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Effectiveness of *Pleurotus eryngii* (King Oyster Mushroom) Extract for Killing Larvae and Attracting Adult Mosquito Vectors in Samut Songkhram Province of Thailand

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Abstract

In this study, we assessed the efficiency of *Pleurotus eryngii* mushroom extract for killing larvae and attracting adult mosquito vectors (*Aedes aegypti* and *Culex sitiens*) in the Samut Songkhram province of Thailand. Five extract concentrations (120, 12, 1.2, 0.12 and 0.012 mg/L) were used for larvicidal tests, while 3 concentrations (100, 10, and 1 mg/L) were examined for adult mosquito attraction. The larvicidal results showed that *P. eryngii* extract did not kill *Ae. aegypti* larvae, while the extract had minimal effect on *Cx. sitiens* larvae except at 1.2 mg/L. *P. eryngii* extract most attracted adult *Ae. aegypti* and *Cx. sitiens* mosquitoes at 10 mg/L, followed by 1 and 100 mg/L, respectively. Further, statistical analysis revealed a significantly different number of mosquitoes that responded to *P. eryngii* extract and octenol. This research demonstrated that this mushroom extract could be developed to attract mosquitoes, although only one concentration (10 mg/L) attracted more than half of all *Ae. aegypti* adults.

Keywords: Killing larvae; Attracting mosquito; *Pleurotus eryngii*; Mosquito vector

Introduction

Mosquitoes carry many human diseases that are major public health issues around the world, especially in tropical and sub-tropical countries [1]. Mosquito-borne diseases include dengue fever, chikungunya, malaria, filariasis, West Nile virus, yellow fever, Zika virus, and Japanese encephalitis, and according to World Health Organization (WHO) estimates, more than one million people die from these diseases every year [2,3]. Thailand has epidemic areas of mosquito-borne diseases, primarily because it is located in a tropical area. In 2017, the Thai Ministry of Public Health reported a total of 65,000 patients suffered from mosquito-borne diseases [4]. This number indicates that these disease are a major problem and should be resolved urgently.

There are two ways to control mosquito vectors, namely reducing the number of larvae or adult mosquitoes. Temephos, a chemical that is not toxic to humans or animals, is widely used on mosquito larvae in all parts of Thailand [5]. However, long-term use of these chemical causes vector resistance. Indeed, temephos resistance has been reported in many areas of Thailand, and this phenomenon makes it difficult to control the mosquito population [6]. Further, temephos use is usually successful only for *Aedes* spp., especially *Ae. aegypti*, since its spawning habits are in household water containers [7]. Other mosquito species, such as *Culex* spp. and *Anopheles* spp., spawn in large water resources in nature. It is unlikely that temephos can or will be used for larvae control.

Octenol (1-octen-3-ol) is a volatile substance that emanates from human sweat and breath. Female mosquitoes use this scent to find bait to suck blood to obtain the blood protein that is required for egg development [8]. Currently, many types of mosquito traps use octenol to increase the efficiency of mosquito attraction. One such trap, the mosquito magnet [9], exhibits high performance, but its expense makes it unpopular in Thailand. It has been reported that octenol can found in mushrooms and it is reportedly toxic to insect larvae [10]. Therefore, it is possible that edible mushrooms in Thailand could be developed for use as larvicides and as substances that attract mosquitoes to replace the expensive synthetic substances on the market.

In this study, we examined the popular and edible king oyster mushroom (*Pleurotus eryngii*), reported to contain octenol as a component [11], to study its efficacy and efficiency for killing larvae and attracting adult mosquito vectors in the laboratory. This study was conducted using prominent mosquito vectors in coastal areas of Samut Songkhram province, including *Ae. aegypti*, a dengue fever vector, and *Cx. sitiens*, a filariasis and Japanese encephalitis vector [12]. We hypothesised that this extract could reduce mosquito numbers in this area and consequently further reduce the number of new patients with mosquito-borne diseases.

Materials and Methods

Mushroom collection

P. eryngii was collected from the Talat Thai market, Khlong Luang district, Pathum Thani province, the center of trade in agricultural products in Thailand (14°4'54.51"N 100°37'53.06"E) in September 2016 (Figure 1). Mushroom samples were transported to the College of Allied Health Sciences (Suan Sunandha University, Samut Songkhram province). Specimens were identified morphologically using the mushroom taxonomic keys [13-16].

