Pak. J. Biotechnol. Vol 15 (3) 641-645 (2018)
www.pjbt.org
ISSN Print: 1812-1837
ISSN Online: 2312-7791

LARVICIDAL AND ADULT ATTRACTANT EFFICIENCY OF THE EDIBLE MUSHROOM *PLEUROTUS PULMONARIUS* ON *AEDES AEGYPTI* AND *CULEX SITIENS* (DIPTERA, CULICIDAE) MOSQUITOES

Tanawat Chaiphongpachara*, Siriluck Jittrabiab and Duangporn Nacapunchai

College of Allied Health Science, Suan Sunandha Rajabhat University, Samut Songkhram 75000, Thailand. E-mail: *tanawat.ch@ssru.ac.th

Article received 24.5.2018, Revised 3.7.2018, Accepted 15.7.2018

ABSTRACT

Mosquito-borne diseases, including malaria, filariasis, Japan encephalitis, chikungunya, dengue fever, yellow fever and other viral diseases, are major public health issues worldwide, especially in tropical and sub-tropical areas. The commercially available edible mushroom *Pleurotus pulmonarius* (Fr.) (Indian oyster) has been reported to contain octenol. Thus, we assessed the larvicidal and adult female attractant activity of *Aedes aegypti (L.)* and *Culex sitiens Wiedemann* of this mushroom. We examined 5 *P. pulmonarius* extract concentrations for larvicidal tests (120, 12, 1.2, 0.12 and 0.012 mg/L) and 3 concentrations for adult mosquito attraction (100, 10, and 1 mg/L). The results showed that *P. pulmonarius* extract did not kill *Ae. aegypti* larvae but had a minimal larvicial effect on *Cx. sitiens*. While *P. djamor* extract attracted adult female *Ae. aegypti* and *Cx. sitiens* mosquitoes, especially at the 10 mg/L concentration, but no significantly mosquitoes of both species were attracted by the extract compared to octenol. However, this mushroom extract attracted more than 50% of *Ae. aegypti* adults.

Keywords: larvicidal, adult mosquito attractant efficiency, *Pleurotus pulmonarius*, *Aedes aegypti*, *Culex sitiens*

INTRODUCTION

Mosquito-borne diseases, including malaria, filariasis, Japanese encephalitis (JE), chikungunya, dengue fever, yellow fever and other viral diseases, are major public health issues worldwide, especially in tropical and sub-tropical areas [Service, 2008]. According to World Health Organization (WHO) estimates, these diseases threaten the health of more than 1 million people worldwide [World Health Organization, 2014], including Thailand [Ministry of Public Health, 2014]. In Thailand, dengue fever and malaria are particularly major problems [Ministry of Public Health, 2016, Chaiphongpachara et al., 2017]. In 2017, the Thai Ministry of Public Health reported that more than 65,000 patients contracted mosquito-borne diseases [Ministry of Public Health, 2017].

There are more than 3,500 mosquito species [Service, 2008]. Male mosquitoes feed only on flower nectar, but female mosquitoes feed on animal and human blood and require a blood meal to produce eggs; blood sucking transmits pathogens to humans [Killick-Kendrick, 1996]. The control programs of mosquito-borne diseases should focus on managing mosquito populations to reduce the risk of infection [Norbert, 2010, Roiz et al., 2012]. Temephos, an organophosphate larvicide, is widely used to reduce larval *Aedes aegypti*, a prominent dengue vector. It effectively controls mosquito larvae in water containers around the house [Chaiphongpachara et al., 2017]. However, long-term use of this chemical causes vector resistance and Thailand has reported *Ae. aegypti* resistance to temephos in many areas. This phenomenon makes it difficult to control the mosquito population [Jirakanjanakit et al., 2007]. Mosquito traps are widely used to reduce adult mosquito numbers [Hock, 2004]. Currently, many mosquito traps, including magnet traps and the CDC light trap, use substances such as octenol and carbon dioxide to increase the efficiency of mosquito attraction [Vezenegho et al., 2014]. However, these traps are expensive and thus not popular in Thailand despite their effectiveness. Octenol (1-Octen-3-ol) is a volatile substance found in human breath and sweat [Xu et al., 2015]. It has the ability to attract biting insects, including biting midges, tsetse flies, tabanids and mosquitoes [Grant & Dickens, 2011]. It has been reported that octenol is usually found in mushrooms [Dijkstra, 1976] and it is toxic to insect larvae [Yin et al., 2015] . Recently, several mushrooms, including *Aspergillus flavus*, *Chrysosporium lobatum*, *Penicillium* spp., *Podospora* spp., *Xylaria nigripes*, *Chlorophyllum* spp., *Steccherinum* spp. and *Thaeogyroporus porentosus*, were reported to exhibit mosquito larvicidal activity [Matasyoh et al., 2011, Mohanty & Prakash, 2009, Govindarajan et al., 2005, Thongwat et al., 2015] Therefore, it is possible that some mushrooms in Thailand could be used to kill larvae and attract adult mosquitoes as an alternative method to control vectors.

