SCREENING FOR MOSQUITO LARVICIDAL ACTIVITY OF THAI MUSHROOM EXTRACTS WITH SPECIAL REFERENCE TO STECCHERINUM SP AGAINST Aedes aegypti (L.) (DIPTERA: CULICIDAE)

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Abstract. For over 50 years, biological control of mosquito larvae has depended mainly on plant extracts, fish, bacteria, protozoa, filamentous fungi, viruses or nematodes. In this study, we screened 143 mushroom samples from 44 confirmed species in Thailand for their mosquito larvicidal activity. One g% (w/v) aqueous extracts of dried powdered mushroom samples were tested against 3rd stage Aedes aegypti larvae. Four mushroom species, namely, Thaeogyroporus porentosus, Xylaria nigripes, Chlorophyllum sp and Stecherinum sp, and two unidentified species showed larvicidal mortality ranging from 10% - 70% and 18% - 90% for 24- and 48-hour exposure time, respectively. Stecherinum sp aqueous crude extract, after 48-hour exposure, did not show any larvicidal activity at 1,000 ppm, whereas that from ethanol, after 24-hour exposure, had 50% and 90% lethal concentration of 203 ppm and 412 ppm, respectively, with higher levels of mortality after 48-hour exposure. This is the first report of mosquito larvicidal properties of Thai mushroom extracts.

Keywords: Aedes aegypti, Stecherinum sp, crude extract, larvicide, mushroom

INTRODUCTION

Aedes aegypti (L.) (Diptera: Culicidae) is the main vector of dengue virus, the cause of dengue and dengue hemorrhagic fevers worldwide (Ratnam et al., 2013). In Thailand, to limit the disease outbreak, temephos (chemical larvicide) has been widely used for a long time against Aedes larvae (Chareonviriyaphap et al., 1999). Although temephos has very good efficacy, its contamination in the environment might be toxic to non-target organisms, including humans. Moreover, resistance to temephos has been reported (Jirakanjanakit et al., 2007; Sornpeng et al., 2009). Therefore, biological control offers an alternative safer method.

Biological agents can kill mosquito larvae in two ways: 1) they are parasites of the larvae, and 2) they are larvicidal substances. As regards the later approach, much attention has been paid to larvic-
Larvicidal activity of mushroom extracts against Ae. aegypti

Vol 46 No. 4 July 2015

Dal substances from living organisms, especially from plants, mostly herbs. Microbial organisms, mostly fungi, produce toxic metabolites against mosquito larvae, viz., metabolites from Aspergillus flavus, Chrysosporium lobatum, Penicillium sp and Podospora sp show larvicidal activity against Culex quinquefasciatus, Anopheles stephensi, Ae. aegypti and Anopheles gambiae mosquitoes, respectively (Govindarajan et al, 2005; Geris et al, 2008; Mohanty and Prakash, 2009; Matasyoh et al, 2011).

In the Fungus Kingdom, mushrooms, mainly belonging to subdivision Basidiomycotina, consist of more than 14,000 species (Lindequist et al, 2005) and traditionally, mushrooms have been used for medical purposes because of their antibacterial (Bender et al, 2003; Lindequist et al, 2005), anti-fungal (Smania et al, 2003), anti-viral (Brandt and Piraino, 2000), anti-tumor (Zaidman et al, 2005; Zhang et al, 2007), anti-allergy (Min et al, 2001), anti-inflammatory (Kim et al, 2003; 2004), and anti-oxidant (Ajith and Janardhanan, 2007) properties. In addition, cordycepin (3′-deoxyadenosine) from fruiting body of Cordyceps militaris has been reported to kill 3rd instar of diamondback moth, Plutella xylostella (Kim et al, 2002). However, few studies have been conducted on mosquito larvicidal activity from mushrooms. A secondary metabolite, (oxiran-2-yl) methylpentanoate, from Cyptotrama asprata mushroom kills Ae. aegypti larvae with LC50 and LC90 values of 1.50 and 1.90 ppm, respectively (Njogu et al, 2009). More recently, Bucker et al (2013) reported larvicidal activity from Pycnoporus sanguineus mushroom against Ae. aegypti and An. nunezovari with LC50 value of 156.8 and 87.2 ppm, respectively. Wild mushroom species, viz, Amanita phalloides, Boletus sp, Lactarius densifolius, Lactarius gymnocarpoides, Russula cellulata and Russula kivuensis demonstrate larvicidal activities against Ae. aegypti, Culex quinquefasciatus and An. gambiae (Chelela et al, 2014).

