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Larvicidal and Adult Mosquito Vector Attractant Activity of *Tremella fuciformis* Berk Mushroom Extract on *Aedes aegypti* (L.) and *Culex sitiens* Wiedemann (Diptera: Culicidae)

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ABSTRACT

In this study, we assessed *Tremella fuciformis* Berk mushroom extract for larvicidal and adult mosquito attractant activity using *Aedes aegypti* (L.) and *Culex sitiens* Wiedemann (Diptera: Culicidae). Five concentrations of extract (120, 12, 1.2, 0.12, and 0.012 mg/L) for larvicidal tests and three concentrations of extract (100, 10, and 1 mg/L) for attraction testing of adult female mosquitoes were used. Results showed no larvicidal activity of *T. fuciformis* extract against *Ae. aegypti* larvae and only slight activity against *Cx. sitiens* larvae, while octenol, tested for comparison, exhibited well larvicidal at the highest concentration (120 mg/L) on *Cx. sitiens* (19.00 ± 1.00 of 20 killed) fair activity on *Ae. aegypti* (9.00 ± 1.00 of 20 killed). At 10 mg/L, the *T. fuciformis* extract attracted the most adults in both species of mosquito, followed by 100 and 1 mg/L, respectively, with *Ae. aegypti* responding better than *Cx. sitiens* at all concentrations. At all concentrations, octenol was a significantly ($p < 0.05$) better attractant than the *T. fuciformis* extract. As an eco-friendly way to increase the efficiency of mosquito traps, *T. fuciformis* extract shows potential.

INTRODUCTION

Mosquito-borne diseases are a major public health issue, especially in tropical and subtropical countries. They include Zika virus disease, malaria, dengue fever, Japanese encephalitis, chikungunya, and filariasis (Service, 2008; World Health Organization, 2016). Globally, more than 1 million people die from these diseases every year (World Health Organization, 2014). In Thailand, mosquito-borne diseases are one of the most important health concerns, requiring urgent resolution. According to Ministry of Public Health of Thailand reports, in 2016 dengue fever had the highest incidence rate at 58.79 cases per one hundred thousand people, followed by malaria, chikungunya, Japanese encephalitis, and filariasis (Ministry of Public Health, 2016). For control of mosquito borne diseases to reduce the risk of infection will focus mosquito population in the endemic area (Roiz *et al.*, 2012) in two ways including reducing the number of both the larval and adult stages of mosquitoes.

Currently, the most common and popular method for controlling mosquitoes is the use of chemical insecticides, but these have harmful effects on humans, animals, and the environment (Nkya *et al.*, 2013). Furthermore, mosquitoes have become resistant to some insecticides, for example, the larvicide temephos (Chaiphongpachara *et al.*, 2017). Thailand has reported mosquito resistance in many areas, resulting in difficulties in controlling the dengue vector population (Pimsamarn *et al.*, 2009).

Mosquito traps are useful tools to reduce mosquito populations (Okumu *et al.*, 2010). Currently, various odors are used for trap optimization, including octenol, carbon dioxide, and lactic acid. These are based on the olfactory attraction of female mosquitoes, which require proteins from the blood of humans or animals for egg production (Takken and Kline, 1989).

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Octenol (1-octen-3-ol) is a volatile organic compound found in the perspiration and breath of humans or animals. It has also been reported that octenol is found in some mushrooms and that it is toxic to insects (Inamdar and Bennett, 2014). Recently, studies have reported that some mushrooms have larvicidal activity including *Aspergillus flavus, Chrysosporium lobatum, Penicillium* spp., *Podospora* spp., *Xylaria nigripes, Chlorophyllum* spp., *Steccherinum* spp. and *Thaeogyroporus porentosus* (Matasyoh *et al.*, 2011; Mohanty and Prakash, 2009; Govindarajan *et al.*, 2005; Thongwat *et al.*, 2015). It is possible, therefore, that certain fungi could have both adult attractant and larvicidal properties, with potential as an alternative method of mosquito control.

We selected *Tremella fuciformis* Berk, an edible fungus that is popular with consumers in Thailand, for assay of larvicidal and adult mosquito attractant activity, and we tested this on *Aedes aegypti* (L.) (a dengue fever vector) and *Culex sitiens* Wiedemann (a filariasis and Japanese encephalitis vector), both major vectors in coastal habitats (Chaiphongpachara and Sumruayphol, 2017).

