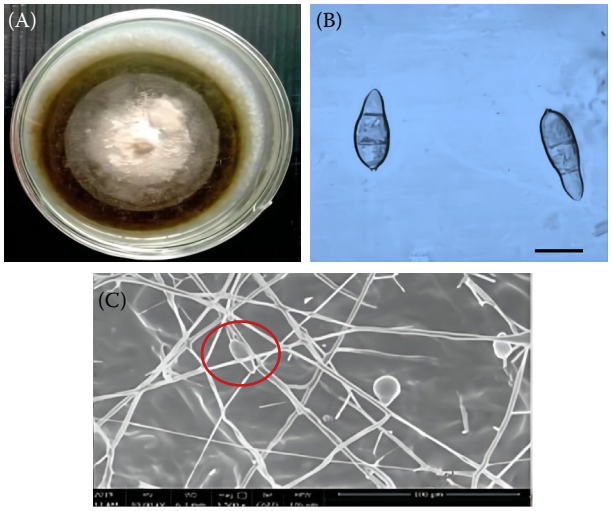

Figure 1. *Magnaporthe oryzae*, (A) a colony on rice flour agar medium at 15 days, (B) conidia under compound microscope and (C) conidia under scanning electron microscopy
Scale bar = 10 µm

phylogeny confirmed the species by the phylogenic tree of taxa to be related to *E. nidulans,* where *C. gloeosporiodes* was the out group (Figure 4).

Chemical elucidation. The separation of crude hexane and ethyl acetate extracts of *E. nidulans* resulted in the isolation of six compounds. Their structures were identified by spectroscopic methods as (A) epishamixanthone, (B) shamixanthone, (C) emericellin, (D) ergosta-6,22-diene-3-ol-5,8-epidioxy-(3β,5α,22*E*), (E) sterigmatocystin and (F) demethylsterigmatocystin as seen in Figure 5.

Dual culture. The results demonstrated that *E. nidulans* is active against *M. oryzae* the rice blast pathogen in the dual culture test. *E. nidulans* inhibited *M. oryzae* in culture in which the colony of *E. nidulans* grew over 60% compared with the pathogen colony (Figure 6). It was also found that the colony of the pathogen in dual culture plates was lower in spore production than the controls.

Bioassay test of metabolites from *E. nidulans* against *M. oryzae*. The result showed that the ethyl acetate crude extract gave the significantly highest spore and colony growth inhibition of 91% and 75%, respectively, followed by the methanol crude extract which inhibited sporulation and colony growth by 88% and 74%, respectively. In addition, the hexane crude extract inhibited sporulation and colony growth by 72% and 69%, respectively. The crude ethyl acetate extract of *E. nidulans* expressed the highest inhibition of spore production with an ED_{50} = 66 µg/mL, followed by the crude

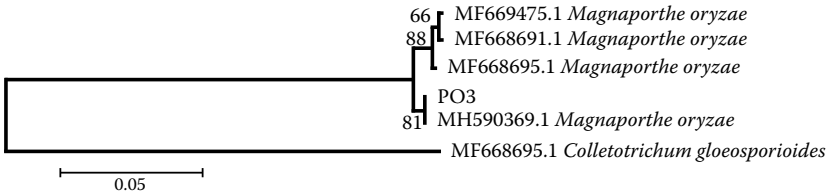

Figure 2. Phylogenic tree of taxa related to *Magnaporthe oryzae* inferred using a neighbour-joining method with the ITS rDNA sequence Bootstrap value is based on 1 000 replicates

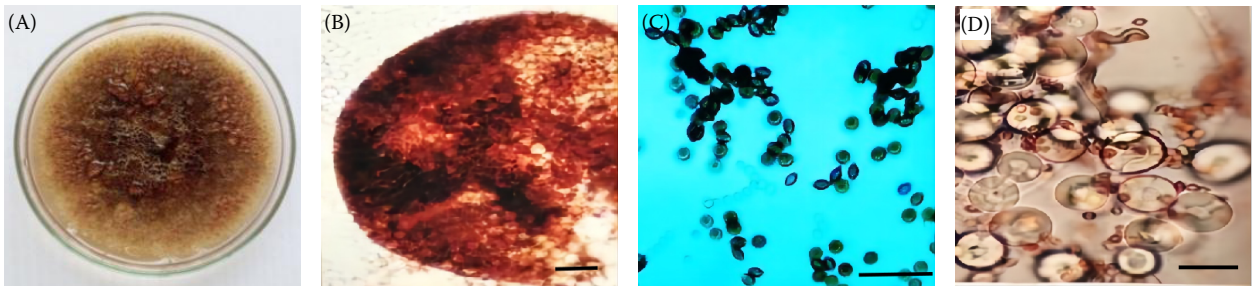

Figure 3. *Emericella nidulans*, (A) colony on PDA after three weeks, (B) cleistothecium, (C) ascospores and (D) hulle cells
Scale bars = 10 µm

