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Insecticidal Activities of Ethyl Acetate Extract of Indonesian Mangrove Fungus *Emericella nidulans* BPPTCC 6038 on *Spodoptera litura*

SILVA ABRAHAM[1]*, ADI BASUKRIADI[2], SUYANTO PAWIROHARSONO[1], AND WELLYZAR SJAMSURIDZAL[2]

[1]*Center for Bioindustrial Technology, Badan Pengkajian dan Penerapan Teknologi, Gedung LAPTIAB, PUSPIPTEK Serpong, Tangerang Selatan 15314, Indonesia;*
[2]*Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Indonesia, Kampus UI Depok, 16424, Indonesia*

Mangrove fungi are known as sources of biological active compounds. The study and the report of secondary metabolites of mangrove fungi as insecticides is very limited in Indonesia. This study assess the insecticidal activities of ethyl acetate extract of Indonesian mangrove fungus *Emericella nidulans* BPPTCC 6038 against *Spodoptera litura* (Lepidoptera, Noctuidae) neonate larvae and pupae. The fungus *E. nidulans* BPPTCC 6038 was isolated from leaves of mangrove *Rhizophora mucronata* and identified based on ITS rDNA sequence data, with the GenBank accession number KP165435, and confirmed with morphological observation. This fungus strain was grown on malt extract broth for 14 days on rotary shaker at 65 rpm, and incubated at room temperature. Mortalities of *S. litura* were observed on larvae fed on artificial diet containing ethyl acetate extract of *E. nidulans* at concentrations of 625-5 000 ppm. The lethal concentration of the extract which causes 50% mortality of larvae (LC_{50} value) was 1 102.27 ppm. The other effects of fungus extract on *S. litura* were decrease in growth rate, longer larval period, inhibition on pupal development and absence in adult emergence. The HPLC analysis of extract showed that the crude extract contained three major compounds. This study provides evidence that the extract of *E. nidulans* possesses insecticidal activities against *S. litura*.

Keywords: *Emericella nidulans*, insecticidal activity, mangrove fungi, *Spodoptera litura*

Kapang mangrove telah dikenal sebagai sumber senyawa aktif. Penelitian dan laporan mengenai aplikasi senyawa metabolit sekunder kapang mangrove sebagai insektisida di Indonesia masih sangat terbatas. Penelitian ini mengkaji aktivitas ekstrak etil asetat kapang mangrove *Emericella nidulans* BPPTCC 6038 terhadap larva neonate dan pupa *Spodoptera litura* (Lepidoptera, Noctuidae). Kapang *E. nidulans* BPPTCC 6038 diisolasi dari daun tumbuhan mangrove *Rhizophora mucronata* dan diidentifikasi berdasarkan data sekuens ITS rDNA dengan kode akses GenBank KP165435. Kapang ditumbuhkan dalam medium malt extract broth selama 14 hari dengan agitasi 65 rpm pada suhu kamar. Mortalitas larva *S. litura* diamati pada perlakuan dengan penambahan ekstrak etil asetat yang dihasilkan oleh *E. nidulans* ke dalam pakan buatan dengan konsentrasi 625-5000 ppm. Konsentrasi ekstrak yang menyebabkan kematian larva sebesar 50% (nilai 50% of *Lethal Concentration* - LC50) adalah 1 102,27 ppm. Pengaruh lain dari ekstrak kapang *E. nidulans* terhadap *S. litura* adalah penurunan laju pertumbuhan, penambahan waktu periode larva, penghambatan pembentukan pupa, dan tidak ada serangga dewasa yang dihasilkan. Hasil HPLC terhadap ekstrak *E. nidulans* menunjukan bahwa ekstrak tersebut terdiri dari beberapa senyawa dengan tiga senyawa utama. Penelitian ini membuktikan bahwa ekstrak yang dihasilkan oleh kapang *E. nidulans* memiliki aktivitas insektisida terhadap *S. litura*.

Kata kunci : aktivitas insektisida, *Emericella nidulans*, kapang mangrove, *Spodoptera litura*

Natural products derived from microorganisms have been used for insect control. Most of the pesticides from microorganisms have been isolated from entomopathogens and the terrestrial environment (Brakhage and Schroeckh 2011). Metabolites from fungal genera, such as *Metarhizium*, *Trichophyton*, *Chrysosporium* and *Lagenidium*, as well as some actinomycetes, and several basidiomycetes, have shown potential insecticidal activity (Bucker *et al*. 2013). Actinomycetes, the Gram positive filamentous bacteria, constituting a significant component of the microbial population in most soils. Among actinomycetes, around 7600 compounds are produced by *Streptomyces* species (Balachandran *et al*. 2015; Velayudam and Murugan 2015).

Marine environment is still under-explored and is considered to be a prolific resource for the isolation of less exploited microorganisms. Recent study on mangrove and marine microorganisms have focused mainly on the discovery of human drugs, whereas limited information about mangrove microorganisms possessing insecticidal activities have been reported

*Corresponding author; Phone: +62-21-7560729, Email:silvaabraham@yahoo.com

(Arasu *et al*. 2013).

