


Final Report

Project Title: Larvicidal activity of mushroom extracts against *Aedes aegypti*, *Culex quinquefasciatus* and *Anopheles minimus* mosquito vectors

By Asst. Prof. Dr. Damrongpan Thongwat

June 2015

Contract No. MRG5680019

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This project granted by the Thailand Research Fund, Office of the Higher Education Commission and Naresuan University

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Project Period: 2 years

Abstract

Because the adverse effects from using synthetic insecticides to control mosquito have been widely reported, bio-materials from living organisms have been considered as an alternative tool for vector control strategy. For over 50 years, biological control of mosquito larvae has mainly scoped on plants, fish, bacteria, protozoa, filamentous fungi, viruses or nematodes. In this study, we reported a mosquito larvicide from an under researched organism, mushroom. 143 mushroom samples (44 confirmed species) of Thailand were screened for their larvicidal activity. 1%w/v aqueous extract of each mushroom sample was tested against the 3rd stage *Ae. aegypti* larvae. From the screening result, 4 identified mushroom species including *Thaeogyroporus porentosus*, *Xylaria nigripes*, *Chlorophyllum* sp., *Steccherinum* sp., and 2 unidentified species were found for the promising activities ranging from 10 - 70% and 18 - 90% larval mortality rates for 24- and 48-hour exposure times, respectively. After that, the *Steccherinum* mushroom was selected for an intensively bioassay against *Ae. aegypti*, *Culex quinquefasciatus* and *Anopheles minimus* larvae following the standard protocol of WHO. An aqueous, hexane and ethanolic crude extracts of *Steccherinum* were prepared in various concentrations up to 1,000 ppm. For *Ae. aegypti* bioassay, after 48-hour post exposure, the aqueous extract did not show any larvicidal activity, while the ethanolic showed superior activity than the hexane with the 24-hour LC_{50} and LC_{90} values of 203.30 and 412.72 ppm, respectively. For *Cx. quinquefasciatus* and *An. minimus* testing, the same activities that the 48-hour showed higher activity than the 24-hour for both the LC_{50} and LC_{90} values were also found. From the finding, we firstly reported the mosquito larvicidal potential from the extracts of Thai mushrooms, especially for *Steccherinum* sp.

Keywords: *Aedes aegypti*, *Culex quinquefasciatus*, *Anopheles minimus*, *Steccherinum*, mushroom

Executive summary

The *Aedes aegypti*, *Culex quinquefasciatus* and *Anopheles minimus* are medically important vectors in Thailand. In this moment, for decreasing the diseases outbreak, especially dengue hemorrhagic fever, Abate (temephos) has widely been used for the mosquito larvae control, mainly for *Aedes* vector. Although temephos is one of the organophosphate insecticides that shows very low toxicity to human, the high dose usage or accidentally intake might cause disorder to human and non-target organisms. Moreover, the continuous application can cause resistance of the vectors. Therefore, biological control, an alternatively safer controlling method, has become to resolve the chemical usage problems. For the mosquito larval biocontrol method, living organisms can kill the larvae via two actions: (1) they are a parasite of the larvae, and (2) they produce larval toxic substances. For the later action, many researches paid attention to extract larvicide substances from living organisms, especially from plants, mostly from herbs. Not only plants can produce the bio-activities substances, but also found from microbe organisms. Most of fungi, a big group of living organisms, can produce toxic metabolites against mosquito larvae.

In the fungus kingdom, mushrooms, mainly belonging to Basidiomycotina Subdivision, consist of more than 14,000 species and some of them can produce toxic metabolite substances. For a long time, mushrooms have been used in medical purposes, because of their antibacterial, antifungal, antiviral, antitumor, antiallergy, anti-inflammatory, and also antioxidant activities. Moreover, a larvicidal activity against agricultural insect pests was also found. However, a few research has been studied for the mosquito larvicidal activity from mushrooms. In 2009, a secondary metabolite, from *Cyptotrama asprata* was reported to kill *Ae. aegypti* larvae. Bucker *et al* (2013) reported the larvicidal activity from *Pycnoporus sanguineus* against *Ae. aegypti* and *Anopheles nuneztovari* mosquitoes. Recently, some wild mushroom species, including *Amanita phalloides*, *Boletus* sp., *Lactarius densifolius*, *Lactarius gymnocarpoides*, *Russula cellulata* and *Russula kivuensis*, were reported for various larvicidal potential against *Ae. aegypti*, *Cx. quinquefasciatus* and *An. gambiae* mosquitoes.

Abundant mushroom species are commonly found from tropical rain forests. Then, it would possible to find some mosquito larvicide producing mushrooms from the areas of Thailand. Our preliminary study indicated that aqueous extracts of some mushrooms likely displayed a promising result. Then, the objectives of this study were to: (1) screen for the mosquito larvicide substance from aqueous mushroom extracts of Thailand, and (2) evaluate the larvicidal efficacy of an aqueous, hexane and ethanolic extracts of selected mushroom species, *Steccherinum*, against the *Ae. aegypti* mosquito.

For the experiments, fresh mushroom samples were collected. Then, genus and/or species identifications were completed following the mushroom taxonomic keys. After identification, the mushroom samples were air-dried, and then ground into powder before analysis. Laboratory strain

Ae. aegypti were used for the larvicidal activity screening of mushroom extracts. 1 g% W/V of aqueous crude mushroom extracts was tested with a hundred of late 3rd-early 4th instar larvae. 200 ml of 1 g% W/V aqueous extract solution from the above was transferred into a plastic bowl and 25 mosquito larvae were gently transferred into the solution. Mortality rates were determined after 24 and 48 hour-exposing times. It was found that, from the identification of all 143 mushroom samples, 136 samples were identified into 46 genera with at least 44 confirmed species, while the 7 remainder samples were unidentified into any genus. After the screening process, 6 of all, including 4 identified samples: *Thaeogyroporus porentosus* (PHK27), *Chlorophyllum* sp. (NU01), *Steccherinum* sp. (CKW03), *Xylaria nigripes* (PW03) and 2 unidentified samples: CKW05 and GSW04, displayed a promising larvicidal efficacy ranging from 10 - 70% and 18 - 90% larval mortality rates for 24- and 48-hour exposure times, respectively. While, the other 135 samples did not showed possibility to contain the mosquito larvicide. Only 0 - 1% and 0 - 2% larval mortality rates were found after 24- and 48-hour detection times, respectively. After the screening process, *Steccherinum* was selected for the intensively larvicidal activity of crude hexane, ethanolic and aqueous extracts because of the quantity limitation of the other samples.

For intensive bioassay studies, dried power of *Steccherinum* sample was extracted with hexane, absolute ethanol and distilled water, sequentially with ratio 1:10 (sample:solvent). The water extracts were concentrated by using a rotary evaporator and lyophilizer while the ethanol and hexane crude extracts were concentrated by using rotary evaporator only. All mosquito species, including *Ae. aegypti*, *Cx. quinquefasciatus* and *An. minimus*, were tested for larvicidal activity of the *Steccherinum* crude extracts following the protocol of WHO. Briefly, each 200 ml of the various concentrations of each crude extract was put into a plastic bowl. Then, twenty-five of late 3rd-early 4th instar larvae of each mosquito species were transferred into the solutions. Mortality rates were determined after 24 and 48 hour-exposing times. The experiments were performed in four replicates, revealing a total of 100 larvae for each concentration of each crude extract. An equal number of control was set up simultaneously with 2 ml of DMSO in 200 ml distilled water. After the testing period, data from all replicates of each crude extract were pooled for the analysis. The mortality data were subjected to the Probit analysis for the LC_{50} and LC_{90} values determination by using the commercial software Ldp Line.

For the results, *Steccherinum* crude extracts showed the larvicidal activity against the *Ae. aegypti* from the hexane and ethanolic extracts, while the aqueous one did not. The ethanolic showed higher activity than the hexane with the LC_{50} values of 203.30 and 304.05 ppm (24-hour)

and 114.12 and 218.45 ppm (48-hour), for ethanolic and hexane extracts, respectively. Identical finding that the ethanolic extract caused higher activity than the hexane was also found for the 24- and 48-hour LC_{90} values detection. For the *Cx. quinquefasciatus* bioassay, the 48-hour showed higher activity than the 24-hour with the LC_{50} and LC_{90} values of 148.67 and 221.38 ppm (48-hour) and 286.33 and 523.39 ppm (24-hour), respectively. For the *An. minimus* bioassay, the 48-hour showed higher activity than the 24-hour with the LC_{50} and LC_{90} values of 119.69 and 387.29 ppm (48-hour) and 316.53 and 1,188 ppm (24-hour), respectively. In this study, the same general finding that the 48-hour activity of both values provided significantly better larvicidal toxicity than the 24-hour exposure time was found from all 3 mosquito species bioassays.

Objectives

1. To screen natural mushroom aqueous extracts for larvicidal activity against *Aedes aegypti*

2. To evaluate larvicidal efficacy of crude hexane, ethanolic and aqueous extracts of selected mushroom (*Steccherinum*) against *Aedes aegypti*, *Culex quinquefasciatus* and *Anopheles minimus* mosquitoes

Research methodology

Mushroom samples collecting

Fresh mushroom samples were collected and kept in a plastic cup and transferred to the laboratory of Department of Microbiology and Parasitology, Faculty of Medical Science, Naresuan University. Macroscopic and microscopic characteristics of all mushroom samples were examined and the Genus and/or Species identifications were completed following the mushroom taxonomic keys (Largent and Thiers, 1977; Largent et al., 1977; Stuntz, 1977; Watling, 1977; Largent, 1986; Largent and Baroni, 1988) under a handle of an experienced researcher of mushroom species identification, Miss Urat Pimonsri (who is the co-author of the output publication), a lecturer of Department of Microbiology and Parasitology, Faculty of Medical Science, Naresuan University, the same place where a voucher specimen was deposited. After identification, the mushroom samples were air-dried in an incubator at 45°C, and then ground into powder by using electric blender before analysis.

Aedes aegypti mosquito collecting and rearing in laboratory

Aedes larvae were carefully collected from breeding places in Muang district of Phitsanulok Province, Thailand by using a plastic pipette and kept in a plastic bottle. The collected larvae were transferred to the laboratory and maintained in a plastic tray contained with 2 liters of tap water. Rearing conditions were 25± 2°C and a 10:14 (L:D) photoperiod. A crushed dog biscuit was used as larval food and the larvae were reared until they pupated. After that, the emerging adults were transferred into a plastic cup covered with a net, and then they were morphologically identified following the illustrated keys to the mosquitoes of Thailand (Rattanarithikul et al., 2010). Identified *Ae. aegypti* mosquitoes were transferred into a mosquito cage (30 x 30 x 30 cm) and provided with 5% sugar mixed with 5% multivitamin syrup solution. Five to 7-day-old females were permitted to feed on a blood meal by using an artificial membrane feeding method (Rutledge et al., 1964). After the blood fed females become gravid, they were allowed to lay egg on filter paper (Whatman No.1). Eggs were air-dried and kept until use. A colony of *Ae. aegypti* was established for producing larval materials.

Larvicidal activity screening of mushroom samples

Two grams of each mushroom powder was suspended into 200 ml of distilled water in 250-ml Erlenmeyer flask. The mushroom powder was extracted by stirring with distilled water at room temperature at 180 rpm for 24 hours on a rotary shaker, and then filtering through a fine net cloth, revealing 1 g% W/V of aqueous crude mushroom extract. Each extract was tested for larvicidal activity with a hundred of late 3rd-early 4th instar larvae of *Ae. aegypti* (from 10.2). Two hundred ml of 1 g% W/V aqueous extract solution from the above was transferred into a plastic bowl and 25 mosquito larvae were gently transferred into the solution. Mortality rates were determined after 24 and 48 hour-exposing times. No food was offered to the larvae during the experimental time. Moribund larvae were counted and added to dead larvae for calculation of mortality. The experiments were replicated fourfold to validate results so that 100 larvae were tested for each mushroom extract. The distilled water was used as control for each experiment. The experiment was done in the same condition of temperature and relative humidity as the mosquito rearing condition. Experimental test that demonstrated more than 20% mortality of control group was discarded and repeated. In cases where the control mortality reaches between 5 and 20%, the mortality observed was corrected by Abbott's formula (Abbott, 1925). The mushroom species that

shows the highest activity (*Steccherinum* sp.) was selected to thoroughly evaluate for larvicidal efficacy in the further experiment.

Crude hexane, ethanolic and aqueous extracts preparation of *Steccherinum* mushroom sample

Dried power of *Steccherinum* sample was extracted with hexane, absolute ethanol and distilled water, sequentially, following the method mention above with ratio 1:10 (sample:solvent). The extraction period was extended to 3 days with continuous stirring on the rotary shaker. The extracted suspension from each solvent was filtered by Whatman No.1 filter paper. The residue was then extracted with two additional samples of same solvent as described above. The combined water extracts were concentrated by using a rotary evaporator and lyophilizer while the ethanol and hexane crude extracts were concentrated by using rotary evaporator only. The resulting crude extracts were kept in a desiccator until they were required for the bioassay.

***Culex quinquefasciatus* mosquito collecting and rearing in the laboratory**

Culex adult mosquitoes were collected from areas of Naresuan University, Phitsanulok Province, Thailand by using an aspirator and kept in a plastic cup covered with net. The collected adults were transferred to the laboratory, and then were morphologically identified following the illustrated keys (Rattanarithikul et al., 2005). Identified *Cx. quinquefasciatus* mosquitoes were transferred into a mosquito cage and provided with 5% sugar mixed with 5% multivitamin syrup solution. The females were permitted to feed on a blood meal. After the blood fed female became gravid, they were allowed to lay egg on suitable water contained in a plastic cup. Eggs were reared to 4^{th} stage larva following the rearing method of McCall and Eaton (2001) with some modifications. Briefly, the larvae were reared in de-chlorinated tap water added with hay infusion to achieve a final concentration of 5%. Dried yeast was added daily as larva food. The late 3^{rd} or early 4^{th} stage larvae were used to evaluate the larvicidal activity of crude *Steccherinum* mushroom extracts.