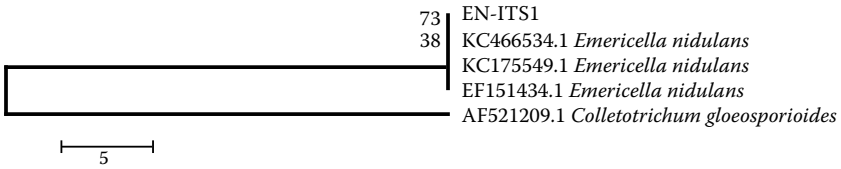

Figure 4. Phylogenic tree of taxa related to *Emericella nidulans* in ferred using a neighbour-joining method with the ITS rDNA sequence Bootstrap value is based on 1 000 replicates

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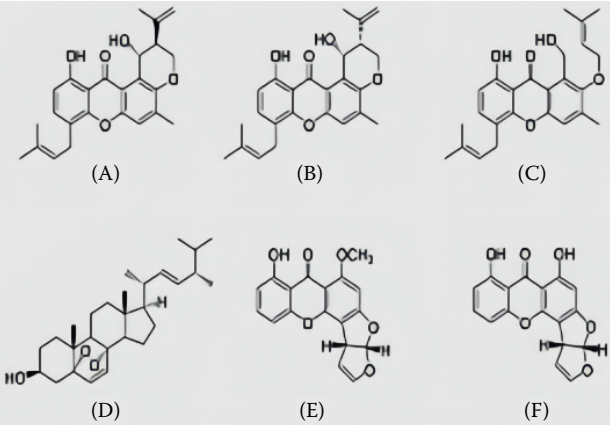

Figure 5. Chemical constituents of *Emericella nidulans*
(A) Epishamixanthone; (B) shamixanthone; (C) emericellin; (D) ergosta-6,22-diene-3-ol-5,8-epidioxy-(3β,5α,22*E*) and (E) sterigmatocystin; while the ethyl acetate extract gave one compound, (F) demethylsterigmatocystin

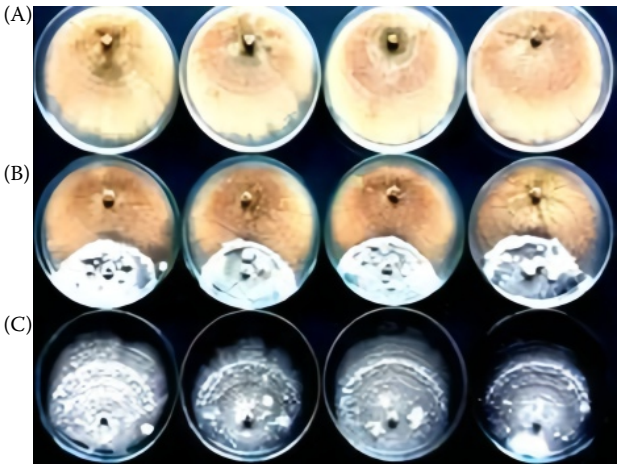

Figure 6. Dual culture test between *Emericella nidulans* and *Magnaporthe oryzae* for 30 days
(A) Antagonist, (B) dual culture, (C) pathogen

methanol extract with an ED_{50} = 85 µg/mL and crude hexane extract with an ED_{50} = 255 µg/mL (Table 1, Figures 7 and 8).