Mangrove fungi are the second group of marine derived fungi which produces new chemical compounds (Chen *et al*. 2011). Mangrove associated fungi provide a broad variety of bioactive secondary metabolites with unique structure, including alkaloids, benzopyranones, chinones, flavonoids, phenolic acids, quinones, steroids, terpenoids, tetralones, xanthones, and others (Joel and Bhimba 2013). New active compounds which isolated from mangrove fungi culture media exhibited toxicity for insects *Helicoverpa armigera* and *Sinergasilus* sp. (Chen *et al*. 2006). Several species of *Aspergillus* fungi from the mangrove plants such as *Aspergillus* sp., *A. flavus*, and *A. niger* have known produce several active compounds (Chaeprasert *et al*. 2010; Chen *et al*. 2011). Abraham *et al*. (2015) reported that five species of *Aspergillus* and *Emericella* isolated from *Rhizophora mucronata* mangrove plant exhibited larvacidal activity on *Artemia salina*, neurotoxicity on *S. litura* larvae and acetylcholinesterase inhibition activity.

Spodoptera litura is the polyphagous insect attacking more than 150 different host species and affect the agricultural crops yield (Arasu *et al*. 2013). *S. litura* plays a major role in damaging the agricultural crops and therefore considered as the most economically important insect pests in many countries including India, Japan, China, and Southeast Asia. The chemical insecticides usually used for controlling these polyphagous insect. The usage of different varieties of chemical insecticides to control insects has resulted in emergence of insecticides resistance in the pests.

Due to this reason, many researchers have involved on invention of alternative control methods for insect's pests. Microbial insecticides are having advantage over chemical pesticides by its highly effective, safe, ecologically acceptable and pose fewer hazards (Arasu *et al*. 2013; Dhanasekaran and Thangaraj 2014). These microbial insecticides tend to be highly selective and established as an alternative to eco-destabilizing chemical insecticides especially against lepidopteran insect (Dhanasekaran and Thangaraj 2014; Arasu *et al.* 2013).

This work explores the presence of mangrove fungal secondary metabolite substances with biological properties against *S. litura* larva with the long term objective for developing them as bioinsecticides. The aim of this work was to evaluate the insecticidal properties of crude ethyl acetate extract of secondary metabolites from the *Emericella nidulans* mangrove fungus against one of the most important polyphagus insect pests, *S. litura*.

MATERIAL AND METHODS

Isolation and Identification of Mangrove Fungus. The leaves of mangrove plant *R. mucronata* were collected in August 14, 2012 at Mangrove Rehabilitation and Ecotourism of Prof. Dr. Sedyatmo Angke Kapuk, Jakarta, Indonesia (Abraham *et al*. 2015). The isolation and identification of mangrove fungus were followed the methods described by Abraham *et al*. (2015). The leaf samples were washed in sterile artificial sea water (Höller 1999) to remove dust and soil particles and were cut into 1 cm × 1 cm segments using a sterile razor blade. The leaf segments were surface sterilized following the procedures described by Ananda and Sridhar (2002). The surface-disinfected leaf segments (1 cm × 1 cm) were pressed on to the surface of PDA medium to ascertain the efficacy of surface sterilization procedure (Schulz *et al*. 1993). Ten gram of sample was added with 100 mL of sterile aquadest and crushed with blander. The sample suspension was placed on the milipore membrane (Sartorius, Gottingen, Germany; pore size 0.45 μm) in Buchner filter apparatus under vacuum condition (Vacuubrand GMBH + CO type ME2, Wertheim, Germany). The milipore membrane with sample on the membrane surface was placed on the modification of SDA media (32.5 g SDA-Oxoid; 500 mL artificial sea water; 0.5 mL from 0.6 g mL^{-1} streptomycin; 0.5 mL from 0.05 g mL^{-1}, tetracycline; 0.5 ml from 0.1 g mL^{-1} dodine; 2.5 m L^{-1} from 0.05 g mL^{-1} cyclohexamide) and incubated at the room temperature until mycelia appeared.

The identification of the fungi was conducted by amplification of the ITS1 and ITS4 region of ribosomal DNA. Fungus DNA was extracted from mycelium using kit from PrepMan™ ultra (Applied Biosystems, Foster City, CA, USA). The obtaining DNA was amplified using the polymerase chain reaction (PCR) apparatus (Bio-Rad MyCycler thermal cycler, Bio-Rad Laboratories, Inc., Hercules, CA, USA) with ITS1 and ITS4 primers (KAPA2G Robust HotStart ReadyMix, Kapa Biosystems, Inc., Wilmington, MA, USA). The amplification cycle consisted of an initial denaturation step of 95 °C for 1 min followed by 40 cycles of (i) denaturation (94 °C for 1 min), (ii) annealing (60 °C for 1 min), and (iii) elongation (72 °C for 1 min), and a final elongation of 72 °C for 5 min (Michaelsen *et al*. 2006). The PCR products were sequenced using an automated multicapillary DNA sequencer (ABI Prism 310 Genetic analyzer, Applied Biosystems, Foster City, CA, USA). The sequences data were aligned with

sequences from the DNA GenBank hosted by NCBI (http://blast.ncbi.nlm.nih.gov) using BLASTP tool. The sequence data of ITS rDNA of the fungus strain was deposited into GenBank under the accession number KP165435. The macroscopic and microscopic observation on fungus morphology was conducted to confirm the molecular identification.