Pleurotus pulmonarius (Fr.) or Indian oyster is a commercially available mushroom species that was reported to contain octenol [Belinky et al., 1994]. We assessed the ability of this mushroom to kill mosquito larvae and attract adult female *Aedes aegypti (L.)* and *Culex sitiens Wiedemann*, two important vectors in Thailand [Chaiphongpachara & Sumruayphol, 2017]. The results of this study could be of great benefit for the discovery of a mushroom extract to further reduce cases of mosquito-borne diseases.

MATERIALS AND METHODS

Collection and extraction of *P. pulmonarius:* *P. pulmonarius* was collected from the Talat Thai market Klong Luang Distinct, Pathum Thani province in Thailand (14°4'54.51"N 100°37' 53.0 6"E) in September 2016 (Figure 1). The mushroom samples were identified at the College of Allied Health Sciences (Suan Sunandha University, Samut Songkhram province), education center using mushroom taxonomic keys [Largent & Thiers, 1977; Largent et al, 1977; Stuntz, 1977; Largent, 1986; Largent & Baroni, 1988]. After mushroom identification, 100 g fresh *P. pulmona rius* were cleaned, dried under a shed at environmental temperatures, ground in a blender and fermented with 95% ethanol at room temperature for 48 hours. The *P. pulmonarius* extract was filtered with Whatman No. 1 filter paper and evaporated using a rotary evaporator. The yields of crude extract were weighed, recorded, dissolved in distilled water for Larvicidal bioassay and in ethanol for adult mosquito attractant bioassay.

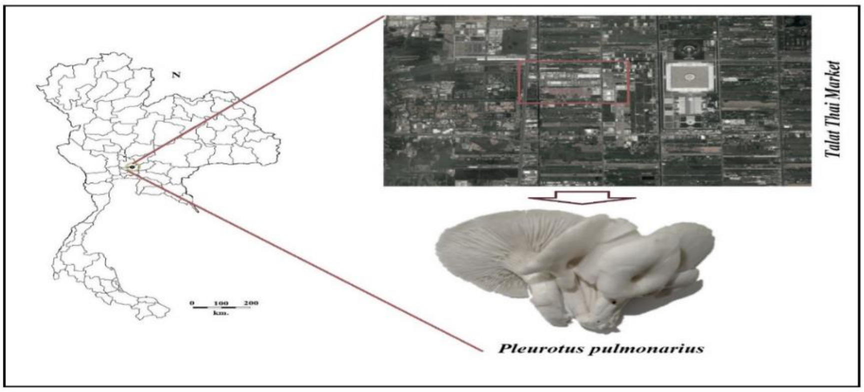


Figure 1: Location of the collection site.

Mosquito rearing: Eggs of the Bora Bora strain of *Ae. aegypti* were obtained from the Faculty of Tropical Medicine (Mahidol University), while *Cx. sitiens* larvae were collected in September 2016 from water sources in coastal areas of Samut Songkhram province (13°23'31.57"N 100°1'59.3 6"E) using a standard mosquito dipper. After that *Ae. aegypti* and *Cx. sitiens* larvae were reared in 700 mL of filtered water in coded plastic trays (25 x 30 x 5 cm) with 0.1 g dog food per day. The mosquito colonies were maintained at 25 ± 2°C with a 10:14 h light:dark cycle. When larvae became pupae, they were transferred to mesh-covered cages (30 × 30 ×30 cm) to facilitate adult emergence.

Larvicidal bioassay: Larvicidal activity of *P. pulmonarius* extract was performed according to WHO standards [WHO, 2016] using 5 extract concentrations (120, 12, 1.2, 0.12 and 0.012 mg/ L). Crude extracts of known concentration were added to 250 mL filtered water in 6-ounce glasses and 20 late third instar or early fourth instar larvae were added. For the control group, we used filtered water with the extraction solvent (ethanol). The number of dead larvae were counted and recorded after 24 hours for each concentration; 3 replicates were performed for each concentration.