A variety of mushroom species commonly are found in tropical rain forests, but little is known concerning mosquito larvicide-producing mushrooms in Thailand. This study reports the screening of aqueous extracts of mushrooms in Thailand for mosquito larvicidal substance, and the evaluation mosquito larvicidal efficacy of aqueous, hexane and ethanol extracts of selected mushroom species against the Ae. aegypti mosquito.

MATERIALS AND METHODS

Mushroom collection and identification

One hundred and forty-three fresh mushroom samples were collected from Chiang Mai, Krabi, Lampang, Nakhon Ratchasima, Nakhon Sawan, Pathum Thani, Phichit, Phitsanulok, Phuket, Pra-chuap Khiri Khan, Sukhothai, Surat Thani and Tak Provinces, Thailand and transferred to the Department of Microbiology and Parasitology, Faculty of Medical Science, Naresuan University, where voucher specimens were deposited. Mushroom samples were identified macroscopically and microscopically following mushroom taxonomic keys (Largent and Thiers, 1977; Largent et al, 1977; Stuntz, 1977; Watling, 1977; Largent, 1986; Largent and Baroni, 1988). Samples were dried at 45°C for 24 hours, then ground into powder using a blender (Single Speed Blender 800G; MRC, Holon, Israel) at 22,000 rpm and stored at 4°C until used.

Mosquito rearing

Laboratory strain Ae. aegypti originally collected from Mueang District, Phitsanulok Province, Thailand was rearred as previously described (Thongwat
et al., 2014). In brief, larvae were reared in tap water, at 25 ± 2°C with 10:14 light:dark photoperiod, and fed with powdered dog biscuits (Adult Complete Nutrition, PEDIGREE®; Mars Petcare, Franklin, TN). After pupation, pupae were transferred to a mosquito cage (30x30x30 cm) and emerging adults were provided with a 5% sugar solution containing 5% multi-vitamin syrup (SEVEN SEAS®; OLIC, Feltham, Middlesex, UK). Five- to 7-day-old females were given a blood meal using an artificial membrane feeding method (Rutledge et al., 1964). Gravid females were allowed to lay eggs on a wet filter paper (Whatman No. 1) and eggs were air-dried for 3 days, then kept in a humidity controlled glass jar until used.

**Larvicidal activity screening**

Two grams of each mushroom powder were suspended in 200 ml of distilled water and agitated at 180 rpm for 24 hours on a rotary shaker (Innova™ 2300; NEW BRUNSWICK SCIENTIFIC, Edison, NJ) at room temperature. Then each suspension was filtered through a fine net cloth and added to 25 3rd instar *Ae. aegypti* larvae. Mortality rate was examined after 24- and 48-hour exposures with no feeding. Experiments were performed in four replicate. Controls contained distilled water.

**Preparation of Steccherinum mushroom crude extracts**

Ten grams of powdered *Steccherinum* sp, sample CKW03, were suspended in 100 ml of hexane and then continuously stirred at 180 rpm for 24 hours on a rotary shaker and filtered as described above. The residue was then extracted with ethanol followed by distilled water as described above. The hexane and ethanol extracts were dried in a rotary the evaporator (BÜCHI Rotavapor® R-205 equipped with BÜCHI Vac® V-500; BÜCHI, Flawil, Switzerland), while water extract was dried by evaporation and lyophilization (Lyotrap LF/LYO/01/1; LTE SCIENTIFIC, Oldham, UK).