Nano particles of *E. nidulans* testing for growth inhibition of *M. oryzae*. The appearance of nano materials were noted through visual observation by the naked eye. It was seen that nano-ENH is yellow, nano-ENE is yellowish orange, and nano-ENM is orange in colour. The diameter of particle sizes from scanning electron micoscopy of nano-ENH, nano-ENE and nano-ENM were

Table 1. Crude extracts of *Emericella nidulans* testing for growth inhibition of *Magnaporthe oryzae* at eight days, and spore production inhibition at 14 days

Crude extracts	Concentration (µg/mL)	Colony diameter (cm)	Growth inhibition (%)	Number of spores (10^5)	Spore inhibition (%)	Effective dose$_{50}$ (µg/mL)
Crude hexane	0	5.00^{a}	–	4.12^{a}	–	255.83
	10	2.89^{d}	42.00^{h}	3.75^{ab}	8.12^{j}	
	50	2.26^{e}	54.75^{g}	3.31^{b}	19.19^{i}	
	100	2.06^{f}	58.75^{f}	2.56^{c}	37.67^{h}	
	500	1.84^{g}	63.00^{e}	1.75^{ef}	57.41^{e}	
	1 000	1.53^{i}	69.25^{c}	1.12^{g}	72.54^{cd}	
Crude ethyl acetate	0	5.00^{a}	–	4.12^{a}	–	66.83
	10	3.43^{c}	31.25^{i}	3.62^{ab}	11.47^{ij}	
	50	1.56^{i}	68.75^{c}	2.18^{cde}	46.87^{fg}	
	100	1.40^{j}	72.00^{b}	1.31^{fg}	68.79^{d}	
	500	1.31^{jk}	73.75^{ab}	0.81^{ghi}	80.49^{bc}	
	1 000	1.22^{k}	75.50^{a}	0.37^{i}	91.02^{a}	
Crude methanol	0	5.00^{a}	–	4.12^{a}	–	85.33
	10	3.82^{b}	23.50^{j}	3.37^{b}	17.63^{i}	
	50	2.02^{f}	59.50^{f}	2.43^{cd}	40.71^{gh}	
	100	1.87^{g}	62.50^{e}	1.93^{de}	52.81^{ef}	
	500	1.71^{h}	65.75^{d}	1.00^{gh}	75.89^{cd}	
	1 000	1.26^{k}	74.75^{a}	0.50^{hi}	88.21^{ab}	
Coefficient of variation (%)	–	2.48	2.28	16.34	–	–

Means followed by a common letter are not significantly different by Duncan's multiple range test at $P \leq 0.05$

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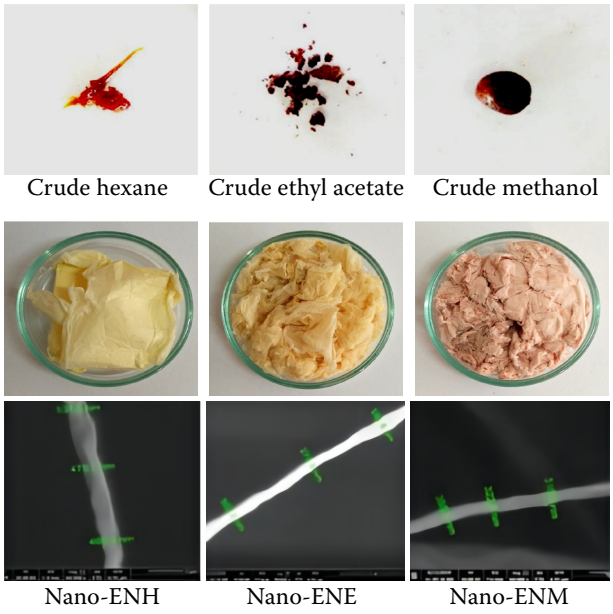

Figure 7. Characteristics of crude hexane, crude ethyl acetate and crude methanol extracts (upper); nanoparticles constructed from *Emericella nidulans* (EN) (middle) and physical appearance of scanning electron micrograph of the nano-particles (lower part)

Nano-ENH – nano particles from crude hexane of EN; Nano-ENE – nano particles from crude ethyl acetate of EN; Nano-ENM – nano particles from crude methanol of EN

averaged as 490 nm, 319 nm and 329 nm, respectively (Figure 7).

It was found that the tested concentrations of nanoparticles from 3–15 µg/mL revealed that the nano-ENE produced a significantly higher spore inhibition (90%) than the nano-ENM and nano-ENH which were 86% and 66%, respectively. The nano-ENE expressed the most antifungal activity against the rice blast pathogen, *M. oryzae,* with ED_{50} = 4.26 µg/mL, followed by nano-ENM with ED_{50} = 4.49 µg/mL, and nano-ENH with ED_{50} = 8.85 µg/mL (Table 2).