***Anopheles minimus* mosquito rearing in the laboratory**

A stenogamous strain of *An. minimus* was kindly supported by Associate Professor Dr. Pradya Somboon, the mentor and co-author of this project. The mosquitoes were reared in the laboratory of Department of Parasitology, Faculty of Medicine, Chiang Mai University, Thailand under the laboratory conditions. Briefly, the larval stage was reared in a plastic tray containing distilled water mixed with natural water (water from natural source, such as a swamp). A powdery

ground tropical fish food was fed to the larvae as food. The larvae were reared until they pupated. After that, the emerging adults were transferred into a mosquito cage and provided with 5% sugar mixed with 5% multivitamin syrup solution. Five to 7-day-old females were permitted to feed on a blood meal. After the females became gravid, they were allowed to lay egg on water in a plastic bowl. Eggs were reared to adults to produce the next generation.

Larvicidal activity evaluation of hexane, ethanolic and aqueous crude extracts

All mosquito species, including *Ae. aegypti*, *Cx. quinquefasciatus* and *An. minimus*, were tested for larvicidal activity of the mushroom crude extracts following the protocol of WHO (2005). The crude extract from each solvent was prepared for a stock solution. For the hexane and ethanolic crude extracts, the volume of stock solution was 20 ml of 1%, obtained by weighing 200 mg of the crude extract and adding 20 ml of dimethylsulphoxide (DMSO). For aqueous crude extract, DMSO was replaced with distilled water. The stock solution was kept in a screw-cap vial, with aluminium foil over the mouth of the vial, in a refrigerator. The series of concentrations were prepared for larvicidal activity testing. For example, 0.2 to 2 ml of stock solution was diluted to 200 ml of distilled water, revealing 10 to 100 ppm of final concentrations. When lower concentration is needed, the stock solution was serially diluted (ten-fold) in DMSO or distilled water (2 ml solution to 18 ml DMSO or distilled water), and then prepared as the protocol mention above, revealing 1 to 10 ppm of final concentrations. When upper concentration is needed, the stock solution was prepared in higher concentration than the previously described. 1,000 ppm of each crude extracts was the highest concentration used for bioassay in this study.

For the range of concentration tested in each crude extract, initially, the larvae were exposed to a wide range of test concentrations and control (2 ml DMSO in 200 ml distilled water) to find out the activity range of the crude extract solution. After that, a narrower range (of 4-5 concentrations, yielding between 10% and 95% mortality in 24h or 48h) was used to determine for the LC_{50} and LC_{90} values.

The mosquito larvicidal activity test was done as described above. Briefly, each 200 ml of the various concentrations of each crude extract was put into a plastic bowl. Then, twenty-five of late 3^{rd}-early 4^{th} instar larvae of each mosquito species were transferred into the solutions. Mortality rates were determined after 24 and 48 hour-exposing times. The experiments were performed in four replicates, revealing a total of 100 larvae for each concentration of each crude extract. An equal number of control was set up simultaneously with 2 ml of DMSO in 200 ml distilled water.

After the testing period, data from all replicates of each crude extract were pooled for the analysis. The mortality data were subjected to the Probit analysis for the LC_{50} and LC_{90} values determination (Finney, 1971) by using the commercial software Ldp Line (*http://embakr.tripod.com/ldpline*).

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Result

For the larvicidal activity screening

From the identification of all 143 mushroom samples, 136 samples were identified into 46 genera with at least 44 confirmed species. While the 7 remainder samples were unidentified into any genus because of the limitation of quantity and complete morphology of the specimen. After the screening for larvicidal activity of all mushroom aqueous extracts (1g% w/v), 6 of all, including 4 identified samples: *Thaeogyroporus porentosus* (PHK27), *Chlorophyllum* sp. (NU01), *Steccherinum* sp. (CKW03), *Xylaria nigripes* (PW03) and 2 unidentified samples: CKW05 and GSW04, displayed a promising larvicidal efficacy ranging from 10 - 70% and 18 - 90% larval mortality rates for 24- and 48-hour exposure times, respectively (Table 1).

For 24-hour exposure time, the highest activity was found from *Th. porentosus* extract with 70% mortality rate, following with *Steccherinum*, *X. nigripes*, and GSW04 samples, with 66, 64 and 52% mortality rates, respectively. While, for 48-hour exposure time, *Steccherinum* caused the highest larval mortality with 90%, following with *X. nigripes*, GSW04 and *Th. porentosus*, with 88, 88 and 70%, respectively. The intensely lower activities (10 and 18% mortality rates, for 24- and 48-hour, respectively) were found from *Chlorophyllum* sp. (NU01) and unidentified CKW05 samples. Furthermore, the other 135 samples did not showed possibility to contain the mosquito larvicide. Only 0 - 1% and 0 - 2% larval mortality rates were found after 24- and 48-hour detection times, respectively. After the screening process, from all 6 mushroom species contained high activities, only *Steccherinum* (Fig 1) was selected for the intensively larvicidal activity of crude hexane, ethanolic and aqueous extracts because of the quantity limitation of the other samples.

Fig 1-Photograph of fresh *Steccherinum* (CKW03) mushroom used in bioassays of this study. Upper pictures are the photographs of growing mushroom in wildlife. Lower pictures are the photographs of mushroom in laboratory after collection.

Table 1-Mushroom species and mortality rates of the 3rd instar larvae *Ae. aegypti* after screening with 1%w/v mushroom aqueous extract for 24 and 48 hours.

No.	Sample	Scientific name	Mortality rate (%)		No.	Sample	Scientific name	Mortality rate (%)	
			24h	48h				24h	48h
1	UMC 01	*Clitocybe* sp.	0	0	53	UMC 53	*Steccherinum* sp.	0	0
2	UMC 02	*Cantharellus cibarius*	0	0	54	UMC 54	*Fomes* sp.	0	0
3	UMC 03	*Lentinus polychrous*	0	0	55	UMC 55	*Ganoderma* sp.	0	0
4	UMC 04	*Abortiporus* sp.	0	0	56	UMC 56	*Ganoderma* sp.	0	0
5	UMC 05	*Schizophyllum commune*	0	0	57	UMC 57	*Fomes* sp.	0	0
6	UMC 06	*Lentinus* sp.	0	0	58	UMC 58	*Ganoderma lucidum*	0	0
7	UMC 07	*Lenzites vespacea*	0	0	59	UMC 59	*Ganoderma lucidum*	0	1
8	UMC 08	*Trametes hirsuta*	0	0	60	UMC 60	*Lentinus polychrous*	0	0
9	UMC 09	*Ganoderma* sp.	0	0	61	UMC 61	*Lentinus polychrous*	0	0
10	UMC 10	*Lentinus squarrosulus*	0	0	62	UMC 62	*Astraeus odoratus*	0	0
11	UMC 11	*Macrocybe crassa*	0	0	63	UMC 63	*Lentinus squarrosulus*	0	0
12	UMC 12	*Rigidoporus* sp.	0	0	64	UMC 64	*Amanita caesarea*	0	0
13	UMC 13	*Bjerkandera* sp.	0	0	65	UMC 65	*Russula rosacea*	0	0
14	UMC 14	*Ganoderma* sp.	0	0	66	UMC 66	*Amanita princeps*	0	0
15	UMC 15	*Pycnoporus sanguineus*	0	0	67	UMC 68	*Amanita princeps*	0	0
16	UMC 16	*Steccherinum* sp.	0	0	68	UMC 69	*Auricularia auricular*	0	0
17	UMC 17	*Ganoderma lucidum*	1	1	69	UMC 70	*Lentinus squarrosulus*	0	0
18	UMC 18	*Trametes* sp.	0	0	70	UMC 71	*Ganoderma applanatum*	0	0
19	UMC 19	*Trametes* sp.	0	0	71	UMC 72	*Polyporus* sp.	0	0
20	UMC 20	*Microporus xanthopus*	0	0	72	UMC 73	*Ganoderma applanatum*	0	0
21	UMC 21	*Podoscypha* sp.	0	0	73	UMC 74	*Pycnoporus sanguineus*	0	0
22	UMC 22	*Laccaria laccata*	0	0	74	UMC 75	*Ganoderma lucidum*	0	0
23	UMC 23	*Macrocybe crassa*	0	0	75	UMC 76	*Polyporus* sp.	0	2
24	UMC 24	*Chlorophyllum molybdites*	0	0	76	UMC 80	*Ganoderma* sp.	0	0
25	UMC 25	*Trametes* sp.	0	0	77	UMC 81	*Ganoderma* sp.	0	0
26	UMC 26	*Polyporus* sp.	0	0	78	UMC 82	*Fomes* sp.	0	0
27	UMC 27	*Trametes* sp.	0	0	79	UMC 83	*Ganoderma lucidum*	0	0
28	UMC 28	*Ganoderma lucidum*	0	0	80	UMC 84	*Ganoderma lucidum*	0	0
29	UMC 29	*Meruliopsis* sp.	0	0	81	UMC 85	*Microporus xanthopus*	0	0

Table 1 (continued).

No.	Sample	Scientific name	Mortality rate (%)		No.	Sample	Scientific name	Mortality rate (%)	
			24h	48h				24h	48h
30	UMC 30	*Lenzites* sp.	0	0	91	PHK 08	*Amanita verna*	0	0
31	UMC 31	*Stereum ostrea*	0	0	92	PHK 09	*Boletus* sp.	0	0
32	UMC 32	*Ganoderma* sp.	0	0	93	PHK 13	*Laccaria laccata*	0	0
33	UMC 33	*Stereum* sp.	0	0	94	PHK 14	*Laccaria* sp.	0	0
34	UMC 34	*Lenzites elegans*	0	0	95	PHK 17	*Termitomyces* sp.	0	0
35	UMC 35	*Ganoderma* sp.	0	0	96	PHK 21	*Hygrocybe* sp.	0	0
36	UMC 36	*Ganoderma lucidum*	0	0	97	PHK 22	*Scleroderma sinnamariense*	0	0
37	UMC 37	*Trametes versicolor*	0	0	98	PHK 23	*Armillaria* sp.	0	0
38	UMC 38	*Macrocybe crassa*	0	0	99	PHK 24	*Polyporus* sp.	0	0
39	UMC 39	*Trametes* sp.	0	0	100	PHK 25	*Stereum ostrea*	0	0
40	UMC 40	*Stereum* sp.	0	0	101	PHK 26	*Russula* sp.	0	0
41	UMC 41	*Trametes* sp.	0	0	102	PHK 27	*Thaeogyroporus porentosus*	70	70
42	UMC 42	*Earliella* sp.	0	0	103	AMC 01	*Russula rosacea*	1	1
43	UMC 43	*Stereum* sp.	0	0	104	AMC 02	*Termitomyces* sp.	0	0
44	UMC 44	*Ganoderma applanatum*	0	0	105	AMC 03	*Russula alboareolata*	0	0
45	UMC 45	*Ganoderma lucidum*	0	0	106	AMC 04	*Russula cyanoxantha*	0	0
46	UMC 46	*Earliella* sp.	0	0	107	AMC 05	*Sentinus* sp.	0	0
47	UMC 47	-*	0	0	108	AMC 06	*Amanita vaginata*	0	0
48	UMC 48	*Ganoderma lucidum*	0	0	109	AMC 07	*Russula densifolia*	1	1
49	UMC 49	*Polyporus* sp.	0	0	110	AMC 08	*Amanita princeps*	0	0
50	UMC 50	*Scytinopogon angulisporus*	0	0	111	AMC 10	*Amanita hemibapha*	0	0
51	UMC 51	*Ganoderma lucidum*	0	0	112	AMC 11	*Amanita princeps*	0	0
52	UMC 52	*Podoscypha* sp.	0	0	113	AMC 12	*Russula* sp.	0	0
82	UMC 86	*Pycnoporus sanguineus*	0	0	114	AMC 13	*Geastrum saccatum*	0	0
83	UMC 87	*Phaeolus* sp.	0	2	115	AMC 15	*Dictyophora indusiata*	0	0
84	UMC 88	*Microporus xanthopus*	0	0	116	AMC 16	*Tremella fuciformis*	0	0
85	PHK 01	*Scleroderma polyrhizum*	0	0	117	NU 01	*Chlorophyllum* sp.	10	18
86	PHK 02	*Boletus chromapes*	0	0	118	NU 02	*Chlorophyllum* sp.	0	0
87	PHK 03	*Lactarius hatsudake*	0	0	119	NU 03	*Chlorophyllum molybdites*	1	1
88	PHK 04	*Boletellus emodensis*	0	0	120	NU 04	*Schizophyllum commune*	0	0
89	PHK 05	*Fomitopsis pinicola*	0	0	121	CKW 01	*Stereum hirsutum*	0	0
90	PHK 07	*Microporus* sp.	0	0	122	CKW 02	*Fomitopsis pinicola*	0	0

Table 1 (continued).

No.	Sample	Scientific name	Mortality rate (%)		No.	Sample	Scientific name	Mortality rate (%)	
			24h	48h				24h	48h
123	CKW 03	*Steccherinum* sp.	66	90	134	PW 01	-*	0	0
124	CKW 04	*Boletus* sp.	0	0	135	PW 02	-*	0	0
125	CKW 05	-*	10	18	136	PW 03	*Xylaria nigripes*	64	88
126	CKW 06	-*	0	0	137	GSW 01	*Scytinopogon angulisporus*	0	0
127	STW 01	*Trametes* sp.	0	0	138	GSW 02	*Ganoderma* sp.	0	0
128	STW 02	*Fomitopsis* sp.	0	0	139	GSW 03	*Daedaleopsis confragosa*	0	0
129	STW 03	-*	0	0	140	GSW 04	-*	52	88
130	MPW 01	*Cyathus striatus*	0	0	141	GSW 05	*Thelephora* sp.	0	0
131	MPW 02	*Mycena* sp.	0	0	142	GSW 06	*Ramaria* sp.	0	0
132	MPW 03	*Cyathus striatus*	0	1	143	GSW 07	*Ganoderma* sp.	0	0
133	MPW 04	*Thelephora penicillata*	1	2					

* Unidentified samples

UMC and AMC samples are collected from several areas of Thailand including Chiang Mai, Krabi, Lampang, Nakhon Ratchasima, Nakhon Sawan, Pathumthani, Phichit, Phitsanulok, Phuket, Prachuap Khiri Khan, Sukhothai, Surat thani and Tak provinces.

PHK, NU, CKW, STW, MPW, PW and GSW samples are collected only from areas of Phitsanulok province.