MATERIALS AND METHODS

Mushroom collection and extract preparation

T. fuciformis mushrooms were purchased during September 2016 from Talat Thai market, Pathum Thani province in Thailand (14°4’54.51”N, 100°37’53.06”E), and then taken to the College of Allied Health Sciences, Suan Sunandha University, Samut Songkhram province education center. They were then washed thoroughly and their identity confirmed by morphological characteristics using taxonomic identification keys (Largent and Thiers, 1977).

The mushrooms were dried in the shade at ambient temperature (~37°C), ground into powder using a blender, and infused in 95% ethanol at room temperature for 48 hours. The mushroom extract was filtrated through Whatman filter paper and dried in a rotary evaporator. The yield of crude extract was recorded, dissolved in methanol for adult mosquito bioassay and distilled water for larvicidal bioassay, and stored at −20°C before investigations in the laboratory.

Mosquito collection and rearing

Eggs of *Ae. aegypti* (WHO susceptible strain Bora-Bora) were supplied by the Faculty of Tropical Medicine, Mahidol University, while *Cx. sitiens* larvae, a coastal vector, and were collected using a standard mosquito dipper from water with a salinity level of more than 0.05 ppt in coastal areas of Samut Songkhram province, Thailand, 200 m inland from the sea. The eggs and larvae were placed separately in trays (25 × 30 × 5 cm) containing filtered water and rearing were carried out at 25 ± 2°C with 10:14 h light: dark cycle with 0.1 g food provided daily. The pupae were transferred to cages (30 × 30 × 30 cm) for adult mosquito emergence.

Larvicidal bioassay

Larvicidal bioassay of the *T. fuciformis* extract was conducted following WHO protocols (WHO, 2016). Five concentrations of mushroom extract were prepared (120, 12, 1.2, 0.12, and 0.012 mg/L) using filtered water in six-ounce glass containers, to which were added 20 late third instar or early fourth instar larvae. After 24 h the number of dead larvae was counted and recorded. All experiments were done in triplicate. For the control group, we used a mixture of filtered water and extraction solvent. Octenol (1-Octanol EMPLURA® from Merck KGaA COMPANY, Darmstadt, Germany) at the same concentrations was used as a benchmark for testing against larvae and ault mosquitoes.

Adult mosquitoes attractant bioassay

Mushroom extract at concentrations of 100, 10, and 1 mg/L following Cilek *et al*. (2011) were used in adult mosquito attraction tests, conducted using a modified Y-tube, according to the method of Geier *et al*. (Geier and Boeckh, 1999). At each concentration level, 20 healthy adult female mosquitoes were tested, with four replicates at each concentration. In this bioassay, one arm of the Y-tube provides a flow of the test substance (the *T. fuciformis* extract) and the other is the control; when all 20 mosquitoes had flown down either one arm or the other, we counted and recorded the results.

Statistical analyses

Numbers of dead larvae and numbers of mosquito attracted were expressed as mean ± S.D. (standard deviation). The T-test was used for comparisons, with differences regarded as significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

Efficacy of *T. fuciformis* extract as a larvicide

At all concentrations, *T. fuciformis* extract showed no larvicidal activity against *Ae. aegypti*, and only a slight (non-significant) effect against *Cx. sitiens*. (Table 1), while Octenol at the highest concentration (120 mg/L) killed nearly all the *Cx sitiens* larvae (19.00 ± 1.00 of 20) and around half the *Ae. aegypti* larvae (9.00 ± 1.00 of 20). In the control groups, none of the *Ae. aegypti* larvae died, and only very few of the *Cx. sitiens* larvae died.

Efficacy of *T. fuciformis* extract for attracting adult mosquitoes

At all concentrations, octenol was a significantly ($p \leq 0.05$) better attractant for *Ae. aegypti* and *Cx. sitiens* than *T. fuciformis* extract (Table 2). Of the three concentrations of attractants tested, the intermediate concentration (10 mg/L) was most effective for both species, followed by 100 and 1 mg/L. *Ae. aegypti* adults were more attracted than *Cx. sitiens* in all tests.