Loss of pathogenicity. The application of nanoparticles of nano-ENH, nano-ENE and nono-ENM on the rice seedlings resulted in no symptoms of infection by the tested pathogen, *M. oryzae*. It was also observed that all nanoparticle treatments, nano-ENH, nano-ENE and nano-ENM, caused the spores to become abnormal in shape (Figure 9).

Phytoalexin production *in vivo*. The results showed that sakuranetin was produced in rice var PLS 2 inoculated with a spore suspension of *M. oryzae* after treatment with nanoparticles of *E. nidulans* at the rate of 15 µg/mL and the chemical fungicide (tricyclazole) for three, five and seven days. The plants treated with the sakuranetin standard, nano-EN and chemical fungicide (tricyclazole) showed spots on TLC plates with the same Rf values by using a combination of chloroform: methyl alcohol (97 : 3) under 365 UV light. The Rf values were found to be 0.09 supporting the presence of sakuranetin (Figure 10). This indicated that nanoparticles of *E. nidulans* and tricyclazole could induce immunity to rice blast disease caused by *M. oryzae*, as it was shown that the inoculated leaves and treated nanoparticles of *E. nidulans* and tricyclazole produced a hypersensitive re-

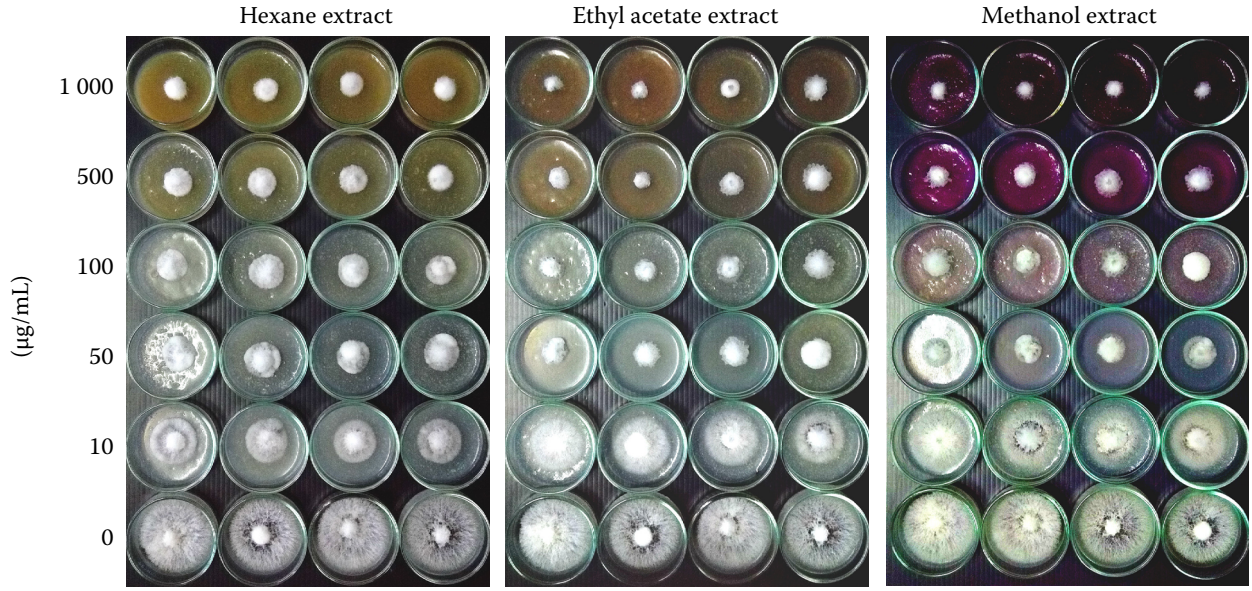

Figure 8. Crude extracts of *Emericella nidulans* tested for inhibition of *Magnaporthe oryzae* growth at eight days and spore production at 14 days

