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Effect of *Pleurotus djamor* (Rumph. ex Fr.) Boedijn Mushroom Extract on Larval and Adult *Aedes aegypti* (L.) and *Culex sitiens* Wiedemann (Diptera: Culicidae) Mosquitoes

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ABSTRACT

In this study, we assessed the efficacy of *Pleurotus djamor* (Rumph. ex Fr.) Boedijn mushroom extracts on *Aedes aegypti* (L.) and *Culex sitiens* Wiedemann mosquitoes (Diptera: Culicidae) larvae and adults. The larvicidal bioassay was conducted according to WHO standardised sensitivity tests. While, we conducted adult mosquito attractant bioassay using a Y-tube. Five concentrations of *P. djamor* extract for larvicidal tests were used (120, 12, 1.2, 0.12 and 0.012 mg/L), while 3 concentrations (10^{-4}, 10^{-5} and 10^{-6} g/mL) were used to examine adult mosquito attraction. In the results, *P. djamor* did not kill *Ae. aegypti* larvae, but it had a minimal larvicidal effect on *Cx. sitiens*. *P. djamor* extract most effectively attracted adult female *Ae. aegypti* and *Cx. sitiens* at 10^{-5} g/mL. However, the extract attracted significantly fewer adults of both species compared to octenol. Still, this mushroom extract attracted 58.30% of adult *Ae. aegypti* at the 10^{-5}g /mL concentration. This finding suggests that *P. djamor* may be developed to further enhance mosquito lure efficiency.

KEY WORDS: *Pleurotus djamor*, *Aedes aegypti*, *Culex sitiens*, larvicidal effect, mosquito attractant effect.

1. INTRODUCTION

Mosquitoes carry a variety of human diseases, including dengue fever, malaria, filariasis and Japanese encephalitis. These diseases are major public health issues in global tropical and subtropical regions (Damapong, 2016; Service, 2008; World Health Organization, 2014). The World Health Organization (WHO) estimates that every year more than 1 million people die, and hundreds of millions of mosquito-borne disease cases occur worldwide (Service, 2008; World Health Organization, 2016). Thailand is one epidemic area of mosquito-borne diseases. In 2017, the Thai Ministry of Public Health reported 65,000 cases of mosquito-borne diseases (Ministry of Public Health, 2017). Thus, mosquito-borne diseases are a major problem that should be resolved urgently. Control of mosquito-borne diseases to reduce the risk of infection should focus on the mosquito population in areas with epidemic outbreaks (Roiz, 2012), including reducing larval and adult mosquito numbers.

The most common method for controlling mosquitoes is the use of chemical insecticides that may be harmful and toxic to humans, animals and the environment. Further, long-term use of these chemicals may cause resistance. Thailand has reported mosquito resistance to the insecticide temephos, a chemical used for larvicidal control of *Aedes* spp. This resistance causes difficulties in controlling the dengue vector population (Jirakanjanakit, 2007).

Mosquito traps are tools used to reduce mosquito populations in nature, including Centers for Disease Control and Prevention (CDC) mosquito light traps and mosquito magnets. Currently, there is interest in using scent to optimise traps. Several compounds, including octenol, carbon dioxide and lactic acid, have been examined to control numbers of female mosquitoes that require human or animal blood for egg production and development (Service, 2008). Octenol (1-octen-3-ol) is a volatile organic compound found in the sweat and breath of humans or animals that attracts female mosquitoes (Beavers, 2004). It is also a natural product derived from linoleic acid, which was first isolated from Matsutake pine mushrooms and was found in other mushrooms (Xu, 2015). In addition, it has been reported that octenol is toxic to *Drosophila* larval metamorphosis (Yin, 2015). Therefore, it is possible that some mushrooms may be used as an alternative method to control larval and adult mosquito vectors.

In this study, we investigated *Pleurotus djamor* (Rumph. ex Fr.) as a commercially available mushroom species and reported source of octenol (Zawirska, 2009). We examined the ability of *P. djamor* extract to kill larvae and attract adult female *Aedes aegypti* (L.) (a dengue fever vector) and *Culex sitiens* Wiedemann (a filariasis and Japanese encephalitis vector) mosquitoes. The results from this study are of great benefit for the discovery of an alternative substance for mosquito vector control to further reduce mosquito-borne disease cases.

2. MATERIALS AND METHODS

Mushroom collection and identification: *P. djamor* was collected from the Talat Thai market, Klong Luang Distinct, Pathum Thani province in Thailand (14°4'54.51"N 100°37'53.06"E) in September 2016 (Figure.1) and sent to the College of Allied Health Sciences, Suan Sunandha Rajabhat University, Samut Songkhram Provincial Education Center. *P. djamor* samples were identified morphologically to confirm their identity according to mushroom taxonomic keys (Largent and Thiers, 1977).