Fermentation and Extraction of Fungus Secondary Metabolite. The fermentation and extraction of fungus metabolites were followed the procedures described by Abraham *et al*. (2015). The culture of fungus strain was cultivated in 500 mL conical flask containing 180 mL fresh ME medium (30 g malt extract; 5 g peptone; 1 000 mL artificial sea water) and incubated for 14 d on rotary shaker (Heidolph Unimax 2010, Heidolph Instruments GmbH & Co., Schwabach, Germany) at 65 rpm and kept at room temperature.

The culture was filtered through Whatman no.1 filter paper to obtain the aqueous filtrate. The aqueous filtrate was extracted with 100 mL of ethyl acetate using separation funnel. The water fraction (upper layer) was collected and re-extracted (three times) with ethyl acetate. The ethyl acetate fraction (bottom layer) was collected and the solvents were removed by vacuum rotary evaporation (Heidolph Laborta 4000-efficient, Heidolph Instruments GmbH & Co., Schwabach, Germany).

Insect Collection and Rearing. Larvae of *S. litura* were collected from the villager's capsicum and corn plant in Bogor (Bojong, Semplak, Leuwi Liang and Ciapus), West Java, Indonesia and reared in a plastic box container and fed with artificial diet (Supriyono, 1997) comprised of 150 g of soya bean (soaked in 460 ml of aquadest for 24 h; 3 g of L-(+)-ascorbic acid; 3 g of nipagine (p-hidroxyibenzoacidethylester), 11 g of dried yeast powder; 180 mg of gentamicyn sulphate; 1 mL of paraformaldehyde; 10 g of agar and 315 mL of aquadest. The laboratory reared larvae were used for bioassay and the *S. litura* culture was maintained throughout the study period.

Larvacidal Activity of The Fungus Secondary Metabolite. Larvacidal activity of fungus secondary metabolite was evaluated using feeding dietary no-choice method described by Supriyono (1997). The freeze dried artificial diet powder (0.735 g) in 2.2 mL of agar solution (78 mg of agar in 2.2 mL of aquadest) supplemented with fungus ethyl acetate extract in different concentrations (5, 4.5, 4, 3.5, 3, 2.5, and 1.25 mg mL^{-1} negative control and deltametrhin 25 g mL^{-1} (Decis, Bayer CropScience, Jakarta, Indonesia) was used as positive control. The experiment was performed at room temperature for 6 d. Larval mortality, larval weight and larval consumption of artificial diet were observed and recorded after 6 d of treatment. The larval growth rate was calculated according formula of Supriyono (1997):

$$\text{Growth rate} = \frac{\text{Average of weight from viable larvae}}{\text{Average of weight from control larvae}} \times 100$$

The larval mortality was calculated according formula of Arivoli and Tennyson (2013) and corrections were made when necessary using Abbott's formula.

$$\text{Per cent larval mortality} = \frac{\text{Number of dead larvae}}{\text{Total number of treated larvae}} \times 100$$

$$\text{Corrected Mortality (\%)} = \left\{ \frac{(\%MT - \%MC)}{(100 - \%MC)} \right\} \times 100$$

where %MT = % larval mortality in treatment; %MC = % larval mortality in control.

Probit analysis (Finney 1971) was conducted to calculate median lethal concentration (Lc_{50}). One-way analysis of variance (ANOVA) was used to compare the treatment means of feeding dietary bioassay to *S. litura* larvae. A post-hoc Tukey's honestly significant difference (HSD) test, with a significance level of $\alpha = 0.05$, was performed when a significant difference between treatment means was detected. All statistical analyses were performed using IBM SPSS Statistics ver. 21 software (IBM Corp., Armonk, NY, USA).

Larval and Pupal Durations. The larvae which survived from larvacidal activity treatment (at 5 000 ppm concentration) were continuously fed with normal artificial diet, without ethyl acetate extracts addition, until they became pupae and adults. The larval growth and mortality were observed every day. The larval duration after the treatment was recorded. Pupal period was calculated from the day of pupation to the day of adult emergence.

Preliminary Chemical Characterization of the Active Extracts. The active ethyl acetate extract were analyzed by High Performance Liquid Chromatography (HPLC, Waters HPLC-UV Vis detector, Waters Co., Milford, MA, USA) according the procedure described by Abraham *et al*. (2015). All amounts of the secondary metabolite extract were loaded onto a C18 column, in 20

μL injection volume. Elution was performed using a linear gradient consisting of double distilled water (ddH_2O) and acetonitrile; an isocratic step was initially employed for 3 min at 85% water, followed by a moderate increase in acetonitrile to reach 100% in 20 min, at a flow rate of 1 mL min^{-1}. The second isocratic step was employed for 5 min with 100% of acetonitrile. To monitor the elution profile of secondary metabolites extract, absorption at 254 nm was used.