**Larvicidal bioassay**

Dose-mortality bioassay against *Ae. aegypti* larvae was conducted following protocols of WHO (2005). In brief, 1 g% (w/v) stock solutions in dimethylsulfoxide (DMSO) of the crude ethanol and hexane extracts or in water for the aqueous extract were serially diluted in water and 200 ml aliquots were added to 25 healthy 3rd instar *Ae. aegypti* larvae. After 24 and 48 hours, mortality rates were recorded. Controls contained either 1% (v/v) DMSO or distilled water alone.

**Data analysis**

The 50 (LC₅₀) and 90% (LC₉₀) lethal concentrations and were determined using Probit analysis (Finney, 1971) with LdP Line® software (Plant Protection Research Institute, Cairo, Egypt). The 95% confidence intervals (CI) of upper and lower fiducial limits were also calculated. Statistical significance is accepted when a p-value is < 0.05.

**RESULTS**

Of 143 mushroom samples, 136 were identified into 46 genera with at least 44 confirmed species. The remaining 7 samples were unidentifiable because of limitation in quantity and incomplete morphology of the specimens. Larvicidal activity of all mushroom aqueous extracts [(1g% (w/v)] showed that 4 identified [*Chlorophyllum* sp (NU01), *Steccherinum* sp (CKW03), *Thaeogyroporus porentosus* (PHK27), and *Xylaria nigripes* (PW03)] and 2 unidentified (CKW05 and GSW04) specimens displayed larvicidal efficacy ranging from 10% - 70% and 18% - 90% lar-
Larvicidal Activity of Mushroom Extracts Against *Ae. aegypti*

Fig 1–Graph showing LC$_{50}$ values of ethanol and hexane crude extracts of *Steccherinum* sp mushroom against *Ae. aegypti* 3rd instar stage ($n = 25$) at 24- and 48-hour exposure. X-axis denotes extract concentration in ppm. Statistically significant differences are indicated by different letters (upper right).

Fig 2–Graph showing LC$_{90}$ values of ethanol and hexane crude extracts of *Steccherinum* sp mushroom against *Ae. aegypti* 3rd instar stage ($n = 25$) at 24- and 48-hour exposure. X-axis denotes extract concentration in ppm. Statistically significant differences are indicated by different letters (upper right).

Val mortality after 24- and 48-hour exposure, respectively (Table 1). For 24-hour exposure, the highest activity was found from *Th. porentosus* (PHK27) extract with 70% mortality rate, following with *Steccherinum* (CKW03), *X. nigripes* (PW03) and GSW04 sample demonstrated 66%, 64% and 52% mortality, respectively, and after 48-hour exposure, larval mortality of 90%, 88%, 88%, and 70% mortality was obtained for *Steccherinum* sp (CKW03), *X. nigripes* (PW03), GSW04 and *Th. porentosus* (PHK27), respectively. Lower larvicidal activities (10% and 18% mortality for 24- and 48-hour exposure, respectively) were found with *Chlorophyllum* sp (NU01) and CKW05 samples, and the other 135 samples showed only 0 - 1% and 0 - 2% larval mortality after 24- and 48-hour exposure, respectively.

Based on the above data *Steccherinum* sp (CKW03) (10 g dried powder) was chosen for serial extraction with hexane, ethanol and water, producing a crude extract yield of 2.29, 8.58 and 18.59 g,
### Table 1

Mushroom species and mortality rates of *Ae. aegypti* 3rd instar larvae (n=25) after exposure to 1% (w/v) mushroom aqueous extracts for 24 and 48 hours.

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<th>Mushroom</th>
<th>Mortality (%)</th>
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Larvicidal Activity of Mushroom Extracts Against *Ae. aegypti*

Table 1 (Continued).

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<td><em>Fomitopsis</em> sp</td>
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<td>MPW 02</td>
<td><em>Mycena</em> sp</td>
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<td><em>Thelephora</em> sp</td>
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</table>

AMC and UMC samples were collected from Chiang Mai, Krabi, Lampang, Nakhon Ratchasima, Nakhon Sawan, Pathum Thani, Phichit, Phitsanulok, Phuket, Prachuap Khiri Khan, Sukhothai, Surat Thani and Tak Provinces, Thailand. CKW, GSW, MPW, NU, PHK, PW, and STW samples were collected only from Phitsanulok Province. NI, not identified.