For the *Ae. aegypti* bioassay

From the 24- and 48-hour bioassays of *Steccherinum* crude extracts, the larvicidal activities were found form the hexane and ethanolic extracts, while the aqueous one did not showed activity with a nil larval mortality rates after testing with extract concentration up to 1,000 ppm for 48 hours. For hexane and ethanolic crude extracts, the ethanolic showed higher activity than the hexane with the LC_{50} values of 203.30 and 304.05 ppm (24-hour) and 114.12 and 218.45 ppm (48-hour), for ethanolic and hexane extracts, respectively. Identical finding that the ethanolic extract caused higher activity than the hexane was also found for the 24- and 48-hour LC_{90} values detection (Table 2).

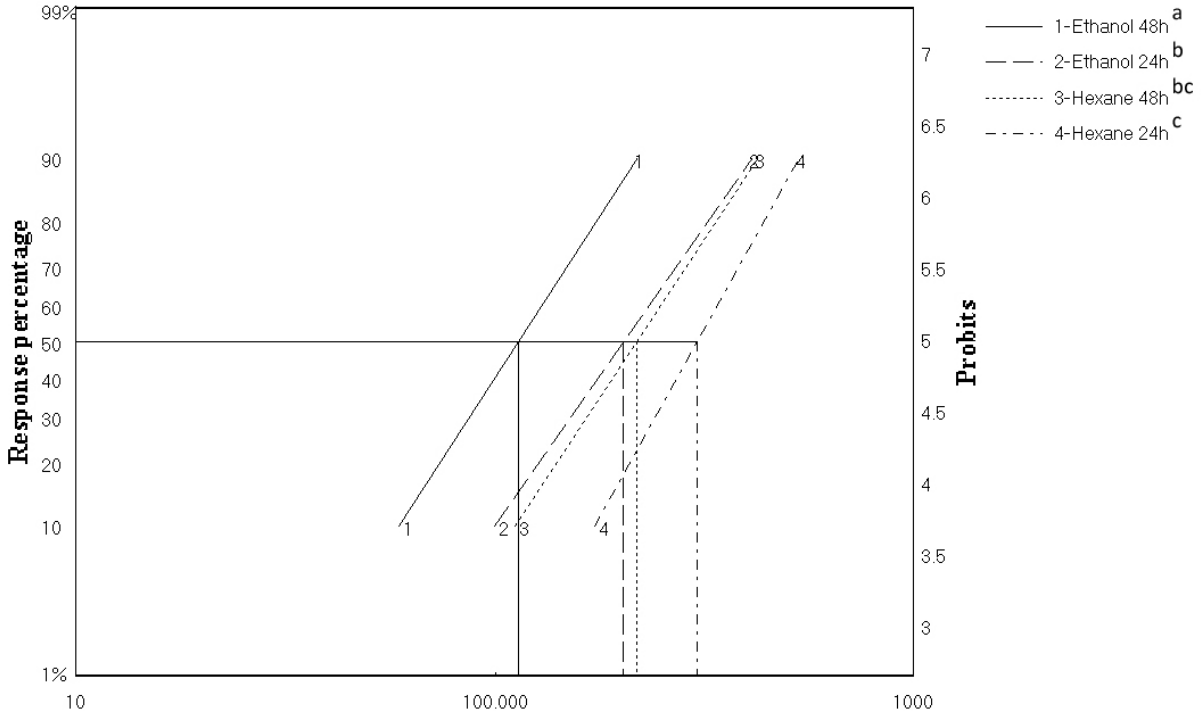
Table 2-Larvicidal activities of the crude hexane and ethanolic *Steccherinum* extracts against the 3rd stage larvae *Ae. aegypti* after 24- and 48-hour exposure times.

Crude extract of *Steccherinum* (ppm)	24-hour					48-hour				
	Larvicidal activity					Larvicidal activity				
	% Mortality (Mean ± SE)	Lethal Concentration with fiducial limits (ppm)		χ^2	Slope ±SE	% Mortality (Mean ± SE)	Lethal Concentration with fiducial limits (ppm)		χ^2	Slope ±SE
		LC_{50}	LC_{90}				LC_{50}	LC_{90}		
Hexane extract										
100	1.00 ± 1.00	304.05	533.40	11.56	4.78 ± 0.40	8.00 ± 1.63	218.45	425.75	0.14	4.42 ± 0.49
200	21.00 ± 3.00	(238.72 - 373.70)	(484.29 - 871.89)			42.00 ± 2.58	(200.65 - 239.28)	(365.84 - 532.79)		
300	41.00 ± 2.52					74.00 ± 3.83				
400	67.00 ± 2.52					100				
500	95.00 ± 1.91					100				
Control	0					1.00 ± 1.00				
Ethanolic extract										
50	1.00 ± 1.00	203.30	412.72	10.49	4.17 ± 0.28	8.00 ± 1.63	114.12	219.71	7.10	4.51 ± 0.33
100	9.00 ± 2.52	(191.27 - 215.39)	(377.34 - 461.18)			37.00 ± 3.42	(105.85 - 122.25)	(200.92 - 245.29)		
150	25.00 ± 2.52					62.00 ± 4.76				
200	51.00 ± 4.43					91.00 ± 1.91				
250	72.00 ± 1.63					96.00 ± 1.63				
300	77.00 ± 1.91					100				
350	85.00 ± 1.91					100				
400	92.00 ± 1.63					100				
Control	0					0				

LC_{50} and LC_{90}: lethal concentration that causes 50% and 90% of mortality, SE: standard error, χ^2: chi-square, ppm: parts per million

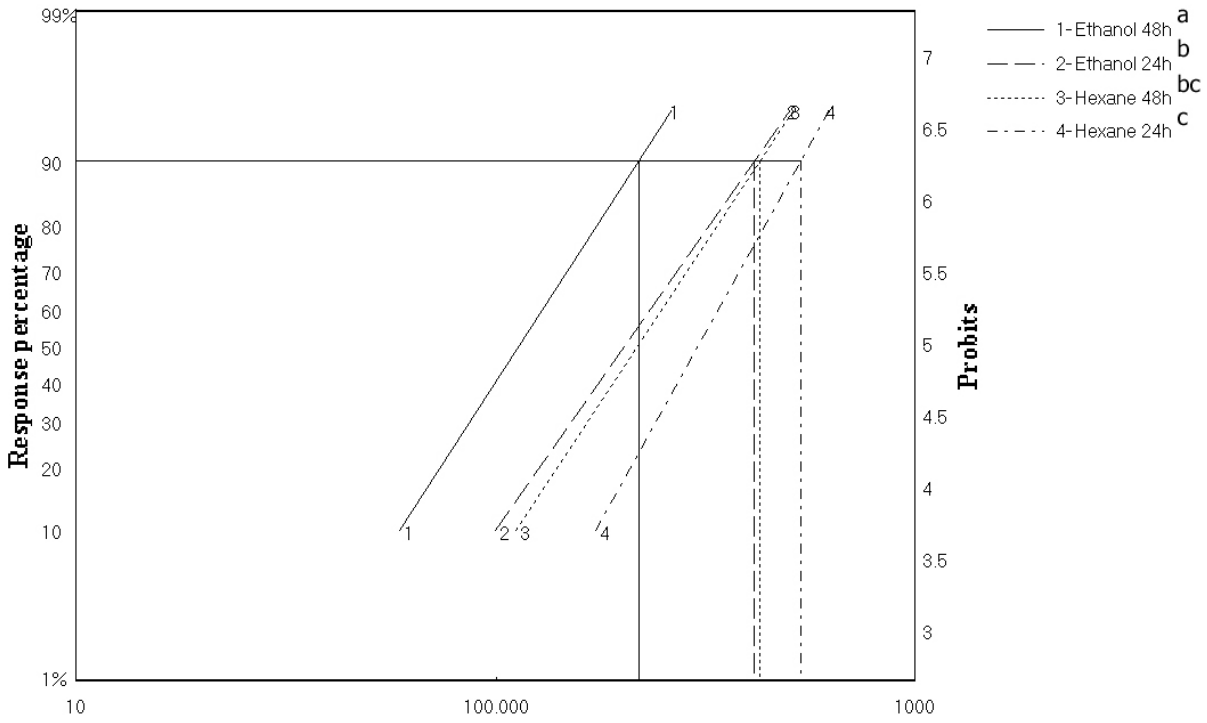
After statistically analysis comparing of the LC_{50} (Fig 2) and LC_{90} (Fig 3) values, the 48-hour activity of the ethanolic extract provided a significantly highest larvicidal toxicity with 114.12 and 219.71 ppm, for the LC_{50} and LC_{90} values, respectively. While the lower activities were found from the 24-hour ethanolic (203.30 and 412.72 ppm), following with the 48-hour hexane (218.45 and 425.75 ppm) and the 24-hour hexane (304.05 and 533.40 ppm) extracts, respectively. From the results, a higher quantity of larvicide substance from *Steccherinum* mushroom was found after extracting with ethanol than hexane and also water.

Fig 2-Graph showing the LC_{50} values of the ethanolic and hexane crude extracts of *Steccherinum* mushroom on the 3rd stage *Ae. aegypti* at 24- and 48-hours exposing time.



Statistically significant differences are indicated by different letter on the ethanolic or hexane extract groups (upper right)

Fig 3- Graph showing the LC_{90} values of the ethanolic and hexane crude extracts of *Steccherinum* mushroom on the 3rd stage *Ae. aegypti* at 24- and 48-hours exposing time.



Statistically significant differences are indicated by different letter on the ethanolic or hexane extract groups (upper right)

For the *Cx. quinquefasciatus* bioassay

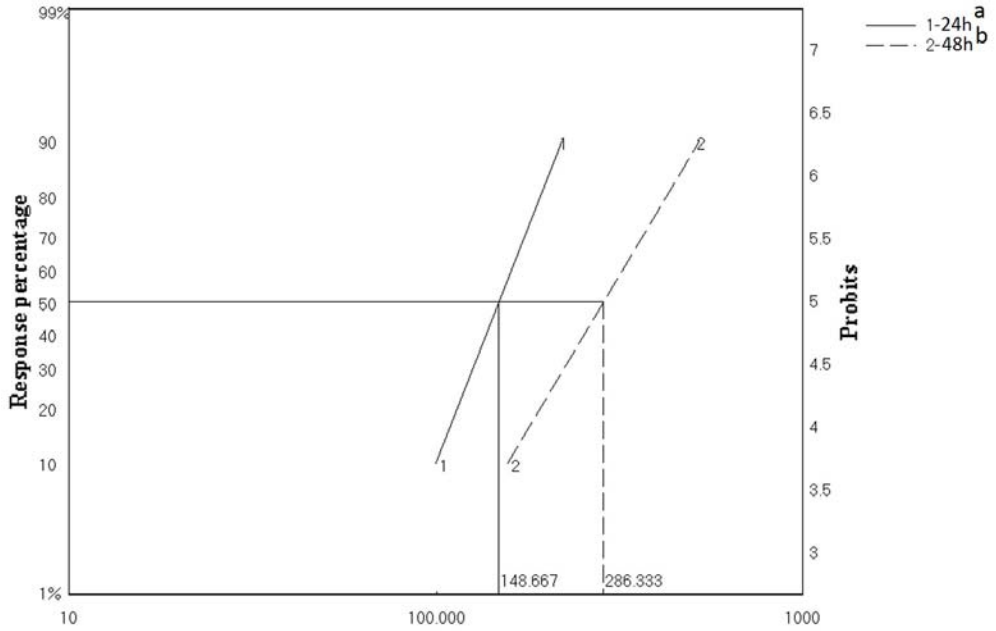
From the 24- and 48-hour 100 – 500 ppm bioassays of *Steccherinum* ethanolic extract, the 48-hour showed higher activity than the 24-hour with the LC_{50} and LC_{90} values of 148.67 and 221.38 ppm (48-hour) and 286.33 and 523.39 ppm (24-hour), respectively (Table 3). After statistically analysis comparing of the LC_{50} (Fig 4) and LC_{90} (Fig 5) values, the 48-hour activity of both values provided significantly better larvicidal toxicity than the 24-hour exposure time. This finding was the same as the 48- hour greater activity of the ethanolic extract against the *Aedes* mosquito.

Table 3-Larvicidal activities of the crude ethanolic *Steccherinum* extracts against the 3rd stage larvae *Cx. quinquefasciatus* after 24- and 48-hour exposure times.

Crude extract of *Steccherinum* (ppm)	24-hour					48-hour				
		Larvicidal activity					Larvicidal activity			
	% Mortality (Mean ± SE)	Lethal Concentration with fiducial limits (ppm)		χ^2	Slope ±SE	% Mortality (Mean ± SE)	Lethal Concentration with fiducial limits (ppm)		χ^2	Slope ±SE
		LC_{50}	LC_{90}				LC_{50}	LC_{90}		
100	3.00 ± 1.00	286.33	523.39	11.34	4.89 ± 0.40	12.00 ± 1.63	148.67	221.38	0.04	7.41 ± 0.64
200	24.00 ± 1.63	(221.87 - 353.03)	(467.86 - 849.77)			83.00 ± 1.91	(138.81 - 158.64)	(204.77 - 244.05)		
300	53.00 ± 1.91					99.00 ± 1.00				
400	67.00 ± 2.52					100				
500	96.00 ± 1.63					100				
Control	1.00 ± 1.00					2.00 ± 1.15				

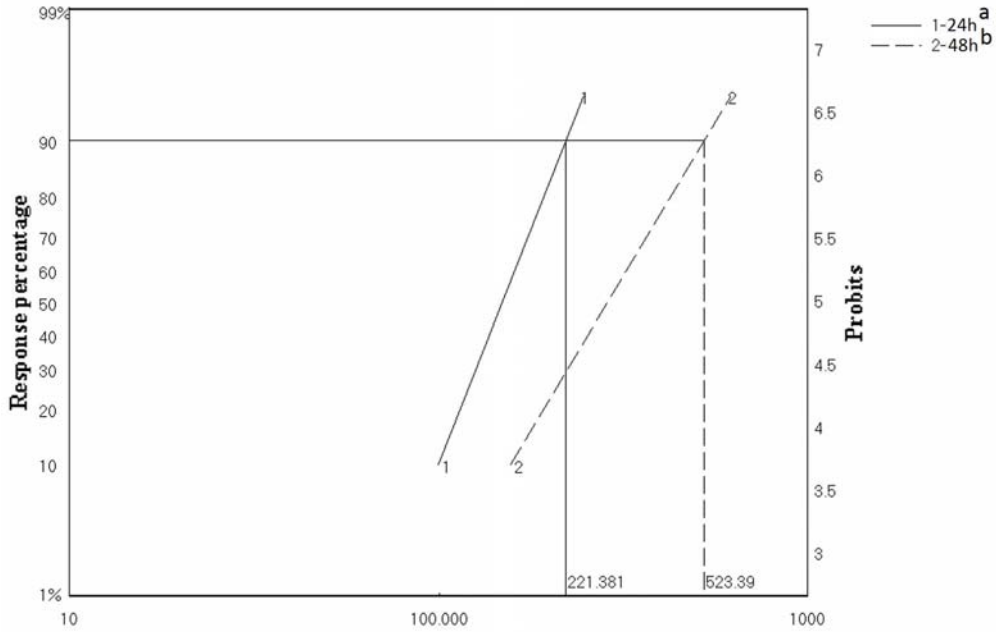
LC_{50} and LC_{90}: lethal concentration that causes 50% and 90% of mortality, SE: standard error, χ^2: chi-square, ppm: parts per million

Fig 4- Graph showing the LC_{50} values of the ethanolic crude extract of *Steccherinum* mushroom on the 3rd stage *Cx. quinquefasciatus* at 24- and 48-hours exposing time.