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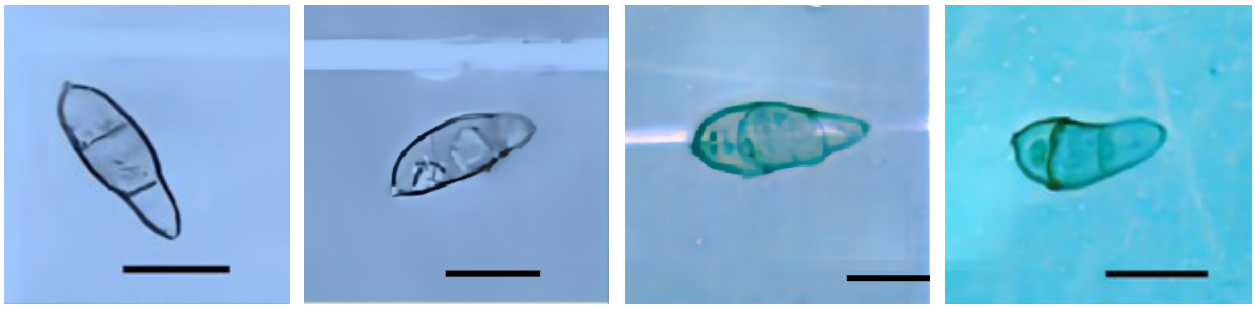
Table 2. Nano particles of *Emericella nidulans* testing for growth inhibition of *Magnaporthe oryzae* at eight days, spore production inhibition at 14 days

Nanopartcles	Concentration (µg/mL)	Colony diameter (cm)	Growth inhibition (%)	Number of spores (10^5)	Spore inhibition (%)	Effective dose$_{50}$ (µg/mL)
From crude hexane	0	5.00^{a1}	–	36.00^{a}	–	8.85
	3	4.98^{ab}	0.25^{i}	28.00^{b}	22.05^{h}	
	5	4.92^{bc}	1.50^{hi}	22.75^{c}	36.50^{f}	
	10	4.82^{e}	3.50^{fg}	18.00^{d}	49.92^{e}	
	15	4.73^{f}	5.25^{de}	12.25^{ef}	66.17^{c}	
From crude ethyl acetate	0	5.00^{a}	–	36.00^{a}	–	4.26
	3	4.94^{abc}	1.00^{hi}	22.25^{c}	38.06^{f}	
	5	4.81^{e}	3.75^{ef}	15.50^{de}	56.88^{d}	
	10	4.28^{i}	14.25^{b}	10.00^{f}	72.27^{b}	
	15	4.04^{j}	19.00^{a}	3.50^{g}	90.31^{a}	
From crude methanol	0	5.00^{a}	–	36.00^{a}	–	4.49
	3	4.89^{cd}	200^{gh}	25.25^{bc}	29.54^{g}	
	5	4.83^{de}	2.50^{fgh}	13.00^{ef}	64.03^{c}	
	10	4.66^{g}	6.75^{d}	9.75^{f}	72.94^{b}	
	15	4.46^{h}	10.75^{c}	5.00^{g}	86.15^{a}	
Coefficient of variation (%)	–	0.92	18.27	10.93	4.55	–

Means followed by a common letter are not significantly different by Duncan's multiple range test at $P \leq 0.05$



Control Nano-ENE Nano-ENH Nano-ENM

Figure 9. The effect of nanoparticles from *Emericella nidulans* to cause distortion in spores of *Magnaporthe oryzae* and resulting in loss of pathogenicity

Nano-ENH – nano particles from crude hexane of *E. nidulans*; Nano-ENE – nano particles from crude ethyl acetate of *E. nidulans*; Nano-ENM – nano particles from crude methanol of *E. nidulans*; scale bars = 10 µm

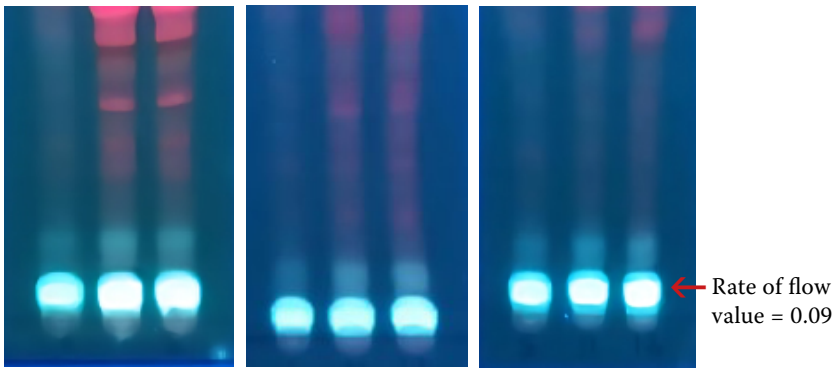


Figure 10. Sakuranetin production after treatment with the nanoparticles of *Emericella nidulans* and chemical fungicide (tricyclazole)

A – sakuranetin standard; B – nanoparticles treatment; C – tricyclazole treatment

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action characterized by reduced-size lesions within three days. However, in the non-treated control lesions of blast disease continued to expand as normal. It was concluded that nanoparticles of *E. nidulans* may possibly be a new microbial elicitor to induce immunity to rice blast disease.