respectively. Ethanol extract shows statistically lower LC$_{50}$ values than hexane extract after 24-hour (203 ppm vs 304 ppm) (Fig 1) and 48-hour (114 vs 218 ppm) exposures (48-hour) (Fig 2 and Table 2). Similar phenomena were observed for LC$_{90}$ values, which are statistically lower than LC$_{50}$ values. However, the aqueous extract lacked larvicidal activity (up to 1,000 ppm) after 48-hour exposure.
Table 2
Larvicidal activities of hexane and ethanol *Steccherinum* sp extracts against *Ae. aegypti* 3rd instar larvae (n=25) after 24- and 48-hour exposure.

<table>
<thead>
<tr>
<th>Steccherinum sp extract (ppm)</th>
<th>24-hour exposure</th>
<th>48-hour exposure</th>
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<tbody>
<tr>
<td></td>
<td>% mortality (mean±SE)</td>
<td>Larvicidal activity</td>
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<td>Lethal concentration with fiducial limits (ppm)</td>
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<tr>
<td></td>
<td>LC$_{50}$</td>
<td>LC$_{90}$</td>
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<tr>
<td>Hexane</td>
<td></td>
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</tr>
<tr>
<td>100</td>
<td>1 ± 1</td>
<td>304</td>
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<tr>
<td>200</td>
<td>21 ± 3</td>
<td>(239 - 374)</td>
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<tr>
<td>300</td>
<td>41 ± 2</td>
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</tr>
<tr>
<td>400</td>
<td>67 ± 2</td>
<td></td>
</tr>
<tr>
<td>500</td>
<td>95 ± 2</td>
<td></td>
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<td>Control</td>
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<tr>
<td>Ethanol</td>
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<tr>
<td>50</td>
<td>1 ± 1</td>
<td>203</td>
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<tr>
<td>100</td>
<td>9 ± 2</td>
<td>(191 - 215)</td>
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<tr>
<td>150</td>
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<tr>
<td>200</td>
<td>51 ± 4</td>
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<td>250</td>
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</table>
DISCUSSION

In this study we show, for the first time to the best of our knowledge, that Thai mushrooms in the genera *Thaeogyroporus* (*T. porentosus*, PHK27), *Chlorophyllum* sp (NU01), *Steccherinum* sp (CKW03) and *Xylaria* (*X. nigripes*, PW03) and 2 unidentified samples (CKW05 and GSW04) contain metabolites with *Ae. aegypti* mosquito larvicidal property. Using sequential extraction with hexane, ethanol and water, the ethanol extract showed superior larvicidal activity over that of hexane, and the aqueous extract lacked activity over the range of time and concentration tested.

Chelela et al (2014) reported that mushrooms in the genera *Amanita* (*A. phalloides*), *Boletus*, *Lactarius* (*L. densifolius*, *L. gymnocarpoides*) and *Russula* (*R. cellulata* and *R. kivuensis*) showed larvicidal activity. However, although we found these genera in our samples but they are of different species and showed little or no toxicity towards *Ae. aegypti* larvae. We did not find *Cryptotrama asprata* earlier reported by Njogu et al (2009).

Ethyl acetate extract of one species of the mushroom genus *Pycnoporus* (*P. sanguineus*) from Manaus, Brazil has a larvicidal activity against *Ae. aegypti* larvae with LC$^{50}$ value of 156.8 ppm at 24-hour exposure (Bucker et al, 2013). However samples of this species from Phitsanulok, Chiang Mai and Krabi Provinces, Thailand showed no larvicidal activity. It is possible that different extraction techniques and/or different geographical locations and habitats might produce different bioactive components. In addition, whether the same morphologically identical mushroom species have the same genetic characteristics needs further investigation.

ACKNOWLEDGEMENTS

The Thailand Research Fund, the Office of Higher Education Commission, Thailand Ministry of Education and Naresuan University (Ref No MRG5680019) supported this study. In addition, the Excellence Center in Insect Vector Study was supported partially by Diamond Research Grant, Faculty of Medicine and the Research Administration Office, Chiang Mai University.

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Larvicide activity of mushroom extracts against Ae. aegypti


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