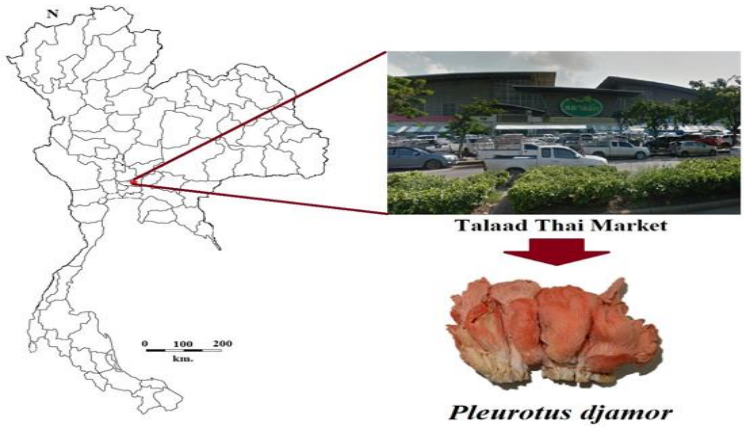

Figure.1. Location of the collection site

Mushroom extraction: After collection and identification, *P. djamor* samples were cleaned, air-dried under a shed at environmental temperatures, ground into powder using a blender and fermented with 95% ethanol at room temperature for 48 h. The *P. djamor* extract was filtered using Whatman No. 1 filter paper and evaporated using a rotary evaporator. Yields of crude extract were weighed, recorded, dissolved in distilled water (for larvicidal bioassay) and in methanol (for adult mosquito attractant bioassay), and stored at -20°C before testing in the laboratory.

Mosquito rearing: Eggs of the Bora Bora strain of *Ae. aegypti* (classified as a WHO susceptible strain) were obtained from the Department of Medical Entomology, Faculty of Tropical Medicine, Mahidol University, while *Cx. sitiens* larvae were collected from water sources in coastal areas of Samut Songkhram province (Thailand) using a standard mosquito dipper. *Ae. aegypti* eggs and *Cx. sitiens* larva were placed in separate coded plastic trays (25 x 30 x 5 cm) that contained filtered water at 25 ± 2°C with a 10:14 hour light:dark cycle, and provided 0.1 g dog food daily. Pupae were transferred to cages (30 x 30 x 30 cm) to facilitate adult emergence.

Larvicidal bioassay: The larvicidal bioassay was conducted according to WHO standardised sensitivity tests (World Health Organization, 2016). Five concentrations of mushroom extract (120, 12, 1.2, 0.12 and 0.012 mg/L) were used. Each concentration and an untreated control group were repeated 3 times with 20 late third instar or early fourth instar larvae per replicate in a 500 mL glass beaker. After 24 h, the number of dead larvae were counted and recorded. For the control group, we mixed filtered water with the extraction solvent in the glass beaker.

Adult mosquito attractant bioassay: Three mushroom extract concentrations (10^{-4}, 10^{-5} and 10^{-6} g/mL) were used to test for adult mosquito attraction. We conducted this bioassay using a Y-tube (Geier and Boeckh, 1999). Twenty healthy adult female mosquitoes per concentration were released into the tube. Each tube has 2 intersections; the left side contained *P. djamor* extract, while the right side contained extraction solvent. When all mosquitoes flew to one end of the tube, we counted and recorded the number; 3 replicates for each concentration were performed.

Statistical analysis: The number of dead larvae and attracted mosquitoes are expressed as mean ± standard deviation (S.D.). The statistical comparison between *P. djamor* and ocetenol treatments were performed using a two-tailed *t*-test for each concentration (p <0.05 was considered statistically significant).

3. RESULTS AND DISCUSSION

Larvicidal efficacy of *P. djamor* extract: *P. djamor* extract did not affect *Ae. aegypti* larvae, while it minimally killed *Cx. sitiens* larvae at all concentrations (Table.1). The highest octenol concentration (120 mg/L) killed almost all *Cx. sitiens* larvae (19.67 ± 0.58), more than *Ae. aegypti* larvae (9.33 ± 4.93). For the control group, no *Ae. aegypti* larvae were killed, but some *Cx. sitiens* larvae were susceptible.