DISCUSSION

M. oryzae and *E. nidulans* were confirmed species by morphology and molecular phylogenetics. The research finding was supported by the works of Soytong and Quimio (1989). It was found that *E. nidulans* exhibited antagonism to *M. oryzae* the causal agent of rice blast for the first time. It was similarly found in other research that *E. rugulosa* ER01 could inhibit *F. oxysporum* f. sp. *lycopersici* causing tomato wilt (Sibounnavong & Soytong 2011).

A crude ethyl acetate extract of *E. nidulans* actively inhibited the spore production of the rice blast pathogen with ED_{50} = 66 µg/mL, followed by crude methanol and crude hexane extracts with ED_{50} = 85 µg/mL and 255 µg/mL, respectively. The control mechanism may involve antibiosis, as similarly report by Moosophon et al. (2009) who found that *E. rugulosa* released the new bicyclo[3.3.1]nona-2,6-diene derivative which has antimalarial (*P. falciparum*) and antimycobacterial (*M. tuberculosis*) activities. This research found that crude extracts and nanoparticles synthesized from *E. nidulans* expressed antifungal activity against the rice blast pathogen.

The chemical constituents of *E. nidulans* have been found to be a control mechanism for antibiosis; sterigmatocystin exhibited activity against breast cancer with ED_{50} = 0.14 µg/mL, followed by small cell lung cancer with ED_{50} = 0.386 µg/mL, and oral human epidermal carcinoma with IC_{50} = 5.59 µg/mL. In the current study, metabolites such as crude extracts and constructed nanoparticles of *E. nidulans* inhibited *M. oryzae* acting as control mechanisms. A similar report by Sibounnavong and Soytong (2011) found that the tajixanthone compound from *E. rugulosa* acted as a control mechanism against *F. oxysporum* f. sp. *lycopersici*, causing wilting of tomato. Furthermore, Moosophon et al. (2009) found that *E. rugulosa* ER01 produces tajixanthone, implying antibiosis against *M. oryzae*.

The particles of nano-ENH, nano-ENE and nano-ENM derived from *E. nidulans* were different in size. The particle diameters of nano-ENH, nano-ENE and nano-ENM were 490 nm, 319 nm and 329 nm, respectively. Wei et al. (2012) reported that the nanofibers constructed by electronspining contained many beads and the diameter averaged about 153.64 nm. That was quite smaller than the result of this current research. Nalwa (2004) and Wang (2000) stated that the nanoparticles' melting point is usually reduced when the particle size reachs the nanometer scale and those nanoparticles still had unchanged nanoparticle properties (Akbari et al. 2011).

As result, the inoculated leaves treated with nanoparticles of *E. nidulans* and tricyclazole resulted in a hypersensitive lesion reaction within three days. Chen et al. (2016) reported rice blast resistance with tricyclazole treatment and the expression of levels of rice defense related genes after tricyclazole treatment. Tricyclazole treatment 24 h after rice blast fungus inoculation could successfully defend against the fungus. It was suggested that tricyclazole may possibly play an important role in immunity to blast disease.

It was demonstrated that nanoparticles from *E. nidulans* produced higher inhibition of *M. oryzae* than crude extracts of *E. nidulans.* The organic nanoparticles can enter pathogens and plant cells faster than the crude extracts. It is possible to develop the nanoparticles for efficient disease management. This investigation was supported by Salata (2004) and Rai and Ingle (2012), who observed that nanoparticles constructed from *E. nidulans* used at low concentrations gave better inhibition of the rice blast pathogen than the crude extracts from *E. nidulans* used at high concentrations. As a result, nanoparticles constructed from *E. nidulans* at the rate of 15 µg/mL were tested as elicitors to induce immunity against rice blast disease. It was found that the negative control did not produce a spot in TLC. The nano-EN and tricyclazole treatments produced spots on TLC plates which were identified as sakuranetin with the Rf value 0.09 after three, five and seven days post inoculation. The same result was reported by Mok (2009) that sakuranetin from rice leaves produced a spot in TLC at Rf 0.09 under 365 UV light demonstrating the presence of sakuranetin.

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