RESULTS

Isolation and Identification of Mangrove Fungus. The macroscopic and microscopic observation on fungus colonies exhibited the sexual structures *i.e* Hulle cells, cleistothecia, ascocarp and ascospores, which confirmed the *Emericella* species, a teleomorphic state of *Aspergillus*. The identification of fungus isolate using ITS1 and ITS4 region produced sequences with at least 700 nucleotide base pairs. The blast result from NCBI shown the isolate sequences posses 100% homology with *E. nidulans* species found in the genbank database. The phylogenetic tree which shows the relationship of species with the other species from genbank is showed in Fig 1.

Larvacidal Activity of The Fungus Secondary Metabolite. In the present study, ethyl acetate extract from secondary metabolite derived from *E. nidulans* revealed insecticidal activity against *S. litura* neonate larvae. Table 1 shows mean of insecticidal activity of ethyl acetate extract from each concentration. The lethal concentration of the ethyl acetate extract which causes 50% mortality of larvae (50% of lethal concentration or LC_{50} value) was 1 102.27 ppm. Fig 2 shows the regression line for the larval mortality induced by each concentration of ethyl acetate extract treatment. The addition of ethyl acetate extract to the artificial diet demonstrated the influence of the extracts to the growth rate and larval mortality. The correlation between the concentration of the extracts to larval growth rate and mortality is showing in Fig 3.

Larval and pupal durations. The ethyl acetate extract demonstrated the influence to larval period, inhibition in pupation and adult emergence process. Tabel 2 shows the influence of the extract to the time to reach pupation, the number of larvae which reach pupation and the percentage of adult emergence.

Preliminary chemical characterization of the active extracts. The HPLC profile from ethyl acetate extract of secondary metabolite produced by *E. nidulans* (Fig 4) were exhibited several peaks with three major peaks. The profilles indicated that the extract containing several compounds with three major compounds.

DISCUSSION

Isolation and Identification of Mangrove Fungus. Based on result from database in NCBI Blast tool for ITS1 and ITS4 sequences, the fungus isolate was identified as *E. nidulans*, teleomorph of *Aspergillus* genus. Several studies reported that *Aspergillus* fungi like *A. flavus*; *A. niger*; *A. versicolor* and *A. nidulans* have been isolated from leaves, twig, and root of several mangroves Rhizophoraceae e.g. *Rhizophora mucronata; R. stylosa* and *R. apiculata* and produce the wide variety of secondary metabolites which have activities against human microbial pathogens; cancer Hep2 and MCF7 cell lines, and acetylcholinesterase inhibition avtivity (Bhimba *et al*. 2012; Chun *et al*. 2013; Abraham *et al*. 2015). The fungus *A. oryzae* obtained from the marine red alga

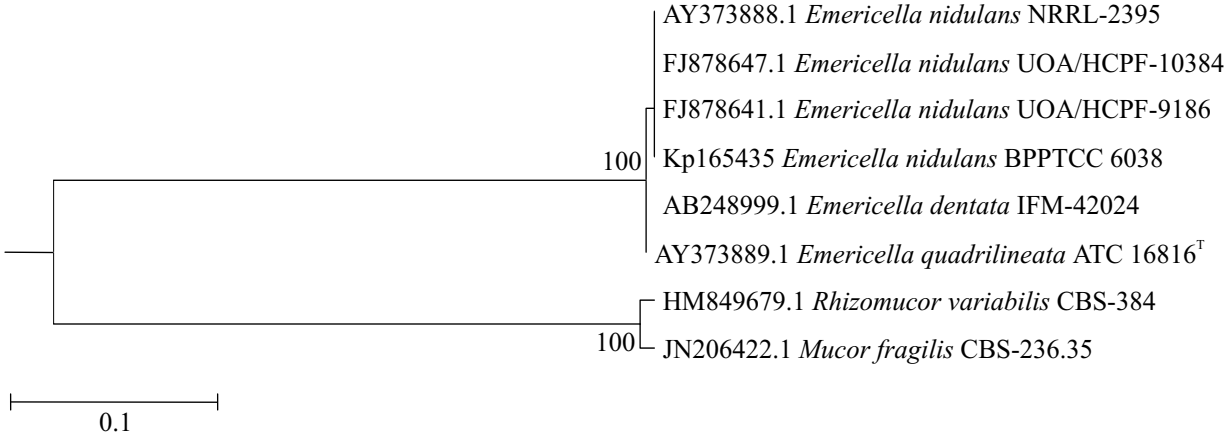

Fig 1 The neighbor-joining phylogenetic tree (shown as a rectangular cladogram) of the mangrove fungus *Emericella nidulans* BPPTCC 6038.