Statistically significant differences are indicated by different letter on the ethanolic extract groups (upper right)

Fig 5- Graph showing the LC_{90} values of the ethanolic crude extract of *Steccherinum* mushroom on the 3rd stage *Cx. quinquefasciatus* at 24- and 48-hours exposing time.

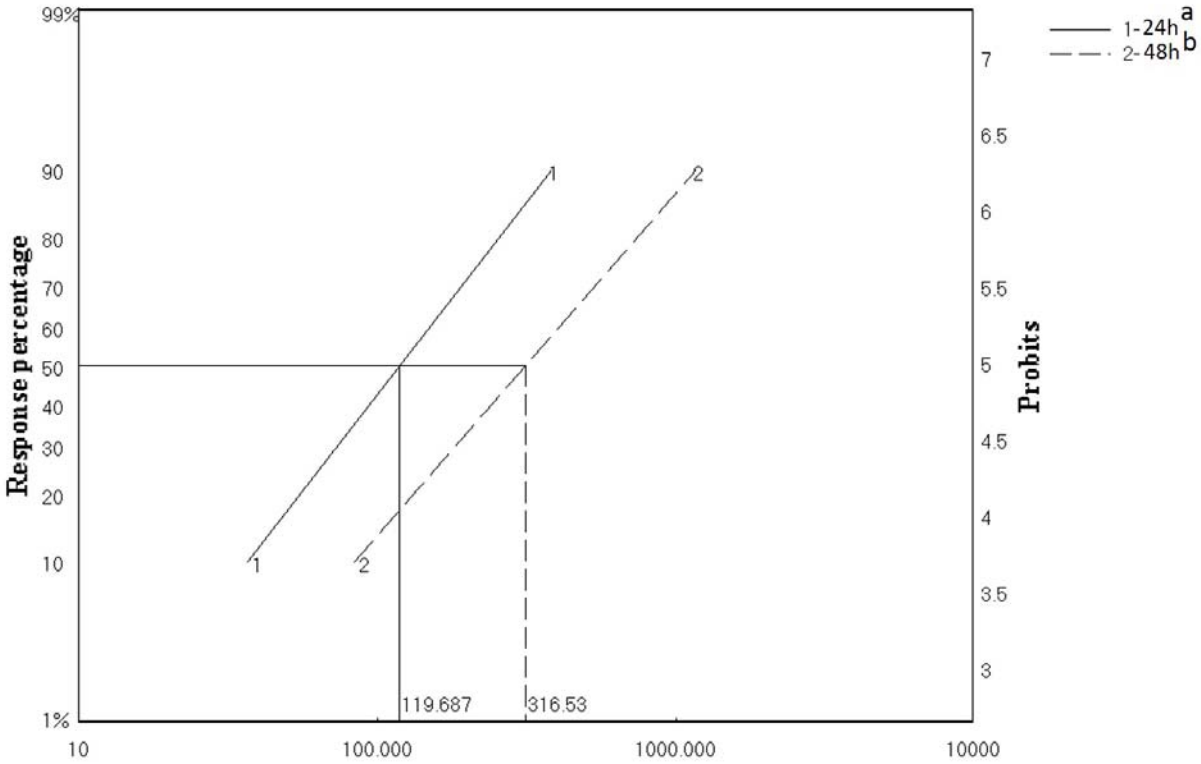


Statistically significant differences are indicated by different letter on the ethanolic extract groups (upper right)

For the *An. minimus* bioassay

From the 24- and 48-hour 10 – 400 ppm bioassays of *Steccherinum* ethanolic extract, the 48-hour showed higher activity than the 24-hour with the LC_{50} and LC_{90} values of 119.69 and 387.29 ppm (48-hour) and 316.53 and 1,188 ppm (24-hour), respectively (Table 4). After statistically analysis comparing of the LC_{50} (Fig 6) and LC_{90} (Fig 7) values, the 48-hour activity of both values provided significantly better larvicidal toxicity than the 24-hour exposure time. It was the same finding with the ethanolic extract bioassays of the *Aedes* and *Culex* mosquitoes of this study.

Fig 6- Graph showing the LC_{50} values of the ethanolic crude extract of *Steccherinum* mushroom on the 3rd stage *An. minimus* at 24- and 48-hours exposing time.



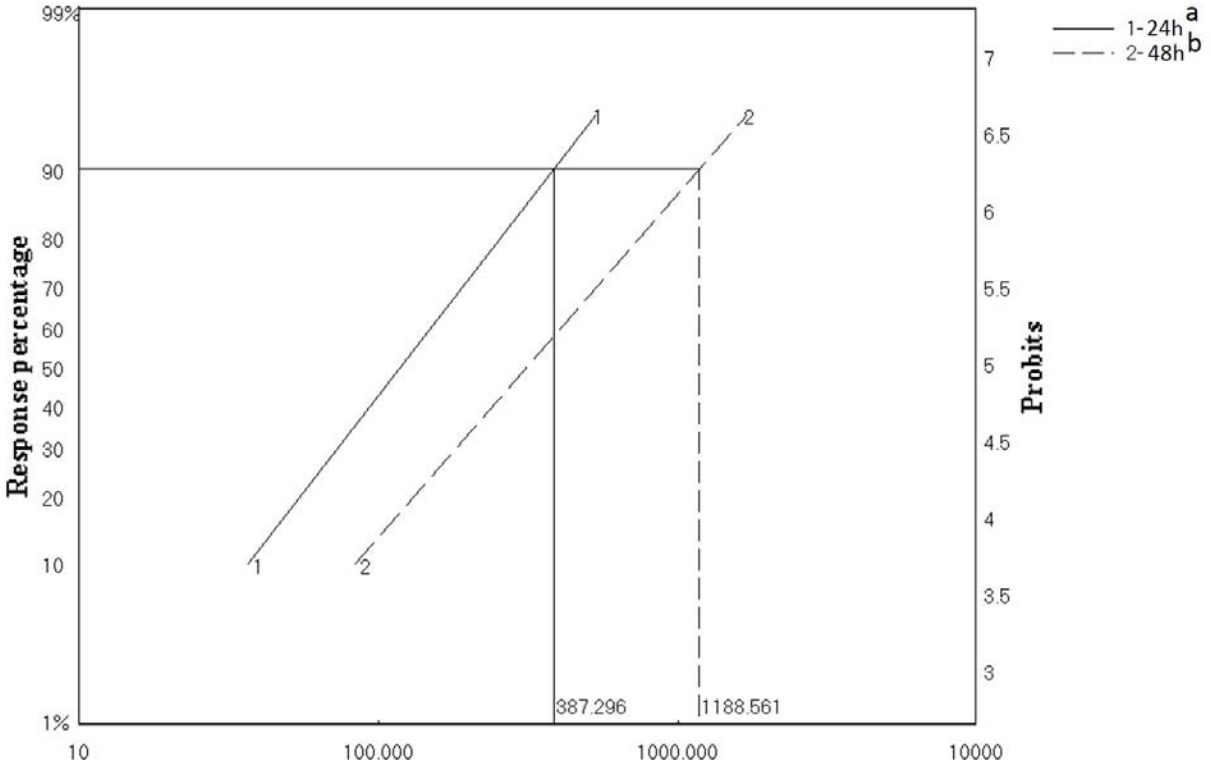
Statistically significant differences are indicated by different letter on the ethanolic extract groups (upper right)

Table 4-Larvicidal activities of the crude ethanolic *Steccherinum* extracts against the 3rd stage larvae *An. minumus* after 24- and 48-hour exposure times.

Crude extract of *Steccherinum* (ppm)	24-hour					48-hour				
	% Mortality (Mean ± SE)	Larvicidal activity				% Mortality (Mean ± SE)	Larvicidal activity			
		Lethal Concentration with fiducial limits (ppm)		χ^2	Slope ±SE		Lethal Concentration with fiducial limits (ppm)		χ^2	Slope ±SE
		LC_{50}	LC_{90}				LC_{50}	LC_{90}		
10	1.00 ± 1.00	316.53	1,188.56	40.68	2.23 ± 0.19	4.00 ± 1.63	119.69	387.29	53.40	2.51 ± 0.14
30	1.00 ± 1.00	(255.88 - 467.46)	(1,075.69 - 2,903.02)			6.00 ± 1.15	(87.03 - 154.02)	(319.51 - 600.04)		
50	1.00 ± 1.00					14.00 ± 2.58				
100	8.00 ± 2.31					35.00 ± 1.91				
150	35.00 ± 1.91					52.00 ± 1.63				
200	35.00 ± 3.00					72.00 ± 2.83				
250	34.00 ± 1.15					80.00 ± 1.63				
300	41.00 ± 1.91					82.00 ± 4.76				
350	56.00 ± 1.63					91.00 ± 1.00				
400	64.00 ± 5.89					98.00 ± 1.15				
Control	0					0				

LC_{50} and LC_{90}: lethal concentration that causes 50% and 90% of mortality, SE: standard error, χ^2: chi-square, ppm: parts per million

Fig 7- Graph showing the LC_{90} values of the ethanolic crude extract of *Steccherinum* mushroom on the 3rd stage *An. minimus* at 24- and 48-hours exposing time.

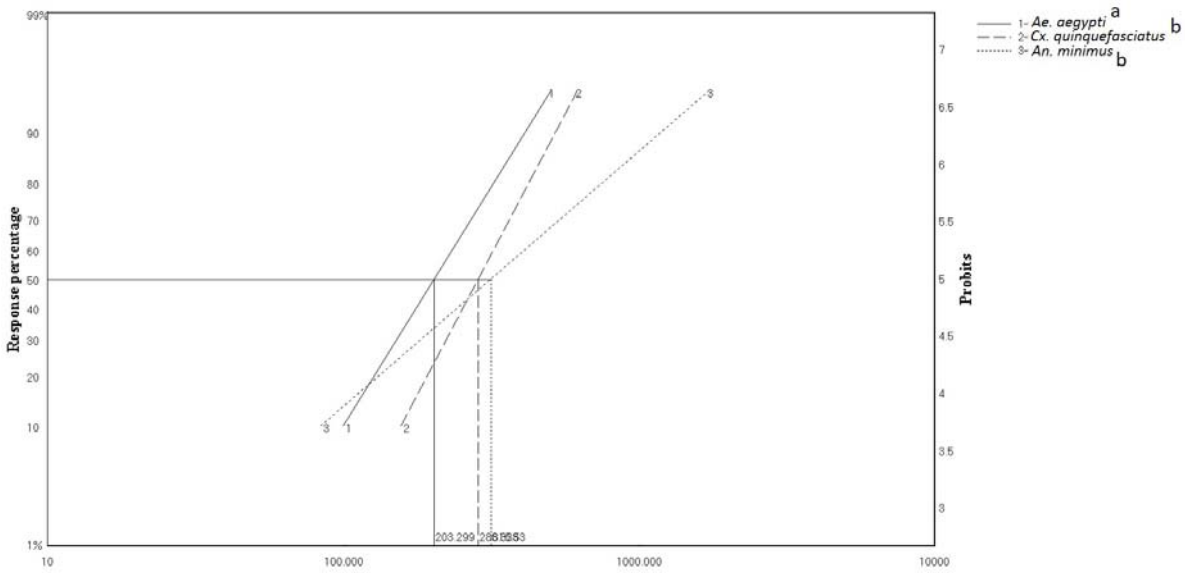


Statistically significant differences are indicated by different letter on the ethanolic extract groups (upper right)

Comparison of ethanolic crude extract against three mosquito species

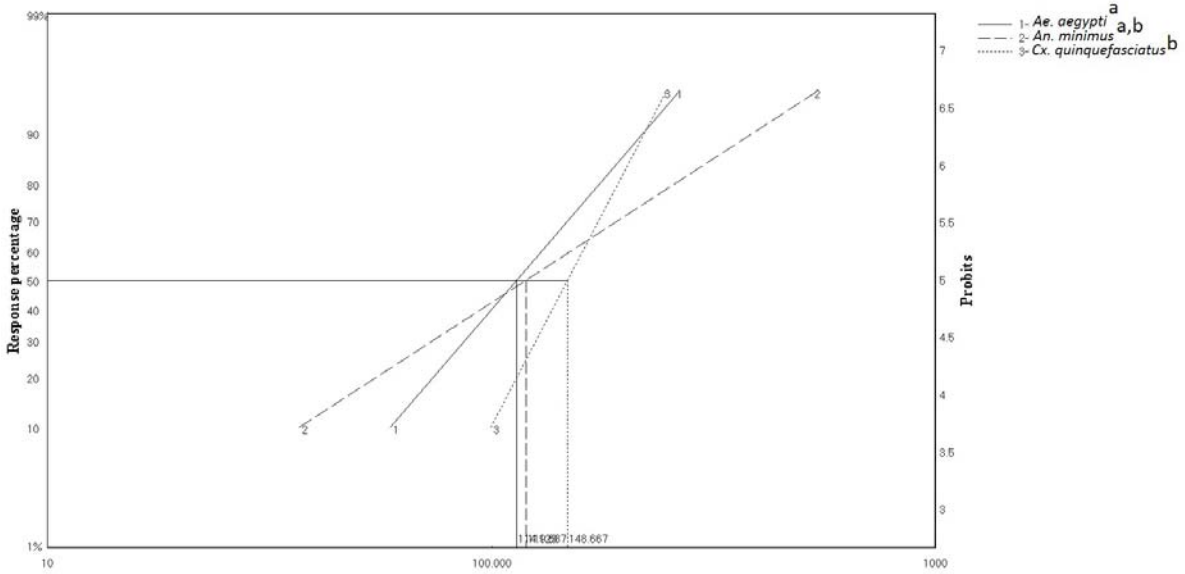
After the statistical analysis of the ethanolic extract bioassays against the 3 studied mosquito species, the LC_{50} values, significantly highest activity of 24-hour exposing time was found from the bioassay against *Ae. aegypti* (203.30 ppm), following with the activities against *Cx. qinquefasciatus* (286.33 ppm) and *An. minimus* (316.53 ppm) which were not significant difference (Fig 8). For the 48-hour, the most susceptible mosquito against the ethanolic extract still was the *Aedes* (114.12 ppm) following with the *Anopheles* (119.69 ppm) without significant difference. The *Culex* revealed the most resistant mosquito against the extract with the LC_{50} value of 148.67 ppm, however, the *Culex* LC_{50} value did not statistically different from the *Anopheles* one, but different from the *Aedes* LC_{50} value (Fig 9).