Adult female mosquito attractant bioassay: We examined adult mosquito attraction using a Y-tube, based on a protocol modified from Geier and Boeckh [1999]. Three concentrations of the *P. pulmonarius* extract (100, 10, and 1 mg/L) were used to examine adult female *Ae. aegypti* and *Cx. sitiens* attraction. Twenty adult female mosquitoes per concentration were released into the Y-tube; the left side contained *P. pulmonarius* extract, while the right side contained ethanol (the extraction solvent). Once all mosquitoes flew to a tube end, we counted and recorded the number. We performed 3 replicates for each concentration.

Statistical analysis: The number of dead larvae or attracted mosquitoes are presented as mean ± standard deviation (S.D.). While statistical comparison of attraction between *P. pulmonarius* extract and octenol was performed using a two-tailed *t*-test that $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Efficacy of *P. pulmonarius* extract to kill mosquito larvae: *P. pulmonarius* extract did not kill *Ae. aegypti* larvae, but it did cause some *Cx. sitiens* larvae death (Table 1). The highest octenol concentration (120 mg/L) killed almost all *Cx. sitiens* larvae (19.50±0.50), but only approximately half *Ae. aegypti* larvae (9.50±2.00; Table 1). The control treatment did not kill any *Ae. aegypti* larvae, but it did cause minimal *Cx. sitiens* larval death.

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

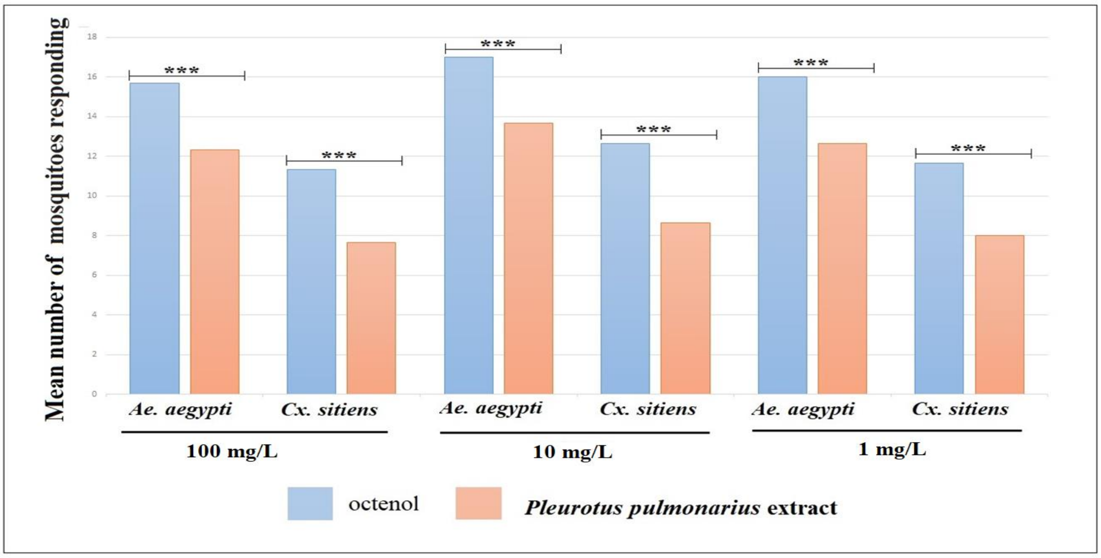
Concentration (mg/L)	n	Mean ± S.D. of dead larvae			
		P. pulmonarius		Octenol	
		Ae. aegypti	*Cx. sitiens*	*Ae. aegypti*	*Cx. sitiens*
120	20	ND	4.50±0.50	9.50±2.00	19.50±0.50
12	20	ND	4.00±1.00	0.33±0.58	9.67±3.06
1.2	20	ND	4.50±2.00	1.00±1.00	6.00±2.00
0.12	20	ND	4.00±2.65	1.00±1.00	5.00±2.65
0.012	20	ND	3.00±1.73	0.33±0.58	5.00±2.65
Control group	20	ND	0.33±0.58	ND	0.67±0.58

ND = No larvae death

Adult female mosquito attraction efficacy of *P. pulmonarius* extract: *P. pulmonarius* extract attracted more *Ae. aegypti* than *Cx. sitiens* females at all concentrations (Table 2). The 10 mg/L concentration attracted the most *Ae. aegypti* and *Cx. sitiens* females (Table 2). Statistical analysis revealed that for both mosquito species, *P. pulmonarius* extract was not significantly different female mosquitoes compared to octenol (Figure 2).