Table 1 Percent larvacidal activity of ethyl acetate extracts from five mangrove fungi against *Spodoptera litura* (mean ± SE)

No.	Fungus extract	Mean of mortality (%) *S. Litura* neonate from different extract concentrations (ppm)							
		5000	4500	4000	3500	3000	2500	1250	625
1.	*E. nidulans* DRM3M3	76.67 ± 12.01 ^{b}	68.33 ± 4.41 ^{ab}	55.00 ± 2.89 ^{ab}	51.67 ± 3.3 ^{ab}	53.33 ± 1.67 ^{ab}	58.33 ± 3.33 ^{ab}	51.67 ± 1.67 ^{ab}	55.00 ± 2.89 ^{ab}
2.	No extract (Control -)		0	0	0	0	0	0	0

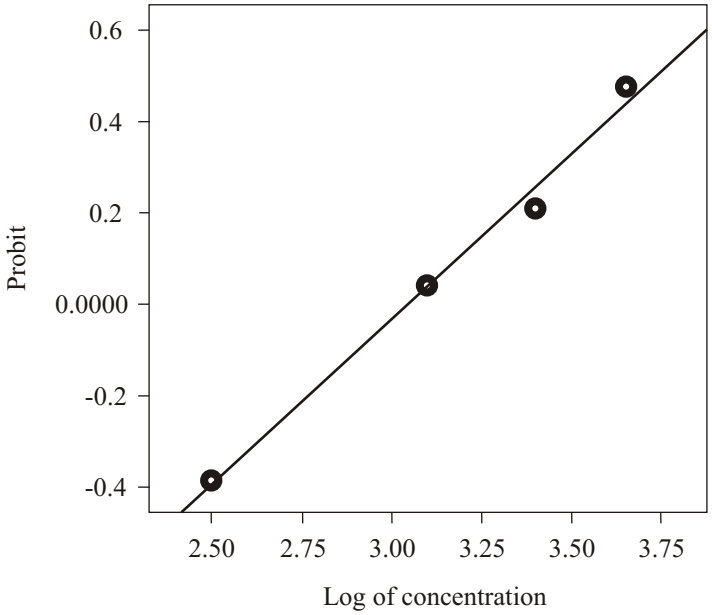

Fig 2 The linear regression of probit mortality against log concentration of ethyl acetate extract from *Emericella nidulans* BPPTCC 6038 ethyl acetate extract on *Spodoptera litura* larvae.

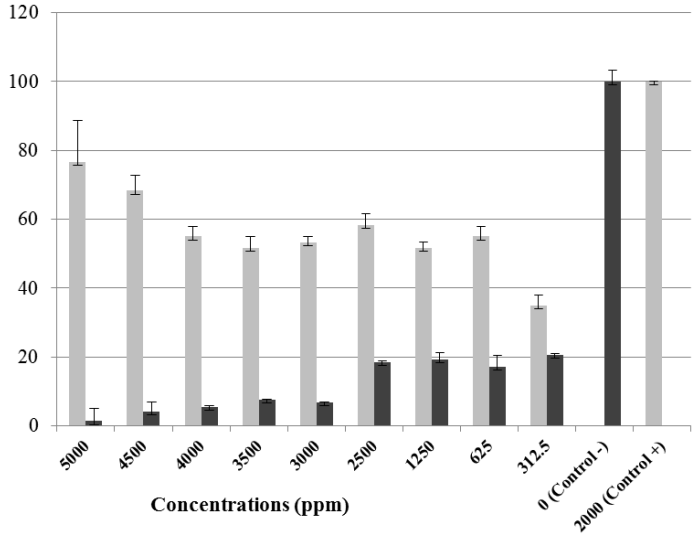

Fig 3 Effects of *Emericella nidulans* BPPTCC 6038 ethyl acetate extract on mortality and growth rate of *Spodoptera litura* neonate larvae. (▒) Mortality (%), (■) growth rate (%).

Table 2 Effects of the *Emericella nidulans* BPPTCC 6038 ethyl acetate extract to *Spodoptera litura* larval and pupal growth and development

No.	Fungus extract	Numbers of larval observed	Larval duration (in days)	Pupal duration (in days)	Adult emergence (%)	
					From larvae	From pupae
1.	*Emericella nidulans* BPPTCC 6038	3	> 23	0	0	0
2.	No extract (Control -)	20	12-17	2-5	90	100

After 6 d treatment with artificial diet containing 5 000 ppm extract.