Fig 8- Graph showing the LC_{50} values of the ethanolic crude extract of *Steccherinum* mushroom on the 3rd stage 3 mosquito species at 24-hours exposing time.



Statistically significant differences are indicated by different letter on the ethanolic extract groups (upper right)

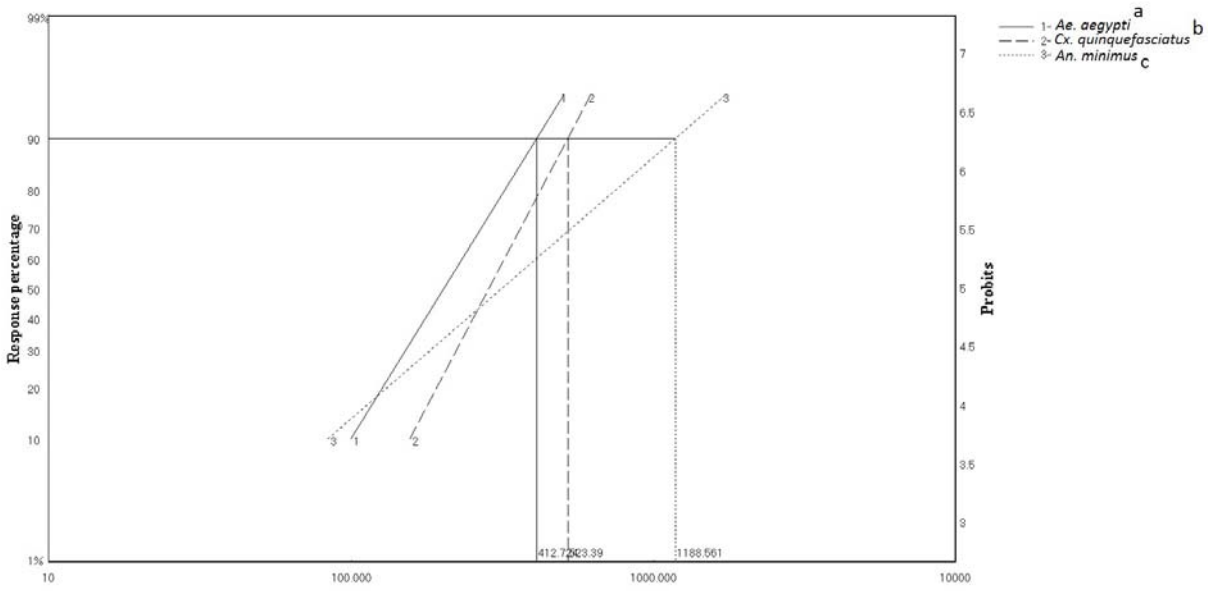
Fig 9- Graph showing the LC_{50} values of the ethanolic crude extract of *Steccherinum* mushroom on the 3rd stage 3 mosquito species at 48-hours exposing time.



Statistically significant differences are indicated by different letter on the ethanolic extract groups (upper right)

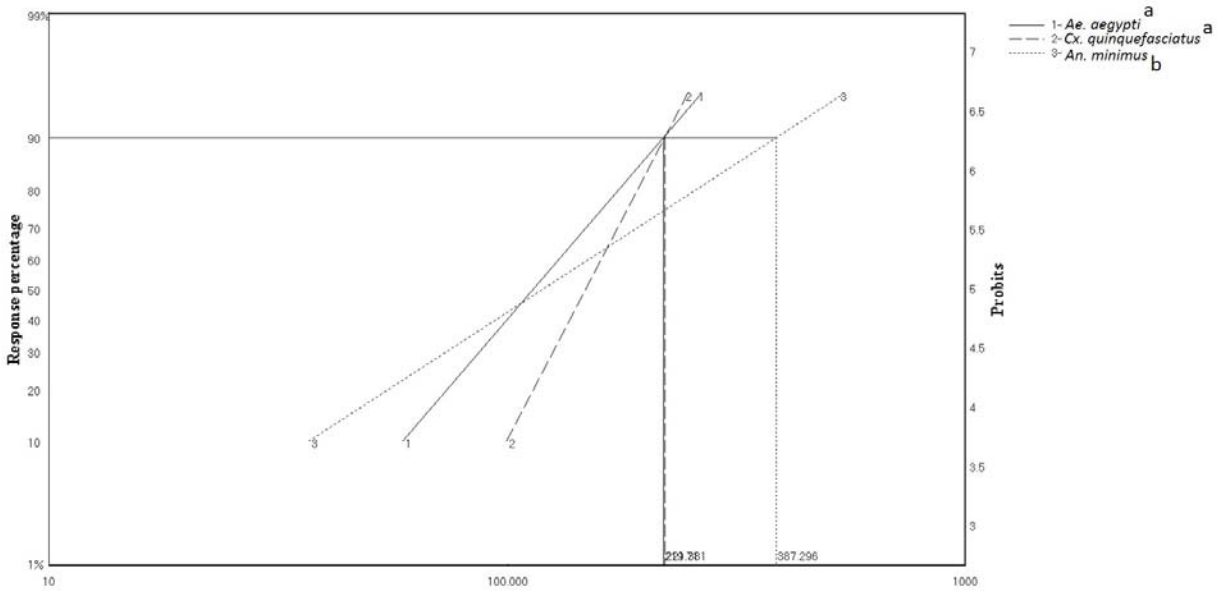
For the LC_{90} value observation, the 24-hour detection time, *Ae. aegypti* larvae was the most susceptible mosquito against the *Steccherinum* ethanolic extract with 412.73 ppm, following with the activities against *Cx. qinquefasciatus* (523.39 ppm) and *An. minimus* (1,188.56 ppm) with significant difference for all 3 mosquito species larval activities (Fig 10). For the 48-hour, the most susceptible mosquito still was the *Aedes* (219.71 ppm) following with the *Culex* (221.38 ppm) without significantly difference. For this time, the *Anopheles* revealed the most resistant mosquito against the extract with the LC_{90} value of 387.30 ppm with statistical difference from the both previous mosquito species (Fig 11).

Fig 10- Graph showing the LC_{90} values of the ethanolic crude extract of *Steccherinum* mushroom on the 3rd stage 3 mosquito species at 24-hours exposing time.



Statistically significant differences are indicated by different letter on the ethanolic extract groups (upper right)

Fig 11- Graph showing the LC_{90} values of the ethanolic crude extract of *Steccherinum* mushroom on the 3rd stage 3 mosquito species at 48-hours exposing time.



Statistically significant differences are indicated by different letter on the ethanolic extract groups (upper right)

Conclusion and Discussion

In conclusion, the screening bioassay results of this study revealed the *Ae. aegypti* larvicide property of the mushroom species from Thailand. At least 4 of 46 identified genus, including *Th. porentosus*, *Chlorophyllum* sp., *Steccherinum* sp. and *X. nigripes*, displayed the promising larvicidal efficacy. After the intensively bioassay investigation against *Ae. aegypti* larvae of *Steccherinum* mushroom. The ethanolic extract showed superior activity with the LC_{50} and LC_{90} values of 203.30 and 412.72 ppm, respectively, after 24-hour exposing time. Moreover, the improved efficacy was examined after 48-hour post larval exposure. The same finding was also found form the *Cx. quinquefasciatus* and *An. minimus* bioassays.

Since the mosquito biological control has been revealed for over 50 years (Jenkins, 1964). The study of mosquito larva killing organisms has mainly scoped on fish, bacteria, protozoa, filamentous fungi, viruses or nematodes (Chapman, 1974; Kamareddine, 2012). For higher fungi, mushroom, there were just the studies of Njogu *et al* (2009), Bucker *et al* (2013) and Chelela *et al* 2014 that reported the larvicidal activity from the extracts of some mushroom species including *C. asprata*, *P. sanguineus*, *A. phalloides*, *Boletus* sp., *L. densifolius*, *L. gymnocarpoides*, *R. cellulata* and *R. kivuensis* against *Ae.*

aegypti, *An. nuneztovari*, *An. gambiae* or *Cx. quinquefasciatus* mosquitoes. These studies were the reports of mosquito larvicide containing mushrooms from Kenya, Brazil and Tanzania. Then, our study was the first report that researched and revealed the mosquito larvicidal activity from mushroom species of Thailand.

From the screening of 143 mushroom samples (>46 genera), most of them did not contain the mosquito larvicide. However, 6 samples including *Th. Porentosus*, *Chlorophyllum* sp., *Steccherinum* sp., *X. nigripes* and 2 unidentified CKW05 and GSW04 samples displayed a promising larvicidal efficacy with >50% *Ae. aegypti* larval mortality rates after 24-hour detection time. All 6 larvicide containing mushrooms could be selected for the further intensive investigation. Unfortunately, because of the quantity limitation of collected samples, only *Steccherinum* mushroom was collected in a large amount. Then, we can investigate only the primary screening results from the left 5 samples. However, the intensive investigation of *Ae. aegypti* larvicidal activity from *Steccherinum* was accomplished. From our results, although the aqueous extract of *Steccherinum* mushroom did not showed any larcividal activity, the hexane and ethanolic extracts provided satisfactory activities with 304.05 and 203.30 ppm LC_{50} values, respectively, at 24-hour detection time. Moreover, after 48 hours, the LC_{50} values reduced to 218.45 and 114.12 ppm.

Compare to the previous studies of mushroom extract against *Ae. aegypti* mosquito, ethyl acetate extracted secondary matabolite, (oxiran-2-yl) methylpentanoate, of *C. asprata* mushroom showed a greater superior activity than *Steccherinum* with the LC_{50} and LC_{90} values at 24-hour exposing time of 1.50 and 1.90 ppm, respectively (Njogu *et al*, 2009). While the ethanolic extract of *Steccherinum* of this study revealed only 203.30 and 412.72 ppm at the same exposing time. It would not be surprised for this assessment because our larvicidal activity finding came from the crude extract, while the Njogu *et al* (2009) came from a pure compound. However, a comparable activity of larvicide was found from the study of *P. sanguineus* mushroom. It was found that ethyl acetate crude extract of *P. sanguineus* revealed the activity against *Ae. aegypti* larvae with the LC_{50} value of 156.8 ppm at 24-hour exposing time (Bucker *et al*, 2013). For the 48-hour mortality rate, because of the literature limitation of the mosquito larvicidal activity from mushroom extract, only the study of Chelela *et al* (2014) was compared. A comparable finding that the 48-hour activity showed improvement than the 24-hour was found form all 6 wild mushrooms, including *A. phalloides*, *Boletus* sp., *L. densifolius*, *L. gymnocarpoides*, *R. cellulata* and *R. kivuensis*, after testing against *Ae. aegypti*, *Cx. quinquefasciatus* or *An. gambiae* larvae. Moreover, the 72-hour observation of Chelela *et al* (2014) found the greater activities than the 48-hour. However, we did not enplaned the observation time to 72 hours, but the same finding of Chelela *et al* (2014) could be predictable.

Comparing of our finding with the same mushroom genus studied by Chelela *et al* (2014), dissimilar finding of all 6 mushroom genus was found. While *A. phalloides*, *Boletus* sp., *L. densifolius*, *L. gymnocarpoides*, *R. cellulata* and *R. kivuensis* of Chelela *et al* (2014) showed the activity against mosquito larvae, the same 6 genus of our study did not. It might because of the different in species level of mushrooms from the two studies. This situation also occurred in our finding during the screening stage, we tested 3 samples of *Steccherinum* sp. including the UMC16, UMC53 and CKW03. Only the CKW03 was found for larvicidal efficacy with very high activities to 66% and 90% *Ae. aegypti* mortality rates, for 24- and 48-hour exposing time, respectively, while the others revealed the zero mortality rate. Then, we concluded that different species of the same mushroom genus could contain a different substance.

For *Cx. quinquefasciatus* and *An. minimus* comparision, the limitation of literatures were found. Chelela *et al* (2014) reported *Cx. quinquefasciatus* larvicide from *Lactarius densifolius* chloroform extract with the LC_{50} values of 445.61 and 327.28 µg/mL (ppm), for 24- and 48-hour detection times, respectively. Compare to our study, *Steccherinum* ethanolic extract showed much greater activity with the LC_{50} values of 286.33 and 148.67 ppm, for 24- and 48-hour detection times, respectively. The larvicidal activity comparison against the *Ae. minimus* could not be done because no literature of mushroom extract against this mosquito was found. However, the LC_{50} values of closely related species, An. nuneztovari and An. gambiae, were reported for 87.2 ppm and 174.93 µg/mL (ppm) after testing with *Pycnoporus sanguineus* and *L. densifolius* mushroom extracts, respectively (Bucker *et al*, 2013; Chelela *et al*, 2014).

In this study, for the first time, we reported the mosquito larvicidal potential of Thai mushroom extracts. Form a huge mushroom diversity, numerous species still be under discovered and researched, then they could be subjected for developing as a potentially alternative larvicide in the mosquito controlling strategy in further.

Referenced cites for the conclusion and discussion

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Output

International Journal Publication

The manuscript of this research has been organizing. It will be submitted to publish in the international journal “The Southeast Asian Journal of Tropical Medicine and Public Health”. The journal is a member of ISI and SCImago Journal Rank indexes.