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

Concentration (mg/L)	n	Mean ± S.D. of mosquito attracted			
		P. pulmonarius		Octenol	
		Ae. aegypti	*Cx. sitiens*	*Ae. aegypti*	*Cx. sitiens*
100	20	12.33±0.57	7.66±0.57	15.67±0.33	11.33±0.57
10	20	13.66±0.57	8.66±0.57	17.00±0.33	12.66±0.57
1	20	12.66±1.00	8.00±0.57	16.00±0.00	11.66±0.57



*** = $p<0.05$

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

Octenol is reportedly toxic to small insects, including mosquitoes [Yin et al., 2015, Inamdar & Bennett, 2014], a finding that is consistent with the results of this experiment. The highest octenol concentration (120 mg/L) killed almost all *Cx. sitiens* larvae (97.50%), but less than half of *Ae. aegypti* larvae (47.50%). However, *P. pulmonarius* extract was slightly effective in killing *Cx. sitiens* larvae but did not kill any *Ae. Aegypti* larvae. These results indicate that *P. pulmonarius* is not useful to control mosquito larvae, a conclusion consistent with Thongwat et al., [2015]. This study screened 143 mushroom species in Thailand against *Ae. aegypti* larvae and found larvicidal activity for only 6 species.

Octenol was reportedly toxic to small insects, it can be used to attract hematophagous insects, including blood-sucking mosquitoes. *P.*

pulmonarius extract attracted 68.30% of *Ae. aegypti* at the 10 mg/L concentration, but only 43.30% of *Cx. sitiens*. These results were consistent with previous research that found 10 mg/L octenol concentration best attracted *Ae*. *albopictus* mosquitoes in the laboratory [Guha et al., 2014]. Octenol was reported to be highly effective against *Aedes* spp., but relatively ineffective against *Culex* spp. in the field [Kline & Mann, 1998]. At all tested concentrations, we noted a significant difference between the numbers of *Ae*. *aegypti* and *Cx*. *sitiens* that responded to *P*. *pulmonarius* extract.

CONCLUSION

Although *P*. *pulmonarius* lacks effective larvicidal activity, this study is the first to reveal the ability of *P*. *pulmonarius* to attract adult female mosquitoes. While *P*. *pulmonarius* extract was less effective in attracting female mosquitoes compared to octenol but it still attracted more than half of the tested *Ae*. *aegypti* females. Previous studies have found that octenol attracted more mosquitoes when used in combination with carbon dioxide [Takken & Kline, 1989]. Thus, future studies could examine *P*. *pulmonarius* extract with carbon dioxide. This extract could represent an alternative inexpensive and eco-friendly means to increase the efficiency of mosquito traps to control vectors.

Acknowledgements

We would like to thank and acknowledge the College of Allied Health Science, Suan Sunandha Rajabhat University, Thailand, for their kind support of our research.

REFERENCES

Belinky, P. A., S. Masaphy, D. Levanon, Y. Hadar and C.G. Dosoretz, Effect of medium composition on 1-octen-3-ol formation in submerged cultures of *Pleurotus pulmonarius*. Appl. Microbiol. Biotechnol. 40(5): 629–633 (1994).

Chaiphongpachara, T., S. Pimsuka, W. Saisanan Na Ayudhaya and W. Wassanasompong, The application of geographic information system in dengue haemorrhagic fever risk assessment in Samut Songkhram province, Thailand. Int. J. of GEOMATE. 12: 53–60 (2017).

Chaiphongpachara, T. and S. Sumruayphol, Species diversity and distribution of mosquito vectors in coastal habitats of Samut Songkhram province, Thailand. Trop Biomed. 34(3): 524–532 (2017).

Chaiphongpachara, T., and L. Moolrat, Insecticide resistance of temephos on *Aedes aegypti* as dengue vector in Samut Songkhram, Thailand. Ann. Trop. Med. Public Health 10: 1439–1442 (2017).

Dijkstra, F.Y., Studies on Mushroom Flavours 3. Some compounds in Fresh,Canned and Dried Edible Mushrooms. Zeitschrift Fuer Lebensmittel -Untersuchung Und -Forschung 160(3): 401–405 (1976).

Geier, M. and J. Boeckh, A new Y-tube olfactometer for mosquitoes to measure the attractiveness of host odours. Entomol. Exp. Appl. 92(1): 9–19 (1999).

Govindarajan, M., A. Jebanesan and D. Reetha, Larvicidal effect of extracellular secondary metabolites of different fungi against the mosquito, *Culex quinquefasciatus* Say. *T*rop. Biomed. 22(1): 1–3 (2005).

Grant, A.J. and J.C. Dickens, Functional characterization of the octenol receptor neuron on the maxillary palps of the yellow fever mosquito. *Aedes aegypti*. *PLoS ONE*. 6(6) (2011).

Guha, L., T. Seenivasagan, S.T. Iqbal, O.P. Agrawal and B.D. Parashar, Behavioral and electrophysiological responses of *Aedes albopictus* to certain acids and alcohols present in human skin emanations. Parasitol Res. 113(10): 3781–3787 (2014).