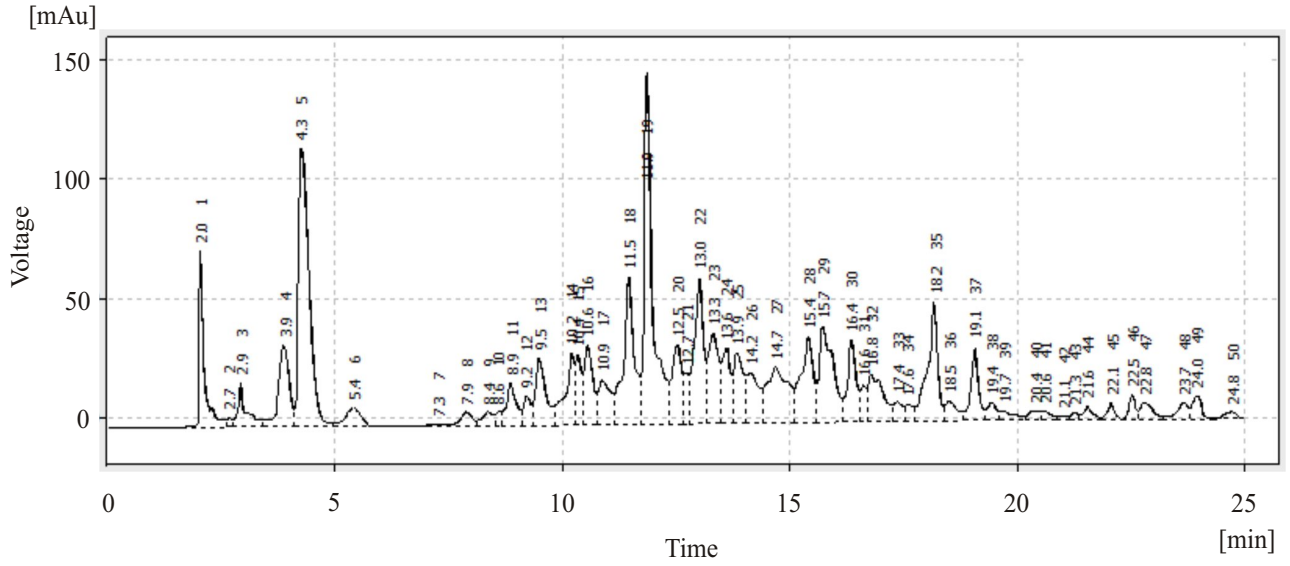

Fig 4 HPLC profile from ethyl acetate extract of secondary metabolites produced by *Emericella nidulans* BPPTCC 6038.

Heterosiphonia japonica produced two new indoloditerpene derivatives, known as tremorgenic mycotoxins, which exhibit potent insecticidal, antiinsectan, and antibiotic activities (Qiao *et al*. 2010). The mangrove fungus *A. oryzae* isolated from *R. mucronata* reported produced secondary metabolite that has larvacidal, insecticidal and acetylcholinesterase inhibition activities (Abraham *et al*. 2015). In marine and estuarine environment, *Aspergillus* also the one of fungal genus that often isolated from the different host, from the marine organisms to mangrove plant (Nofiani *et al.* 2012).

The endophytic fungus *Emericella* sp. isolated from the mangrove plant *Aegiceras corniculatum* led to isolation of six isoindolones derivatives termed as emerimidine A and B and emeriphenolicins A and D, and six previously reported compounds named aspernidine A and B, austin, austinol, dehydroaustin, and acetoxydehydroaustin with antiviral activity detected in almost compounds (Zhang *et al*. 2011). The asexual state of *E. nidulans*, *A. nidulans* which isolated from fresh leaves of the mangrove plant *Rhizophora stylosa* produced six new dihydroquinolone derivatives along with the related aflaquinolone A and a part of those compounds shown toxic activity against *Artemia salina* (Chun *et al*. 2013). Two strains of *E. nidulans* which isolated from root and leaves of the mangrove plant *R. mucronata* also shown toxic activity against *A. salina* (Abraham *et al*. 2015).

Larvacidal Activity of The Fungus Secondary Metabolite. In the present study, ethyl acetate extract from secondary metabolite derived from *E.nidulans* revealed strong larvacidal activity (76.67%) against *S. litura* neonate larva at 5 000 ppm concentration and was statistically significant over controTtable 1). The ethyl acetate extract from *E. nidulans* secondary metabolite exhibited constant insecticidal activity for almost concentrations ranges, caused at least 55% larval mortality from 625 ppm to 4 000 ppm (Table 1 and Fig 2). The study conducted by Abraham *et al*. (2015) reported that ethyl acetate extract from two strains of *E. nidulans* culture filtrate shown acute toxicity on *S.litura* larvae. It's probably due to toxic substances containing in the *E. nidulans* secondary

metabolite. Several studies indicate that mostly *E. nidulans* strains produced styrigmatocystin, the most highly toxic, mutagenic, and carcinogenic compounds which natural products known (Frisvad *et al*. 2004). The study conducted by Matasyoh *et al*. (2011) reported that strerigmatocystin had larvacidal activity against *Anopheles gambiae* third instars larvae.

The LC_{50} value of the extract was 1 102.27 ppm (Fig 2), relatively equivalent with LC_{50} values of commercial insecticides chlorpyrifos and deltamethrin (4 180 ppm and 3 990 ppm respectively) against *S. litura* III instar larvae (Tong *et al*. 2013). High larval mortality normally indicates potential insecticidal activity of fungal secondary metabolite extract. Secondary metabolite compounds act as insecticides by poisoning or by production of toxic molecules after ingestion (Jeyasankar *et al*. 2014). Larval mortality may be attributed to direct insecticidal action (as a contact poison) or due to feeding inhibition or gustatory repellency or impairment in the food assimilation (Jeyasankar *et al*. 2014).

The growth rate of *S. litura* neonate larva treated with the fungus extract were lower than the control and generally exhibited the tendency to increased proportionally with the decreased of concentrations. The larval growth rate demonstrated the exception in 2 500 ppm and 1 250 ppm concentrations, the growth rate tend to increased compared with the lower concentrations (Fig 3). The increasing of growth rate indicated the attractant substances in the extracts that induced larva to eat the artificial diet containing those substances. The further study, *e.g*. choice test and detection of volatile compound in the extract which attracts the larva to feed, is required to confirm the possibilities of attractant substances in the extract.