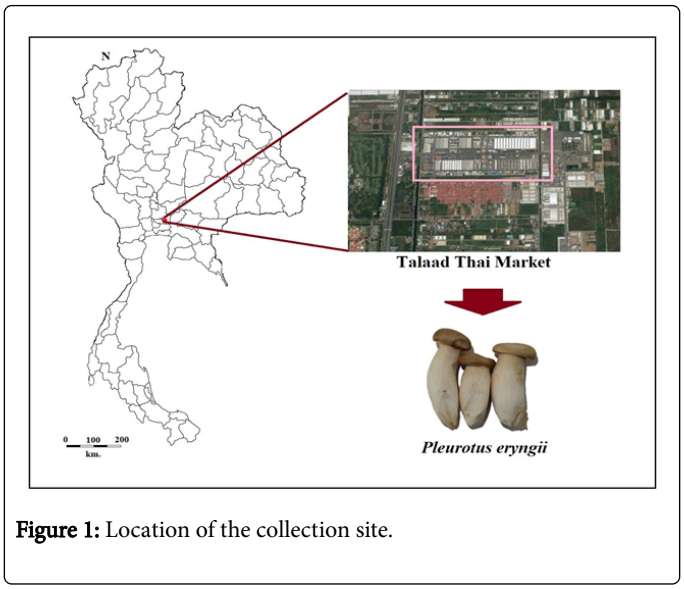


Figure 1: Location of the collection site.

Mushroom extraction

P. eryngii samples were cut into small pieces and fermented with 95% ethanol at room temperature for 48 h. The mushroom extract was filtered and evaporated under reduced pressure at 50°C using a rotary evaporator to obtain the crude extract. The crude extract was then dried by freeze dehydration at -85°C for approximately 24 h. The yields of *P. eryngii* extract were weighed, recorded, dissolved in ethanol and stored at -20°C before laboratory testing.

Mosquito rearing

Ae. aegypti (Bola Bola strain, F171) were obtained from the Faculty of Tropical Medicine (Mahidol University). *Cx. sitiens* were collected from the coastal area of Samut Songkhram province, which is 200 m away from the sea (13°23'31.57"N 100°1'59.36"E), using a standard mosquito dipper in a water source with a salinity level of more than 0.05 parts per thousand (ppt). *Ae. aegypti* eggs and *Cx. sitiens* larvae were placed in separate trays (25 × 30 × 5 cm) that contained filtered water; 0.1 g dog food was provided daily. When eggs or larvae reached the pupae stage, they were transferred to cages (30 × 30 × 30 cm) to facilitate adult emergence.

Bioassay for *P. eryngii* extract larvicidal effect

Larvicidal tests were modified according to WHO [17]. Five concentrations of extract were used (120, 12, 1.2, 0.12 and 0.012 mg/L). Filtered water containing the appropriate concentration of the substance was added to 6 ounce glasses and 20 late third instar or early forth instar larvae were added. After 24 h, the number of dead larvae was recorded. For the control group, only solvent was added to the filtered water in the test glass. Each concentration was repeated 3 times.

Bioassay to test *P. eryngii* extract adult mosquito attraction

Three concentrations of mushroom extract were used to test adult mosquito attraction (100, 10, and 1 mg/L). We conducted this bioassay using a modified Y tube, according to Geier et al. [18], using 20 mosquitoes per concentration. Mosquitoes were released into the tube, which has 2 sides: the left contained *P. eryngii* extract and the right contained solvent. The number of mosquitoes that flew to each end was counted and recorded. This experiment was repeated 3 times for each concentration.

Data analysis

The number of dead larvae or attracted mosquitoes are expressed as the mean ± standard deviation (S.D.). Statistical comparison for adult attraction between *P. eryngii* extract and octenol was performed using a two-tailed t-test. $p < 0.05$ was considered statistically significant.

Results

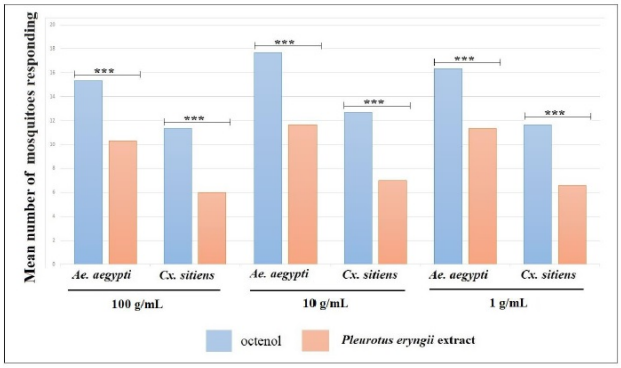
P. eryngii extract had no effect on *Ae. aegypti* larvae, while the extract minimally killed *Cx. sitiens* larvae at all concentrations except 1.2 mg/L (Table 1). The highest octenol concentration (120 mg/L) killed less *Ae. aegypti* than *Cx. sitiens* larvae (9.33 ± 4.93 compared to 19.67 ± 0.58, respectively). For the *P. eryngii* control group, no larval death was observed, while the octenol control group caused slight larval death for *Ae. aegypti* (0.33 ± 0.58; Table 1) and *Cx. sitiens* (1.33 ± 0.58; Table 1).