We studied the larvicidal effect and adult female mosquito attractant activity of *T. fuciformis* mushroom extract on *Ae. aegypti* and *Cx. sitiens*. There are reports of the toxicity of octenol to small insects (Inamdar and Bennett, 2014) consistent with the results of this study; here, the highest concentration (120 mg/L) killed almost all *Cx. sitiens* larvae (19.00 ± 1.00 of 20) and about half the *Ae. aegypti* larvae (9.00 ± 1.00 of 20). However, the *T. fuciformis* mushroom extract was not effective against *Ae. aegypti* larvae and only very slightly effective on *Cx. sitiens* larvae and our results indicate that it is unlikely that *T. fuciformis* extracts as prepared in this study could be used to control mosquito larvae. This result is consistent with that of Thongwat *et al*. (2015), who screened 143 species of mushroom against *Ae. aegypti* larvae in the laboratory and found larvicidal activity in only *Thaeogyroporus porentosus*, *Xylaria nigripes, Chlorophyllum* spp., *Steccherinum* spp., and two unidentified species.

Table 1: Mean number of dead larvae of *Ae. aegypti* and *Cx. sitiens.*

Concentrations, (mg/L)	n	Number of dead mosquito larvae			
		Ae. aegypti		*Cx. sitiens*	
		T. fuciformis extract Mean ± S.D.	Octenol Mean ± S.D.	*T. fuciformis* extract Mean ± S.D.	Octenol Mean ± S.D.
120	20	ND[a]	9.00 ± 1.00[b]	1.00 ± 0.00[a]	19.00 ± 1.00[b]
12	20	ND[a]	0.33 ± 0.58[b]	1.00 ± 1.00a	9.33 ± 0.33[b]
1.2	20	ND[a]	0.33 ± 0.58[b]	0.67 ± 0.58[a]	6.00 ± 1.00[b]
0.12	20	ND[a]	1.00 ± 1.00[b]	0.67 ± 0.58[a]	5.00 ± 2.65[b]
0.012	20	ND[a]	0.33 ± 0.58[b]	1.00 ± 0.00[a]	1.00 ± 1.00[a]
Control group	20	ND	ND	0.67 ± 0.58	1.33 ± 0.33

ND = No deaths, and the same letter in the row between *T. fuciformis* extract and octenol in each concentration is not significantly different at $p \leq 0.05$.

Table 2: Mean number of responding adult *Ae. aegypti* and *Cx. sitiens.*

Concentrations, (mg/L)	n	Attracted mosquito			
		Ae. aegypti		*Cx. sitiens*	
		T. fuciformis extract Mean ± S.D.	Octenol Mean ± S.D.	*T. fuciformis* extract Mean ± S.D.	Octenol Mean ± S.D.
100	20	11.33 ± 0.57[a]	15.00 ± 1.00[b]	5.66 ± 0.57[a]	11.33 ± 0.33[b]
10	20	13.33 ± 0.57[a]	17.33 ± 0.57[b]	7.00 ± 0.00[a]	12.00 ± 0.00[b]
1	20	12.33 ± 0.57[a]	16.00 ± 0.00[b]	6.66 ± 0.57[a]	11.66 ± 0.57[b]

The same letter in the row between *T. fuciformis* extract and octenol in each concentration is not significantly different at $p \leq 0.05$.

Apart from octanol's toxicity to aquatic insects, it is a substance that can be used to attract mosquitoes. Octenol is a powerful attractant for female mosquitoes looking for a blood meal, and several studies have reported that octenol is contained in mushrooms, including *T. fuciformis* (Mau *et al.*, 1997). The *T. fuciformis* extract used in our study attracted more than 60% of *Ae. aegypti* at a concentration of 10 mg/L, though at the same concentration the rate of attraction for *Cx. sitiens* was poorer at around 35%. These results are consistent with previous research that found that octenol at a concentration of 10 mg/L was best for attracting mosquitoes, with *Aedes* spp. more attracted than *Culex* spp. (Cilek *et al.*, 2011). In this study, the *T. fuciformis* extract produced a significantly lower attraction response than octenol at all concentrations ($p \leq 0.05$), a difference that may arise from the concentration of bioactive substances in the *T. fuciformis* mushroom.

CONCLUSION

This experiment is the first to reveal the ability of *T. fuciformis* mushroom extract to control mosquitoes. Although the performance of *T. fuciformis* extract is not equal to that of octanol, it was effective in attracting more than half of all mosquitoes in the laboratory tests and could be an eco-friendly way to increase the efficiency of mosquito traps.

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