Larval and Pupal Durations. After treatment with ethyl acetate extract, the larval developmental period were increased significantly Table 2), mostly all of the larvae were not able to go into further instars. The interference of toxic substances in the moulting process triggers the larval duration. Larval developmental period was increased in treatment (more than 23 d) when compared to the control (12 to 17 d). The pupation process was inhibited and eventually there was no imago emergence from the metamorphosis process. In general, prolonged in larval duration was directly proportional to the increase in pupacidal activities (Arasu *et al*. 2013).

The extension of larval period, failure in pupation and adult emergence could either be due to the presence of toxic ingredients in the extract or the imbalances between growth stimulating and growth inhibiting hormones (Arivoli and Tennyson 2013). Insect growth regulation properties of fungal secondary metabolite extracts are very unique in nature, since insect growth regulator works on juvenile hormone. The enzyme ecdysone plays a major role in shedding of old skin and the phenomenon is called ecdysis or moulting (Packiam and Ignacimuthu 2012). When the active fungal compounds enter into the body of the larvae, the activity of ecdysone is suppressed and the larva fails to moult, remaining in the larval stage and ultimately dying. The inhibition to the larval growth and development might be due to the interference of toxic substances that were present in the ethyl acetate extracts on the growth and developmental processes of the test insect.

Preliminary Chemical Characterization of The Active Extracts. HPLC profile of ethyl acetate extracts from *E. nidulans* which shown insecticidal activities exhibited several peaks and three major peaks (Fig 4). The profile indicated the several substances with three major substances in the extract. Each peak of ethyl acetate extract could be represented the single compound which has different toxicity and its need the further study to identified the each compound and examine toxicities degree of each fraction to obtain the best toxic fraction. Through the optimization of fermentation and extraction process, the quantity of the toxic fraction could be increased so the insecticidal activity of the crude extract also could be increased.

In conclusion, ethyl acetate extracts of *E. nidulans* secondary metabolite showed insecticidal activity and inhibition on larval growth and development on *S. litura*. The results obtained from the present investigation suggests that further studies on isolation and identification of the active insecticidal compound and on mode of action needs to develop the promising *E. nidulans* secondary metabolite as an alternative method or tool for the control of *S. litura*.

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REFERENCES

Abraham S, Basukriadi A, Pawiroharsono S, Sjamsuridzal W. 2015. Insecticidal activity of ethyl acetate extracts from culture filtrates of mangrove fungal endophytes. Mycobiology 43(2):132-149. doi:10.5941/MYCO.2015.43.2.137.

Ananda K, Sridhar KR. 2002. Diversity of endophytic fungi in the roots of mangrove species on the west coast of India. Can J Microbiol. 48:871-878. doi:10.1139/W02-080.

Arasu MV, Al-Dhabi NB, Saritha V, Duraipandiyan V, Muthukumar V, Kim SJ. 2013. Antifeedant, larvicidal and growth inhibitory bioactivities of novel polyketide metabolite isolated from *Streptomyces* sp. AP-123 against *Helicoverpa armigera* and *Spodoptera litura*. BMC Microbiol. 13:105-111. doi: 10.1186/1471-2180-13-105.

Arivoli S, Tennyson S. 2013. Antifeedant activity, developmental indices and morphogenetic variations of plant extracts against *Spodoptera litura* (Fab) (Lepidoptera: Noctuidae). J Entomol Zool Stud. 1(4):87-96.

Balachandran C, Duraipandiyan V, Emi N, Ignacimuthu S. 2015. Antimicrobial and cytotoxic properties of *Streptomyces* sp. (ERINLG-51) isolated from Southern Western Ghats. South Indian J Bio Sci. 1(1):7-14.

Bhimba BV, Franco DAAD, Mathew JM, Jose GM, Joel EL, Thangaraj M. 2012. Anticancer and antimicrobial activity of mangrove derived fungi *Hypocrea lixii* VB1. Chin J Nat Med. 10(1):77-80. doi:10.3724/SP.J.1009.2012.00077.

Brakhage AA, Schroeckh V. 2011. Fungal secondary metabolites-strategies to activate silent gene clusters. Fungal Gene Biol. 48:15-22. doi:10.1016/j.fgb.2010.04.004.

Bücker A, Bücker DCF, de Souza AQL, da Gama AM, Rodrigues-Filho E, da Costa FM, Nunez CV, Silva CA, Tadei WP. 2013. Larvicidal effects of endophytic and basidiomycete fungus extracts on *Aedes* and *Anopheles* larvae (Diptera, Culicidae). Rev Soc Bras Med Trop. 46(4):411-419. doi:10.1590/0037-8682-0063-2013.

Chaeprasert S, Piapukiew J, Whalley AJS, Sihanonth P. 2010. Endophytic fungi from mangrove plant species of Thailand: their antimicrobial and anticancer potentials. Bot Mar. 53:555-564. doi:10.1515/BOT.2010.074.

Chen G, Lin Y, Vrijmoed LLP, Fong WF. 2006. A new isochromanan from the marine endophytic fungus 1893. Chem Nat Compd. 42(2):63-72. doi:10.1007/s10600-006-0062-1.