International conference

A part of this research was presented as “Poster Presentation” in an international conference “The 10th International Mycological Congress (IMC10)”, 4-8 August 2014, Queen Sirikit National Convention Center (QSNCC), Bangkok, Thailand.

Appendix

Manuscript for publishing in "The Southeast Asian Journal of Tropical Medicine and Public Health"

SCREENING FOR MOSQUITO LARVICIDAL ACTIVITY OF THAI MUSHROOM EXTRACTS WITH SPECIAL REFRENCE TO *STECCHERINUM* SP. AGAINST *AEDES AEGYPTI* (L.) (DIPTERA: CULICIDAE)

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Short title: LARVICIDAL ACTIVITY OF MUSHROOM EXTRACTS AGAINST *AE. AEGYPTI*

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Keywords: *Aedes aegypti*, *Steccherinum*, crude extract, larvicide, mushroom

Abstract. For over 50 years, biological control of mosquito larvae has mainly scoped on plants, fish, bacteria, protozoa, filamentous fungi, viruses or nematodes. In this study, we screened 143 mushroom samples (44 confirmed species) from Thailand for their mosquito larvicidal activity. 1 g%w/v of aqueous extract of dried powdered mushroom sample was tested against the 3rd stage *Aedes aegypti* larvae. Four identified mushroom species, including *Thaeogyroporus porentosus*, *Xylaria nigripes*, *Chlorophyllum* sp. and *Steccherinum* sp., and 2 unidentified species showed larvicidal activity with mortality rates ranging from 10 - 70% and 18 - 90% for 24- and 48-hour exposure times, respectively. After that, the *Steccherinum* mushroom was selected for an intensively bioassay following the standard protocol of WHO. An aqueous, hexane and ethanolic crude extracts of *Steccherinum* were prepared in various concentrations up to 1,000 ppm. After 48-hour post exposure, the aqueous extract did not show any larvicidal activity, while the ethanolic showed superior activity than the hexane with the 24-hour LC_{50} and LC_{90} values of 203.30 and 412.72 ppm, respectively. General finding that the 48-hour activities of the extracts were higher than the 24-hour's was observed. According to the finding, we are the first to report the mosquito larvicidal potential from the extracts of Thai mushrooms.

INTRODUCTION

Aedes aegypti (L.) (Diptera: Culicidae) is the main vector of dengue virus causing dengue and dengue hemorrhagic fevers worldwide (Ratnam *et al*, 2013). In Thailand, for limiting the disease outbreak, temephos (larvicide chemical agent) has widely been used for *Aedes* larvae controlling for a long time (Chareonviriyaphap *et al*, 1999). Although temephos reveals a very good efficacy to control the larvae, unappropriated apply into the environment might cause disorder to non-targeted organisms, including human. Moreover, resistance to temephos has been increasingly reported (Jirakanjanakit *et al*, 2007; Sornpeng *et al*, 2009). Therefore, biological control, an alternatively safer controlling method, is potentially used to resolve the chemical usage problem.

Biological agents can kill mosquito larvae in two ways: (1) they are a parasite of the larvae, and (2) they produce larval toxic substances. For the later action, many researches paid attention to extract larvicide substances from living organisms, especially from plants, mostly from herbs. Recently, extracts of *Ixora coccinea* and *Allamanda violacea* have been shown to have larvicidal activity against *Ae. aegypti*. The LC_{50} values of *I. coccinea* flower and leaf and *A. violacea* leaf extracts were 139.6, 150.7 and 218.9 mg/L, respectively (Suryawanshi *et al*, 2015). Not only plants can produce the bio-activities substances, but also the microbe organisms. Most fungi, a big group of living organisms, can produce toxic metabolites against mosquito larvae. For examples, the metabolites from *Aspergillus flavus, Chrysosporium lobatum, Penicillium* sp. and *Podospora* sp. showed the 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 Basidiomycotina Subdivision, consist of more than 14,000 species (Lindequist *et al*, 2005) and some of them can produce toxic metabolite substances. For a long time, mushrooms have been used in medical purposes because of their antibacterial (Bender *et al*, 2003; Lindequist *et al*, 2005), antifungal (Smania *et al*, 2003), antiviral (Brandt and Piraino, 2000), antitumor (Zaidman *et al*, 2005; Zhang *et al*, 2007), antiallergy (Min *et al*, 2001), anti-inflammatory (Kim *et al*, 2003; 2004), and also antioxidant (Ajith and Janardhanan, 2007) activities. Moreover, a larvicidal activity against agricultural insect pests was also found. For example, cordycepin (3'-deoxyadenosine) from fruiting body of *Cordyceps militaris* was reported to kill 3rd instar of diamondback moth, *Plutella xylostella* (Kim *et al*, 2002). However, a few researches have been studied for the mosquito larvicidal activity from mushrooms. In 2009, a secondary metabolite, (oxiran-2-yl) methylpentanoate, from *Cyptotrama asprata* mushroom was reported to kill *Ae. aegypti* larvae with the LC_{50} and LC_{90} values of 1.50 and 1.90 ppm, respectively (Njogu *et al*, 2009). Bucker *et al* (2013) reported the larvicidal activity from *Pycnoporus sanguineus* mushroom against *Ae. aegypti* and *An. nuneztovari* mosquitoes with the LC_{50} values of 156.8 and 87.2 ppm, respectively. Recently, some wild mushroom species, including *Amanita phalloides*, *Boletus* sp., *Lactarius densifolius*, *Lactarius gymnocarpoides*, *Russula cellulata* and *Russula kivuensis*, were reported for various larvicidal potential against *Ae. aegypti*, *Culex quinquefasciatus* and *An. gambiae* mosquitoes (Chelela *et al*, 2014).

Abundant mushroom species are commonly found in tropical rain forests, but little is known about mosquito-larvicide producing mushrooms in Thailand. Our preliminary study indicated that aqueous extracts of some mushrooms displayed a promising result. Then, the objectives of this study were to: (1) screen for the mosquito larvicide substance from aqueous mushroom extracts of Thailand, and (2) evaluate the larvicidal efficacy of an aqueous, hexane and ethanolic extracts of selected mushroom species, *Steccherinum*, against the *Ae. aegypti* mosquito. The exploring of new mosquito larvicide producing mushrooms was reported in this study.

MATERIALS AND METHODS

Mushrooms material collection identification

143 fresh mushroom samples were collected from several provinces of Thailand, including Chiang Mai, Krabi, Lampang, Nakhon Ratchasima, Nakhon Sawan, Pathumthani, Phichit, Phitsanulok, Phuket, Prachuap Khiri Khan, Sukhothai, Surat thani and Tak. They were kept in a plastic cup, and then transferred to the laboratory of Department of Microbiology and Parasitology, Faculty of Medical Science, Naresuan University, the same place that voucher specimens were deposited. They were identified macroscopically and microscopically following the mushroom taxonomic keys (Largent and Thiers, 1977; Largent *et al*, 1977; Stuntz, 1977; Watling, 1977; Largent, 1986; Largent and Baroni, 1988). After the identification processes, the mushroom samples were air-dried in a hot air oven at 45°C for 24 hours, and ground into powder by using an electric blender (Single Speed Blenders 800G, MRC Ltd., Israel) at 22,000 rpm. The dried powder of each mushroom species was kept in 4°C refrigerator until use for the mosquito larvicidal activity screening.

Mosquito colonization

Laboratory strain *Ae. aegypti* that originally collected from breeding places in Mueang district of Phitsanulok province, Thailand were used. The rearing protocols were performed according to Thongwat *et al* (2014). Briefly, the larvae were reared with tap water provided with a powder of dog biscuits (Adult Complete Nutrition, PEDIGREE®, Mars Petcare [Thailand] Co., Ltd., USA). After pupation, the pupae were transferred to a mosquito cage (30x30x30 cm) and the emerging adults adults were provided with a 5% sugar solution mixed with 5% multivitamin syrup (Multi-Vitamin Syrup, SEVEN SEAS®, OLIC [Thailand] Limited, UK). Five to 7-day-old females were permitted to take a blood meal by using an artificial membrane feeding method (Rutledge *et al*, 1964). The gravid females were allowed to lay eggs on a wet filter paper (Whatman N^{o} 1). Those eggs were air-dried for 3 days, and then kept in a humidity controlling glass jar until being required. The rearing room was set up at 25± 2°C with 10:14 (L:D) photoperiod.

Larvicidal activity screening

Two grams of each mushroom powder were suspended into 200 mL of distilled water in 250-mL Erlenmeyer flask, and then continuously stirred at 180 rpm for 24 hours on a rotary shaker (Innova™ 2300, NEW BRUNSWICK SCIENTIFIC, USA) at room temperature. After that, the extracted suspension was filtered through a fine net cloth, revealing 1 g% w/v of aqueous crude mushroom extract. The extract was put into a plastic bowl followed by twenty-five 3rd instar *Ae. aegypti* larvae. Mortality rates were examined after 24- and 48-hour exposure times. No food was offered to the larvae during the experimental time. The experiments were performed in four replicates. An equal number of controls was set up simultaneously with 200 mL of distilled water alone.

Crude extracts preparation of *Steccherinum* mushroom

The sample CKW03 (*Steccherinum* sp.) were selected for further study because it showed the highest mortality (see below) and the remaining quantity was sufficient to be tested. The dried powder (100.85 g) was extracted following the previous method of Thongwat *et al* (2014). Briefly, the powder was sequentially extracted with hexane, ethanol and distilled water. Ten grams of powder were suspended into 100 mL of hexane, and then continuously stirred at 180 rpm for 24 hours on the shaker. After that, the extracted suspension was filtered with a Whatman N^{o} 1 filter paper. The residue will be then extracted with ethanol and then distilled water. The hexane and ethanol solvents were removed from the extracts by the evaporation (BÜCHI Rotavapor® R-205 with BÜCHI Vac® V-500, BÜCHI, Switzerland), while the water was removed by the evaporation and lyophilization (Lyotrap LF/LYO/01/1, LTE SCIENTIFIC, UK). The crude extract yields were 2.29, 8.58 and 18.59 g for hexane, ethanolic and aqueous, respectively.

Larvicidal bioassay

The dose-response bioassay against *Ae. aegypti* larvae was done following the protocol of WHO (2005) with minor modifications. Briefly, 1g% w/v stock solution of the crude extracts were prepared by weighing 200 mg of the extract and adding 20 mL of suitable solvent, dimethylsulphoxide (DMSO) for the hexane/ ethanolic extracts and distilled water for the aqueous extract. After that, a series of concentrations were prepared for the larvicidal activity testing. Two-hundred mL of various concentrations of each extract was put into a plastic bowl, and then healthy twenty-five 3rd instar *Ae. aegypti* larvae were transferred into the solutions, in which 4 replicates were done. After 24 and 48 hours, the mortality rates were examined. An equal number of controls was made simultaneously with 2 mL of DMSO in 198 mL of distilled water for the hexane/ethanolic extracts and 200 mL of distilled water alone for the aqueous extract.

Data analysis

The 50% and 90% lethal concentration (LC_{50} and LC_{90}) values were determined following the Probit analysis (Finney, 1971) by using the commercial LdP Line® software (Cairo, EGY: Plant Protection Research Institute). The 95% Confidence Intervals (CI) of upper and lower fiducial limits were also calculated. Differences below the probability level of 0.05 were considered statistically significant of the LC_{50} and LC_{90} values comparison.

RESULTS

Of 143 mushroom samples, 136 were identified into 46 genera with at least 44 confirmed species. The 7 remainder samples were unidentifiable into any genus because of the limitation of quantity and complete morphology of the specimen. After the screening for larvicidal activity of all mushroom aqueous extracts (1g% w/v), 6 of all, including 4 identified samples, *Thaeogyroporus porentosus* (PHK27), *Chlorophyllum* sp. (NU01), *Steccherinum* sp. (CKW03) and *Xylaria nigripes* (PW03) and 2 unidentified samples (CKW05 and GSW04) displayed a promising larvicidal efficacy ranging from 10 - 70% and 18 - 90% larval mortality rates for 24- and 48-hour exposure times, respectively (Table 1). For 24-hour exposure time, the highest activity was found from *Th. Porentosus* (PHK27) extract with 70% mortality rate, following with *Steccherinum* (CKW03), *X. nigripes* (PW03), and GSW04 samples, with 66, 64 and 52% mortality rates, respectively. Meanwhile, for 48-hour exposure time, *Steccherinum* (CKW03) caused the highest larval mortality (90%), followed by *X. nigripes* (PW03), GSW04 and *Th. Porentosus* (PHK27), with 88%, 88% and 70%, respectively. The relatively lower activities (10% and 18% mortality rates, for 24- and 48-hour, respectively) were found from *Chlorophyllum* sp. (NU01) and unidentified CKW05 samples. The other 135 samples showed only 0 - 1% and 0 - 2% larval mortality rates after 24- and 48-hour exposer times, respectively.

From the 24- and 48-hour bioassays of *Steccherinum* (CKW03) crude extracts, the larvicidal activities were found from the hexane and ethanolic extracts, while the aqueous one showed no mortality after testing with extract concentration up to 1,000 ppm for 48 hours. The ethanolic extract showed higher activity than the hexane extract with the LC_{50} values of 203.30 and 304.05 ppm (24-hour), and 114.12 and 218.45 ppm (48-hour), for ethanolic and hexane extracts, respectively (Table 2). The LC_{90} values of the ethanolic extract were also lower than the hexane extract. Statistical analysis of the LC_{50} (Fig 1) and LC_{90} (Fig 2) values revealed that the 48-hour activity of the ethanolic extract provided a significantly highest larvicidal toxicity with 114.12 and 219.71 ppm, for the LC_{50} and LC_{90} values, respectively. Lower activities were found from the 24-hour ethanolic (203.30 and 412.72 ppm), following with the 48-hour hexane (218.45 and 425.75 ppm) and the 24-hour hexane (304.05 and 533.40 ppm) extracts, respectively. From the results, a higher quantity of larvicide substance from *Steccherinum* mushroom was found after extracting with ethanol than hexane and also water.