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

Concentrations (mg/L)	n	*P. djamor*		Octenol	
		Ae. aegypti	*Cx. sitiens*	*Ae. aegypti*	*Cx. sitiens*
120	20	ND	4.00±0.00	9.33±4.93	19.67±0.58
12	20	ND	5.25±2.65	0.33±0.58	9.67±3.06
1.2	20	ND	5.00±1.73	ND	6.00±2.00
0.12	20	ND	4.00±2.00	1.00±1.00	5.00±2.65
0.012	20	ND	4.00±2.65	0.33±0.58	5.00±2.65
Control group	20	ND	0.67±0.58	ND	0.33±0.58

ND = No larvae death

Adult mosquito attraction efficacy of *P. djamor* extract: A concentration of 10^{-5}g/mL crude *P. djamor* extract mL attracted the most adult female *Ae. aegypti* and *Cx. sitiens* (Table.2). Female *Ae. aegypti* were more attracted to this extract compared to *Cx. sitiens* females at all concentrations. Statistical comparison between *P. djamor* extract and octenol showed no difference at all concentrations (Figure.2).

Table.2. Mean number of adult *Ae. aegypti* and *Cx. sitiens* mosquitoes attracted to *P. djamor* extract or octenol

Concentration (g/mL)	n	*P. djamor*		Octenol	
		Ae. aegypti	*Cx. sitiens*	*Ae. aegypti*	*Cx. sitiens*
10^{-4}	20	10.33±0.57	6.00±1.00	15.33±0.57	11.33±0.57
10^{-5}	20	11.66±0.57	7.33±0.57	17.33±0.57	12.66±0.33
10^{-6}	20	11.00±0.00	6.33±1.15	16.33±0.57	11.66±0.33

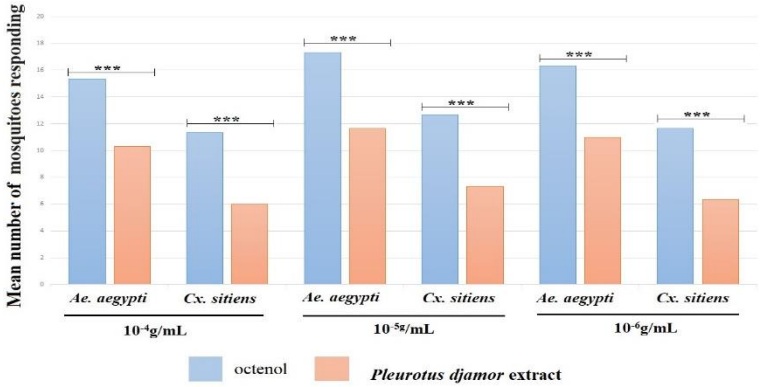

Figure.2. Statistical comparison between the mean numbers of mosquitoes attracted by *P. djamor* extract or octenol

*** = $p<0.05$

In this study, we examined the larvicidal effect and adult female mosquito attractant ability of *P. djamor* extracts for *Ae. aegypti* and *Cx. sitiens*. Octenol was reported to be toxic to small insects (Yin, 2015). Our results showed that the highest octenol concentration (120 mg/L) killed almost all *Cx. sitiens* larvae (19.67±0.58) but only half of the *Ae. aegypti* larvae (9.33±4.93). Comparatively, *P. djamor* extract caused less *Cx. sitiens* larval death than ocetenol and no *Ae. aegypti* death. These results indicate that it would be difficult to use *P. djamor* extract to control mosquito larvae numbers. This finding is consistent with Thongwat (2015), a study that screened 143 mushroom species against *Ae. aegypti* larvae in the laboratory and found larvicidal activity in only approximately 4% of the tested mushrooms, namely *Thaeogyroporus porentosus*, *Xylaria nigripes*, *Chlorophyllum* spp., *Steccherinum* spp. and two unidentified species (Thongwat, 2015).

Octenol is a powerful scent used to attract blood-sucking female mosquitoes. Several studies reported that mushrooms, including *P. djamor*, contained octenol (Zawirska, 2009). Here, *P. djamor* extract attracted 58.30% of adult female *Ae. aegypti*, but only 36.65% of *Cx. sitiens* females at the 10^{-5}g /mL concentration. This effect is consistent with previous research that found 10^{-5} g/mL octenol effectively attracted mosquitoes (Guha, 2014). It has also been reported that octenol is highly effective in *Aedes* mosquitoes, but has little effect on *Culex* spp. However, at all tested concentrations, we noted a significant difference in the number of *Ae. aegypti* and *Cx. sitiens* mosquitoes that responded to the *P. djamor* extract and octenol.

4. CONCLUSIONS

Our experiment is the first to reveal the ability of *P. djamor* mushrooms to attract adult female mosquitoes. Although the attractant ability of *P. djamor* extract is less than octenol, it still attracted more than half of the tested *Ae. aegypti* females. This extract could be an inexpensive and eco-friendly alternative to increase the efficiency of mosquito traps for vector control.

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