Chen Y, MaoW , Tao H, Zhu W, Qi X, Chen Y, Li H, Zhao C, Yang Y, Hou Y, Wang C, Li N. 2011. Structural characterization and antioxidant properties of an exopolysaccharide produced by the mangrove endophytic fungus *Aspergillus* sp. Y16. Bioresour Technol. 102:8179-8184. doi:10.1016/j.biortech.2011.06.048.

Chun YA, Xiao ML, Han L, Chun SL, Ming HW, Gang MX, Bin GW. 2013. 4-Phenyl-3,4-dihydroquinolone derivatives from *Aspergillus nidulans* MA-143, an endophytic fungus isolated from the mmangrove plant *Rhizophora stylosa.* J Nat Prod. 76(10):1896-1901. doi:10.1021/np4004646.

Dhanasekaran D, Thangaraj R. 2014. Microbial secondary metabolites are an alternative approaches against insect vector to prevent zoonotic diseases. Asian Pac J Trop Dis. 4(4):253-261. doi:10.1016/S2222-1808(14)60569-7.

Finney D J. 1971. Probit Analysis. 3th edition. London: Cambridge University Press.

Frisvad JC, Samson RA, Smedsgaard J. 2004. *Emericella astellata*, a new producer of aflatoxin B1, B2 and sterigmatocystin. Lett Appl Microbiol. 38:440-445. doi:10.1111/j.1472-765X.2004.01520.x.

Höller U. 1999. Isolation, biological activity and secondary metabolite investigations of marine-derived fungi and selected host sponge [dissertation]. Braunschweig (ID): Universität Carolo-Wilhelmina.

Jeyasankar A, Premalatha S, Elumalai K. 2014. Antifeedant and insecticidal activities of selected plant extracts against epilachna beetle, *Henosepilachna vigintioctopunctata* (Coleoptera: Coccinellidae). Adv Entomol. 2(1):14-19. doi:10.4236/ae.2014.21003.

Joel EL, BV Bhimba. 2013. Biological activity of secondary metabolites isolated from mangrove fungi *Neurospora crassa*. J Environ Biol. 34:729-732.

Matasyoh JC, Dittrich B, Schueffler A, Laatsch H. 2011. Larvicidal activity of metabolites from the endophytic *Podospora* sp. against the malaria vector *Anopheles gambiae*. Parasitol Res. 108:561-566. doi:10.1007/s00436-010-2098-1.

Michaelsen A, Pinzari F, Ripka K, Lubitz W, Pinar G. 2006. Application of molecular techniques for identification of fungal communities colonizing paper material. Int Biodet Biodeg. 58:133-141. doi:10.1016/j.ibiod.2006.06.019.

Nofiani R, Brilliantoro R, Ardiningsih P. 2012. Effect of culture media and incubation time on biological activities from ethyl acetate extract of marine fungus, *Aspergillus brevipes* Rk06. Drug Inv Today. 4(6):381-384.

Packiam SM, Ignacimuthu S. 2012. Effect of PONNEEM# on *Spodoptera litura* (Fab.) and its compatibility with *Trichogramma chilonis.* Braz Arch Biol Technol. 5(2):291-298. doi:10.1590/S1516-89132012000200016.

Qiao MF, Ji NY, Liu XH, Li K, Zhu QM, Xue QZ. 2010. Indoloditerpenes from an algicolous isolate of *Aspergillus oryzae.* Bioorg Med Chem Lett. 20:5677-5680. doi:10.1016/j.bmcl.2010.08.024.

Schulz B, Wanke U, Drager S, Aust HJ. 1993. Endophytes from herbaceous plants and shrubs: effectiveness of surface sterilization methods. Mycol Res. (97):1447-1450.

Supriyono A. 1997. Biologische aktive naturstoffe aus

tropischen schämmen Südostasiens [dissertation]. Würzburg (ID): Bayerischen Julius-Maximilians-Universität.

Tavares WS, Cruz I, Petacci F, Freitas SS, Serrão JE, Zanuncio JC. 2011. Insecticide activity of piperine: toxicity to eggs of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) and *Diatraea saccharalis.* (Lepidoptera: Pyralidae) and phytotoxicity on several vegetables. J Med Plants Res. 5(21):5301-5306.

Tong H, Su Q, Zhou X, Bai L. 2013. Field resistance of *Spodoptera litura* (Lepidoptera: Noctuidae) to organophosphates, pyrethroids, carbamates and four newer chemistry insecticides in Hunan, China. J Pest Sci. 86: 599-609. doi:10.1007/s10340-013-0505-y.

Velayudam S, Murugan K. 2015. Sequential optimization approach for enhanced production of antimicrobial compound from *Streptomyces rochei* BKM-4. South Indian J Bio Sci. 1(2):72-79.

Zhang G, Sun S, Zhu T, Lin Z, Gu J, Li D, Gu Q. 2011. Antiviral isoindolone derivatives from an endophytic fungus *Emericella* sp. associated with *Aegiceras corniculatum.* Phytochemistry. 72:1436-1442. doi:10.1016/j.phytochem.2011.04.014.