DISCUSSION

Since the mosquito biological control has been revealed for over 50 years (Jenkins, 1964). The study of mosquito larva killing organisms has mainly scoped on fish, bacteria, protozoa, filamentous fungi, viruses or nematodes (Chapman, 1974; Kamareddine, 2012). For higher fungi, mushroom, there were just the studies of Njogu *et al* (2009), Bucker *et al* (2013) and Chelela *et al* 2014 that reported the larvicidal activity from the extracts of some mushroom species including *C. asprata*, *P. sanguineus*, *A. phalloides*, *Boletus* sp., *L. densifolius*, *L. gymnocarpoides*, *R. cellulata* and *R. kivuensis* against *Ae. aegypti*, *An. nuneztovari*, *An. gambiae* or *Cx. quinquefasciatus* mosquitoes. These studies were the reports of mosquito larvicide containing mushrooms from Kenya, Brazil and Tanzania. Then, our study was the first report that researched and revealed the mosquito larvicidal activity from mushroom species of Thailand.

From the screening of 143 mushroom samples (>46 genera), most of them did not contain the mosquito larvicide. However, 6 samples including *Th. Porentosus*, *Chlorophyllum* sp., *Steccherinum* sp., *X. nigripes* and 2 unidentified CKW05 and GSW04 samples displayed a promising larvicidal efficacy with >50% *Ae. aegypti* larval mortality rates after 24-hour detection time. All 6 larvicide containing mushrooms could be selected for the further intensive investigation. Unfortunately, because of the quantity limitation of collected samples, only *Steccherinum* mushroom was collected in a large amount. Then, we can investigate only the primary screening results from the left 5 samples. However, the intensive investigation of *Ae. aegypti* larvicidal activity from *Steccherinum* was accomplished. From our results, although the aqueous extract of *Steccherinum* mushroom did not showed any larcividal activity, the hexane and ethanolic extracts

provided satisfactory activities with 304.05 and 203.30 ppm LC_{50} values, respectively, at 24-hour detection time. Moreover, after 48 hours, the LC_{50} values reduced to 218.45 and 114.12 ppm.

Compare to the previous studies of mushroom extract against *Ae. aegypti* mosquito, ethyl acetate extracted secondary matabolite, (oxiran-2-yl) methylpentanoate, of *C. asprata* mushroom showed a greater superior activity than *Steccherinum* with the LC_{50} and LC_{90} values at 24-hour exposing time of 1.50 and 1.90 ppm, respectively (Njogu *et al*, 2009). While the ethanolic extract of *Steccherinum* of this study revealed only 203.30 and 412.72 ppm at the same exposing time. It would not be surprised for this assessment because our larvicidal activity finding came from the crude extract, while the Njogu *et al* (2009) came from a pure compound. However, a comparable activity of larvicide was found from the study of *P. sanguineus* mushroom. It was found that ethyl acetate crude extract of *P. sanguineus* revealed the activity against *Ae. aegypti* larvae with the LC_{50} value of 156.8 ppm at 24-hour exposing time (Bucker *et al*, 2013). For the 48-hour mortality rate, because of the literature limitation of the mosquito larvicidal activity from mushroom extract, only the study of Chelela *et al* (2014) was compared. A comparable finding that the 48-hour activity showed improvement than the 24-hour was found form all 6 wild mushrooms, including *A. phalloides*, *Boletus* sp., *L. densifolius*, *L. gymnocarpoides*, *R. cellulata* and *R. kivuensis*, after testing against *Ae. aegypti*, *Cx. quinquefasciatus* or *An. gambiae* larvae. Moreover, the 72-hour observation of Chelela *et al* (2014) found the greater activities than the 48-hour. However, we did not enplaned the observation time to 72 hours, but the same finding of Chelela *et al* (2014) could be predictable.

Comparing of our finding with the same mushroom genus studied by Chelela *et al* (2014), dissimilar finding of all 6 mushroom genus was found. While *A. phalloides*, *Boletus* sp., *L. densifolius*, *L. gymnocarpoides*, *R. cellulata* and *R. kivuensis* of Chelela *et al* (2014) showed the activity against mosquito larvae, the same 6 genus of our study did not. It might because of the different in species level of mushrooms from the two studies. This situation also occurred in our finding during the screening stage, we tested 3 samples of *Steccherinum* sp. including the UMC16, UMC53 and CKW03. Only the CKW03 was found for larvicidal efficacy with very high activities to 66% and 90% *Ae. aegypti* mortality rates, for 24- and 48-hour exposing time, respectively, while the others revealed the zero mortality rate. Then, we concluded that different species of the same mushroom genus could contain a different substance.

In conclusion, the screening bioassay results of this study revealed the *Ae.aegypti* larvicide property of the mushroom species from Thailand. At least 4 of 46 identified genus, including *Th. porentosus*, *Chlorophyllum* sp., *Steccherinum* sp. and *X. nigripes*, displayed the promising larvicidal efficacy. After the intensively bioassay investigation against *Ae. aegypti* larvae of *Steccherinum* mushroom. The ethanolic extract showed superior activity with the LC_{50} and LC_{90} values of 203.30 and 412.72 ppm, respectively, after 24-hour exposing time. Moreover, the improved efficacy was examined after 48-hour post larval exposure. In this study, for the first time, we reported the mosquito larvicidal potential of Thai mushroom extracts. Form a huge mushroom diversity, numerous species still be under discovered and researched, then they could be subjected for developing as a potentially alternative larvicide in the mosquito controlling strategy in further.

ACKNOWLEDGEMENTS

The Thailand Research Fund, Office of the Higher Education Commission and Naresuan University (Ref N^{o} MRG5680019) financially supported this study.

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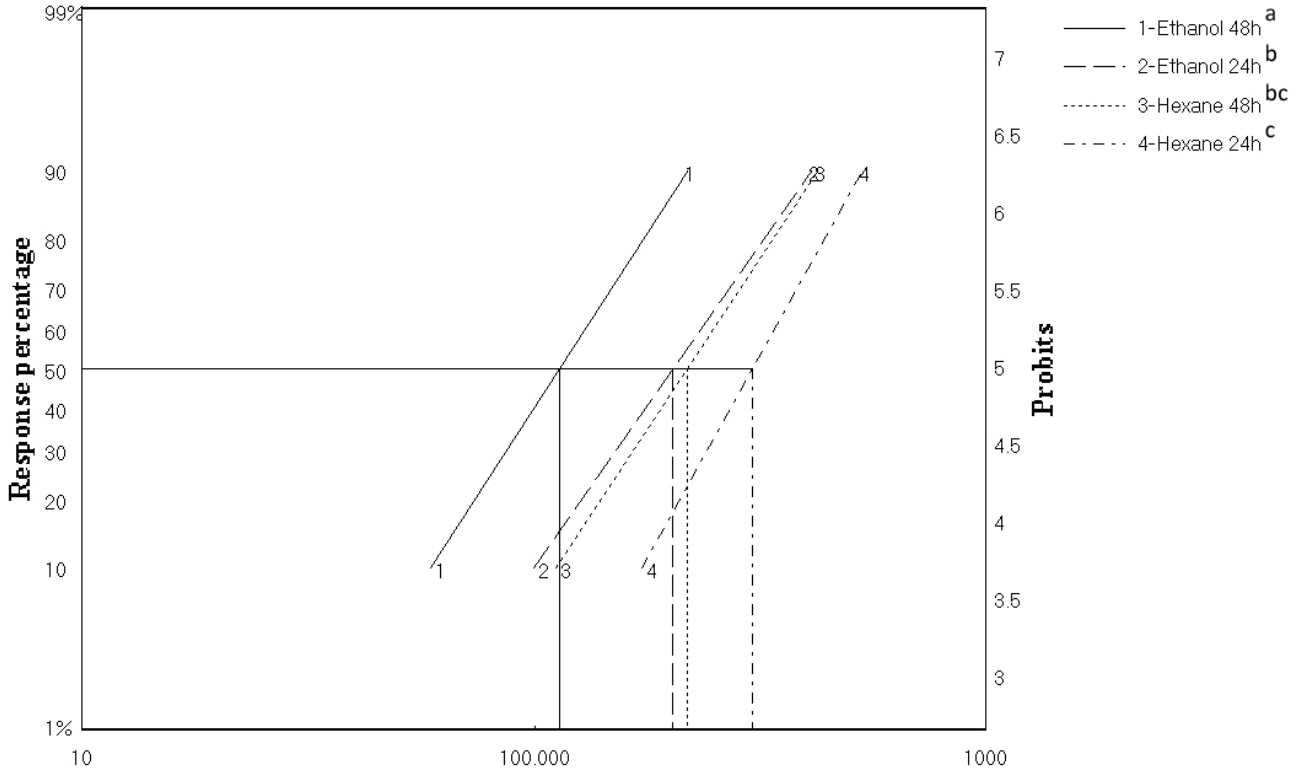
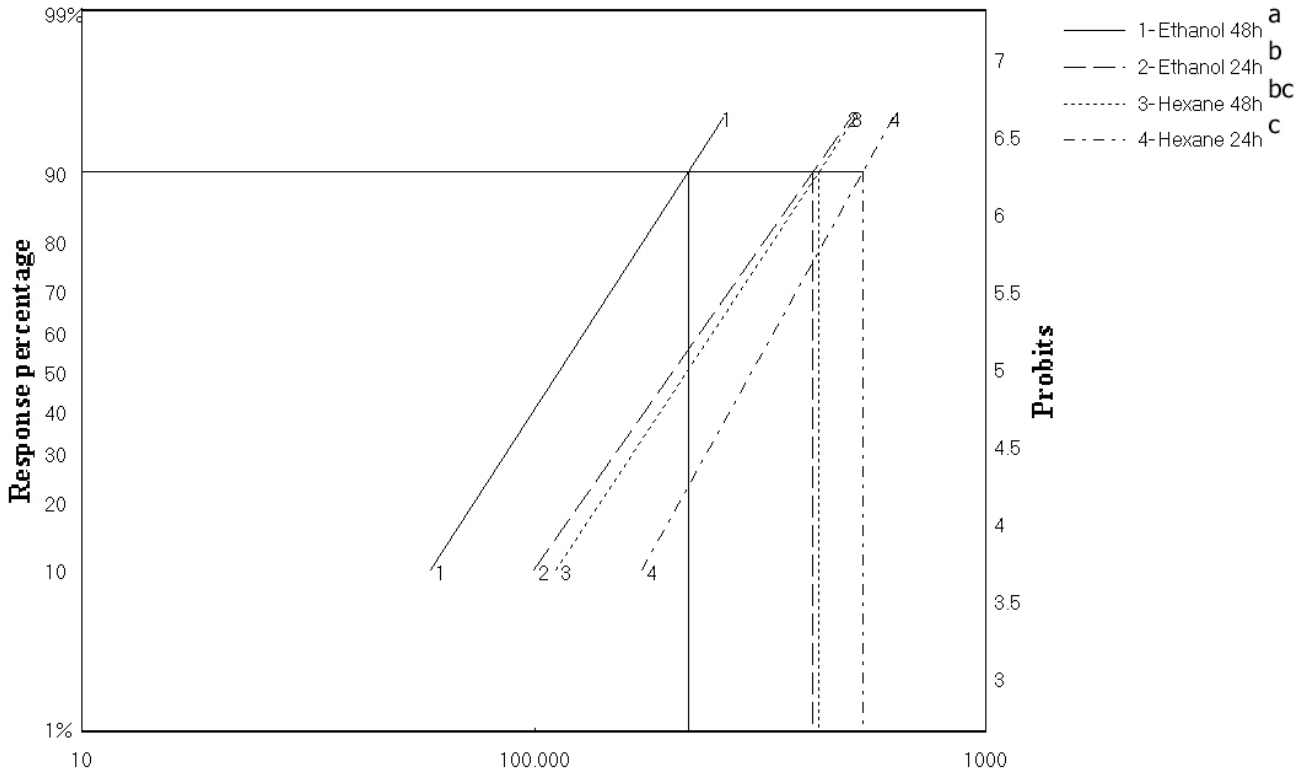
Fig 1-Graph showing the LC_{50} values of the ethanolic and hexane crude extracts of *Steccherinum* mushroom on the 3rd stage *Ae. aegypti* at 24- and 48-hours exposing time.



Statistically significant differences are indicated by different letter on the ethanolic or hexane extract groups (upper right)

Fig 2- Graph showing the LC_{90} values of the ethanolic and hexane crude extracts of *Steccherinum* mushroom on the 3rd stage *Ae. aegypti* at 24- and 48-hours exposing time.



Statistically significant differences are indicated by different letter on the ethanolic or hexane extract groups (upper right)

Table 1

Mushroom species and mortality rates of the 3rd instar larvae *Ae. aegypti* after screening with 1%w/v mushroom aqueous extract for 24 and 48 hours.