Concentration (mg/L)	n	Mean ± S.D. number of dead larvae			
		P. eryngii		Octenol	
		Ae. aegypti	*Cx. sitiens*	*Ae. Aegypti*	*Cx. sitiens*
120	20	0.00 ± 0.00	1.33 ± 1.53	9.33 ± 4.93	19.67 ± 0.58
12	20	0.00 ± 0.00	1.67 ± 0.58	0.33 ± 0.58	9.67 ± 3.06
1.2	20	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
0.12	20	0.00 ± 0.00	0.67 ± 0.58	1.00 ± 1.00	5.00 ± 2.65
0.012	20	0.00 ± 0.00	0.67 ± 0.58	0.33 ± 0.58	5.00 ± 2.65

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

The 10 g/mL concentration of *P. eryngii* extract attracted the most adult *Ae. aegypti* and *Cx. sitiens* mosquitoes, followed by 1 and 100 g/mL concentrations, respectively. The 10 g/mL octenol concentration also attracted the most adults for both species (Figure 2). At all concentrations, *P. eryngii* extract attracted more *Ae. aegypti* than *Cx. sitiens* adults (Figure 2). However, compared to all octenol

concentrations, *P. eryngii* extract attracted significantly fewer adult mosquitoes for both species (Figure 2).



*** = $p<0.05$.

Figure 2: Statistical comparison between the mean numbers of mosquitoes attracted by *P. eryngii* extract or octenol.

Discussion

In this study, we investigated the effectiveness of *P. eryngii* mushroom extract for killing larvae and attracting adult mosquitoes. We found that *P. eryngii* extract was ineffective in killing *Ae. aegypti* larvae, but it appeared mildly larvicidal toward *Cx. sitiens* at certain concentrations. However, the minimal *Cx. sitiens* larval death may be due to our use of larvae from nature (rather than a laboratory strain), since the number of larval deaths in the extract and control groups were similar. Thus, *P. eryngii* extract is not suitable to kill mosquito larvae in the field. This finding is consistent with previous research from Thongwat et al. [19] who screened the ability of 143 mushroom species in Thailand to kill mosquito larvae and found them to be almost entirely ineffective. There were only 6 mushroom species with potential larvicidal activity, all of which were wild, expensive and seasonal. Thus, it appears quite difficult to further develop a product to control mosquito larvae based on mushroom extracts.

For adult mosquito control, mosquito traps are an effective control option with substantial interest worldwide. Currently, mosquito trap development focuses on optimising traps to avoid polluting the environment [20]. The use of odor in traps is one of the most important tools used to increase the effectiveness of mosquito control. Octenol is a powerful mosquito attractant [21], but it is expensive. It attracts mosquitoes because it emanates from humans and animals that would serve as prey for blood-feeding female mosquitoes [22]. Several studies have documented that mushrooms, including *P. eryngii*, contain octenol [11]. The results in this study showed that 10 g/mL *P. eryngii* extract most effectively attracted adult mosquitoes. This finding is consistent with other octenol research that demonstrated higher octenol concentrations did not necessarily attract mosquitoes more efficaciously than lower concentrations [8]. Since the insect odor system has a specific odor range, it varies according to mosquito species.

Our research found that *P. eryngii* extract attracted *Ae. aegypti* better than *Cx. sitiens* at all concentrations. This finding is similar to previous research by Cilek et al. [23] that reported that octanol attracted *Ae. albopictus* better than *Cx. quinquefasciatus*. However, at all concentrations *P. eryngii* extract attracted significantly fewer adult mosquitoes than octenol. Nevertheless, at 10 g/mL, the extract attracted more than half of all *Ae. aegypti* mosquitoes (58.33%).

Conclusion

These finding is the first to demonstrate that *P. eryngii* extract could possibly be developed to further enhance mosquito lure efficiency. Although the performance of *P. eryngii* extract is different with octanol but extract was effective in attracting more than half of all mosquitoes in the laboratory. The advantages of this extract are an inexpensive and eco-friendly way to increase the efficiency of mosquito traps.

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