Hock, J.W., CDC Miniature Light Trap. Experts in Insect Sampling (2004).

Inamdar, A.A. and J.W. Bennett, A common fungal volatile organic compound induces a nitric oxide mediated inflammatory response in *Drosophila melanogaster*. Sci. Rep. 4 (2014).

Jirakanjanakit, N., S. Saengtharatip, P. Rongnoparut, S. Duchon, C. Bellec and S. Yoksan, Trend of Temephos Resistance in *Aedes* (Stegomyia) Mosquitoes in Thailand During 2003–2005. Environ. Entomol. 36(3): 506–511 (2007)

Killick-Kendrick, R., Medical entomology for students (1996).

Kline, D.L. and M.O. Mann, Evaluation of butanone, carbon dioxide, and 1-octen-3-OL as attractants for mosquitoes associated with north central Florida bay and cypress swamps. J. Am. Mosq. Control Assoc. 14(3): 289–297 (1998).

Largent, D.L., How to identify mushrooms to genus I: macroscopic features. Eureka: Eureka Printing (1986).

Largent, D.L. and T.J. Baroni, How to identify mushrooms to genus VI: modern genera. Eureka: Eureka Printing (1988).

Largent, D.L., Johnson, D. and R. Watling, How to identify mushrooms to genus III: microscopic features. Eureka: Eureka Printing (1977).

Largent, D.L. and H.D. Thiers, How to identify mushrooms to genus II: field identification of genera. Eureka: Eureka Printing (1977).

Matasyoh, J.C., B. Dittrich, A. Schueffler and H. Laatsch, Larvicidal activity of metabolites

from the endophytic *Podospora* sp. against the malaria vector *Anopheles gambiae*. Parasitol. Res. 108(3): 561–566 (2011).
Ministry of Public Health, Thailand. Annual report. http://www.thaivbd.org/n/home; Accessed 15 Dec 2017 (2014).
Ministry of Public Health, Thailand. Annual report. http://www.thaivbd.org/n/home; Accessed 15 Dec 2017 (2016).
Ministry of Public Health, Thailand. Annual report. http://www.thaivbd.org/n/home; Accessed 15 Dec 2017 (2017).
Mohanty, S.S. and S. Prakash, Effects of culture media on larvicidal property of secondary metabolites of mosquito pathogenic fungus *Chrysosporium lobatum* (Moniliales: Moniliaceae). Acta Trop. 109(1): 50–54 (2009).
Norbert, B., Mosquitoes and Their Control. Springer (2010).
Roiz, D., Roussel, M., Munõz, J., Ruiz, S., Soriguer, R. and J. Figuerola, Efficacy of mosquito traps for collecting potential west nile mosquito vectors in a natural mediterranean wetland. Am. J. Trop. Med. Hyg. 86(4): 642–648 (2012).
Service, M., Medical entomology for students, fourth edition (2008).
Stuntz, D.E., How to identify mushrooms to genus IV: key to families and genera. Eureka: Eureka Printing (1977).
Takken, W. and D.L. Kline, Carbon dioxide and 1-octen-3-ol as mosquito attractants. J. Am. Mosq. Control Assoc. 5(3): 311–316 (1989).
Thongwat, D., U. Pimolsri, and P. Somboon, Screening for mosquito larvicidal activity of thai mushroom extracts with special reference to *Steccherinum* sp against *Aedes aegypti* (l.) (diptera: culicidae). Southeast Asian J. Trop. Med. Public Health 46(4): 586–595 (2015).
Vezenegho, S.B., A. Adde, P. Gaborit, R. Carinci, J. Issaly, V. Pommier De Santi, I. Dusfour, S. Briolant, and R. Girod, Mosquito magnet® liberty plus trap baited with octenol confirmed best candidate for *Anopheles* surveillance and proved promising in predicting risk of malaria transmission in French Guiana. Malar. J. 13 (2014).
WHO. Monitoring and Managing Insecticide Resistance in *Aedes* Mosquito Populations (2016).
WHO. WHO | Vector-borne diseases. World Health Organization (2014).
Xu, P., F. Zhu, G.K. Buss and W.S. Leal, 1-Octen-3-ol - the attractant that repels. *F1000 Research* 4: 156. (2015).
Yin, G., S. Padhi, S. Lee, R. Hung, G. Zhao and J.W. Bennett, Effects of Three Volatile Oxylipins on Colony Development in Two Species of Fungi and on 1-octen-3-ol Larval Metamorphosis. *Curr. Microbiol.* 71(3): 347–356 (2015).