No.	Sample	Scientific name	Mortality rate (%)		No.	Sample	Scientific name	Mortality rate (%)	
			24h	48h				24h	48h
1	UMC 01	*Clitocybe* sp.	0	0	53	UMC 53	*Steccherinum* sp.	0	0
2	UMC 02	*Cantharellus cibarius*	0	0	54	UMC 54	*Fomes* sp.	0	0
3	UMC 03	*Lentinus polychrous*	0	0	55	UMC 55	*Ganoderma* sp.	0	0
4	UMC 04	*Abortiporus* sp.	0	0	56	UMC 56	*Ganoderma* sp.	0	0
5	UMC 05	*Schizophyllum commune*	0	0	57	UMC 57	*Fomes* sp.	0	0
6	UMC 06	*Lentinus* sp.	0	0	58	UMC 58	*Ganoderma lucidum*	0	0
7	UMC 07	*Lenzites vespacea*	0	0	59	UMC 59	*Ganoderma lucidum*	0	1
8	UMC 08	*Trametes hirsuta*	0	0	60	UMC 60	*Lentinus polychrous*	0	0
9	UMC 09	*Ganoderma* sp.	0	0	61	UMC 61	*Lentinus polychrous*	0	0
10	UMC 10	*Lentinus squarrosulus*	0	0	62	UMC 62	*Astraeus odoratus*	0	0
11	UMC 11	*Macrocybe crassa*	0	0	63	UMC 63	*Lentinus squarrosulus*	0	0
12	UMC 12	*Rigidoporus* sp.	0	0	64	UMC 64	*Amanita caesarea*	0	0
13	UMC 13	*Bjerkandera* sp.	0	0	65	UMC 65	*Russula rosacea*	0	0
14	UMC 14	*Ganoderma* sp.	0	0	66	UMC 66	*Amanita princeps*	0	0
15	UMC 15	*Pycnoporus sanguineus*	0	0	67	UMC 68	*Amanita princeps*	0	0
16	UMC 16	*Steccherinum* sp.	0	0	68	UMC 69	*Auricularia auricular*	0	0
17	UMC 17	*Ganoderma lucidum*	1	1	69	UMC 70	*Lentinus squarrosulus*	0	0
18	UMC 18	*Trametes* sp.	0	0	70	UMC 71	*Ganoderma applanatum*	0	0
19	UMC 19	*Trametes* sp.	0	0	71	UMC 72	*Polyporus* sp.	0	0
20	UMC 20	*Microporus xanthopus*	0	0	72	UMC 73	*Ganoderma applanatum*	0	0
21	UMC 21	*Podoscypha* sp.	0	0	73	UMC 74	*Pycnoporus sanguineus*	0	0
22	UMC 22	*Laccaria laccata*	0	0	74	UMC 75	*Ganoderma lucidum*	0	0
23	UMC 23	*Macrocybe crassa*	0	0	75	UMC 76	*Polyporus* sp.	0	2
24	UMC 24	*Chlorophyllum molybdites*	0	0	76	UMC 80	*Ganoderma* sp.	0	0
25	UMC 25	*Trametes* sp.	0	0	77	UMC 81	*Ganoderma* sp.	0	0
26	UMC 26	*Polyporus* sp.	0	0	78	UMC 82	*Fomes* sp.	0	0
27	UMC 27	*Trametes* sp.	0	0	79	UMC 83	*Ganoderma lucidum*	0	0
28	UMC 28	*Ganoderma lucidum*	0	0	80	UMC 84	*Ganoderma lucidum*	0	0
29	UMC 29	*Meruliopsis* sp.	0	0	81	UMC 85	*Microporus xanthopus*	0	0
30	UMC 30	*Lenzites* sp.	0	0	82	UMC 86	*Pycnoporus sanguineus*	0	0
31	UMC 31	*Stereum ostrea*	0	0	83	UMC 87	*Phaeolus* sp.	0	2
32	UMC 32	*Ganoderma* sp.	0	0	84	UMC 88	*Microporus xanthopus*	0	0
33	UMC 33	*Stereum* sp.	0	0	85	PHK 01	*Scleroderma polyrhizum*	0	0
34	UMC 34	*Lenzites elegans*	0	0	86	PHK 02	*Boletus chromapes*	0	0
35	UMC 35	*Ganoderma* sp.	0	0	87	PHK 03	*Lactarius hatsudake*	0	0
36	UMC 36	*Ganoderma lucidum*	0	0	88	PHK 04	*Boletellus emodensis*	0	0
37	UMC 37	*Trametes versicolor*	0	0	89	PHK 05	*Fomitopsis pinicola*	0	0
38	UMC 38	*Macrocybe crassa*	0	0	90	PHK 07	*Microporus* sp.	0	0
39	UMC 39	*Trametes* sp.	0	0	91	PHK 08	*Amanita verna*	0	0
40	UMC 40	*Stereum* sp.	0	0	92	PHK 09	*Boletus* sp.	0	0
41	UMC 41	*Trametes* sp.	0	0	93	PHK 13	*Laccaria laccata*	0	0
42	UMC 42	*Earliella* sp.	0	0	94	PHK 14	*Laccaria* sp.	0	0
43	UMC 43	*Stereum* sp.	0	0	95	PHK 17	*Termitomyces* sp.	0	0
44	UMC 44	*Ganoderma applanatum*	0	0	96	PHK 21	*Hygrocybe* sp.	0	0
45	UMC 45	*Ganoderma lucidum*	0	0	97	PHK 22	*Scleroderma sinnamariense*	0	0
46	UMC 46	*Earliella* sp.	0	0	98	PHK 23	*Armillaria* sp.	0	0
47	UMC 47	-*	0	0	99	PHK 24	*Polyporus* sp.	0	0
48	UMC 48	*Ganoderma lucidum*	0	0	100	PHK 25	*Stereum ostrea*	0	0
49	UMC 49	*Polyporus* sp.	0	0	101	PHK 26	*Russula* sp.	0	0
50	UMC 50	*Scytinopogon angulisporus*	0	0	102	PHK 27	*Thaeogyroporus porentosus*	70	70
51	UMC 51	*Ganoderma lucidum*	0	0	103	AMC 01	*Russula rosacea*	1	1
52	UMC 52	*Podoscypha* sp.	0	0	104	AMC 02	*Termitomyces* sp.	0	0

Table 1 (continued).

No.	Sample	Scientific name	Mortality rate (%)		No.	Sample	Scientific name	Mortality rate (%)	
			24h	48h				24h	48h
105	AMC 03	*Russula alboareolata*	0	0	125	CKW 05	-*	10	18
106	AMC 04	*Russula cyanoxantha*	0	0	126	CKW 06	-*	0	0
107	AMC 05	*Sentinus* sp.	0	0	127	STW 01	*Trametes* sp.	0	0
108	AMC 06	*Amanita vaginata*	0	0	128	STW 02	*Fomitopsis* sp.	0	0
109	AMC 07	*Russula densifolia*	1	1	129	STW 03	-*	0	0
110	AMC 08	*Amanita princeps*	0	0	130	MPW 01	*Cyathus striatus*	0	0
111	AMC 10	*Amanita hemibapha*	0	0	131	MPW 02	*Mycena* sp.	0	0
112	AMC 11	*Amanita princeps*	0	0	132	MPW 03	*Cyathus striatus*	0	1
113	AMC 12	*Russula* sp.	0	0	133	MPW 04	*Thelephora penicillata*	1	2
114	AMC 13	*Geastrum saccatum*	0	0	134	PW 01	-*	0	0
115	AMC 15	*Dictyophora indusiata*	0	0	135	PW 02	-*	0	0
116	AMC 16	*Tremella fuciformis*	0	0	136	PW 03	*Xylaria nigripes*	64	88
117	NU 01	*Chlorophyllum* sp.	10	18	137	GSW 01	*Scytinopogon angulisporus*	0	0
118	NU 02	*Chlorophyllum* sp.	0	0	138	GSW 02	*Ganoderma* sp.	0	0
119	NU 03	*Chlorophyllum molybdites*	1	1	139	GSW 03	*Daedaleopsis confragosa*	0	0
120	NU 04	*Schizophyllum commune*	0	0	140	GSW 04	-*	52	88
121	CKW 01	*Stereum hirsutum*	0	0	141	GSW 05	*Thelephora* sp.	0	0
122	CKW 02	*Fomitopsis pinicola*	0	0	142	GSW 06	*Ramaria* sp.	0	0
123	CKW 03	*Steccherinum* sp.	66	90	143	GSW 07	*Ganoderma* sp.	0	0
124	CKW 04	*Boletus* sp.	0	0					

* Unidentified samples

UMC and AMC samples are collected from several areas of Thailand including Chiang Mai, Krabi, Lampang, Nakhon Ratchasima, Nakhon Sawan, Pathumthani, Phichit, Phitsanulok, Phuket, Prachuap Khiri Khan, Sukhothai, Surat thani and Tak provinces.

PHK, NU, CKW, STW, MPW, PW and GSW samples are collected only from areas of Phitsanulok province.

Table 2

Larvicidal activities of the crude hexane and ethanolic *Steccherinum* extracts against the 3rd stage larvae *Ae. aegypti* after 24- and 48-hour exposure times.

Crude extract of *Steccherinum* (ppm)	24-hour					48-hour				
	% Mortality (Mean ± SE)	Larvicidal activity				% Mortality (Mean ± SE)	Larvicidal activity			
		Lethal Concentration with fiducial limits (ppm)		χ^2	Slope ±SE		Lethal Concentration with fiducial limits (ppm)		χ^2	Slope ±SE
		LC_{50}	LC_{90}				LC_{50}	LC_{90}		
Hexane extract										
100	1.00 ± 1.00	304.05	533.40	11.56	4.78 ± 0.40	8.00 ± 1.63	218.45	425.75	0.14	4.42 ± 0.49
200	21.00 ± 3.00	(238.72 - 373.70)	(484.29 - 871.89)			42.00 ± 2.58	(200.65 - 239.28)	(365.84 - 532.79)		
300	41.00 ± 2.52					74.00 ± 3.83				
400	67.00 ± 2.52					100				
500	95.00 ± 1.91					100				
Control	0					1.00 ± 1.00				
Ethanolic extract										
50	1.00 ± 1.00	203.30	412.72	10.49	4.17 ± 0.28	8.00 ± 1.63	114.12	219.71	7.10	4.51 ± 0.33
100	9.00 ± 2.52	(191.27 - 215.39)	(377.34 - 461.18)			37.00 ± 3.42	(105.85 - 122.25)	(200.92 - 245.29)		
150	25.00 ± 2.52					62.00 ± 4.76				
200	51.00 ± 4.43					91.00 ± 1.91				
250	72.00 ± 1.63					96.00 ± 1.63				
300	77.00 ± 1.91					100				
350	85.00 ± 1.91					100				
400	92.00 ± 1.63					100				
Control	0					0				

LC_{50} and LC_{90}: lethal concentration that causes 50% and 90% of mortality, SE: standard error, χ^2: chi-square, ppm: parts per million

Poster Presentation in "The 10th International Mycological Congress (IMC10)"

Scientific Theme : 7 Biotechnology and Applied Aspects
Session : 7.2 Biotechnological exploitation of basidiomycetes

Account ID IMC1080
Abstract ID ABS0537

Screening for larvicidal activity of Thai mushroom aqueous extracts against *Aedes aegypti* mosquito

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Because the adverse effects from using synthetic insecticides to control mosquito have been widely reported, bio-materials from living organisms have been considered as an alternative tool. Although various substances produced from plants and also fungi have been reported having mosquito larvicidal activities, most of them have a limitation of use. Therefore, it is of interest to explore new substances. Mushrooms have more than 14,000 species. Some can produce metabolite substances along with antibacterial, antiviral, antitumor, anti-inflammatory and antioxidant activities. The insecticidal activity however has been an under-researched area. In this study, 145 mushroom samples were collected from areas across Thailand and then extracted with distilled water (1% w/v). The mushroom extracts were screened for their larvicidal activity against *Aedes aegypti* mosquito following the WHO protocol. Twenty-five mosquito larvae were transferred into 100 mL of each mushroom extracts, and then the mortality was observed after 24 h of exposing time. Each experiment was replicated for 4 times. It was found that 8 of 145 samples, including PHK27, NU01, NU02, CKW03, CKW05, PW01, PW03 and GSW04 displayed the larvicidal efficacy ranging from 10 to 70% of larval mortality rates. According to the preliminary screening result, this was the first finding for mosquito larvicidal activity from mushroom extract in Thailand. It indicated the possibility to use mushroom as the bio-material for the mosquito control. However, further studies such as the extraction with other solvents, the larvicidal testing with more mosquito species and also the causes of larval death, should be done.

Keywords: mushroom, larvicidal activity, *Aedes aegypti*, aqueous extract, mosquito control

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SCREENING FOR LARVICIDAL ACTIVITY OF THAI MUSHROOM AQUEOUS EXTRACTS AGAINST *AEDES AEGYPTI* MOSQUITO

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INTRODUCTION

Temephos has wildly been used as larvicide to control the *Aedes aegypti*, the most important vector transmits dengue pathogen in many countries including Thailand[1]. Although it is an organophosphate that shows very low toxicity to human, the adverse effects from using synthetic insecticides to control mosquito have been widely reported especially the causing of insecticide resistant vectors[2]. Then, bio-materials from living organisms have been considered as an alternative tool. Mushrooms have more than 14,000 species. Some can produce metabolite substances along with antibacterial, antiviral, antitumor, anti-inflammatory and antioxidant activities[2,3,4,5,6]. The insecticidal activity however has been an under-researched area. There was only one study reported that a secondary metabolite, (oxiran-2-yl)methylpentanoate, extracted from *Cyptotrama asprata* mushroom can kill *Ae. aegypti* larvae with the LC_{50} and LC_{90} values of 1.50 and 1.90 ppm, respectively[7]. Then, it was interesting to screen for the larvicidal efficacy from mushrooms of Thailand.

MATERIALS & METHODS

145 mushroom samples were collected from areas across Thailand and then extracted with distilled water. The extracts were screened for larvicidal activity against a laboratory *Ae. aegypti* strain following the WHO protocol[8]. Briefly, 2 grams of each mushroom powder was suspended into 200 mL of distilled water and extracted by stirring with distilled water at 180 rpm for 24 hours, and then filtering through a fine net cloth, revealing 1 g% w/v of aqueous crude mushroom extract. 25 mosquito larvae were transferred into 200 mL of each mushroom extracts, and then the mortality was observed after 24-hour exposing time. Each experiment was replicated for 4 times.

RESULTS

From 145 mushroom extracts screening, 6 of all samples, including **PHK27**, **NU01**, **CKW03**, **CKW05**, **PW03** and **GSW04** displayed a promising larvicidal efficacy ranging from 10 to 70% of larval mortality rates. ***The highest activity was found from PHK27 sample with 70% mortality rate***, following with CKW03, PW03 and GSW04, with 66%, 64% and 52%, respectively. While, the intensely lower activities (10% mortality rate) were found from NU01 and CKW05. The other 139 samples did not showed the possibility to have the larvicidal activity. Zero to 4% mosquito mortality rates were found after 24-hour detection time. The morphological photographs of some mushroom samples are showed in Fig 1 and 2.

Fig 1. Photographs of the tested mosquito species and some mushrooms displaying a promising larvicidal activity

Fig 2. Photographs of some mushrooms indicating a negative result for mosquito larvicide screening

DISCUSSION

From the literature, it was the only one report from Kenya for the *Ae. aegypti* larvicidal activity from mushroom. A secondary metabolite extracted from *C. asprata* showed very high activity with LC_{50} of just 1.50 ppm[7]. Then, this was a second report for recording the mosquito larvicidal activity from mushroom extract. ***Although just 6 of total 145 samples were found to display the larvicidal activity, the positive outcome of this study indicated the possibility to use mushroom as the bio-material for the mosquito control***. However, further studies such as the testing with more mosquito species and also the causes of larval death, should be investigated.

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ACKNOWLEDGEMENT

This study was supported by The Thailand Research Fund, Office of the Higher Education Commission and Naresuan University. (TRF grant number: MRG5680019)