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Efficacies of five edible mushroom extracts as odor baits for resting boxes to attract mosquito vectors: A field study in Samut Songkhram Province, Thailand

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Abstract. Effective trapping of adult mosquitoes in the wild can reduce the spread of deadly human pathogens, such as *Plasmodium* species causing malaria. The efficacy of this strategy depends on the capacity of the trap to attract and retain insects, and specific odorants such as octenol in mushrooms are strong attractants for mosquitoes. In this study, we assessed the efficiency of a resting box baited with five different extracts from local edible mushrooms, *Pleurotus ostreatus*, *Thaeogyroporus porentosus*, *Volvariella volvacea*, *Pleurotus sajor-caju*, and *Lentinus edodes*, for attracting mosquito vectors in Samut Songkhram Province, Thailand. Extracts were used in identical "resting box" at 50, 100, and 200 mg/mL per box. Compared to the unbaited resting box (control), only traps containing 200 mg/mL of *L. edodes* extract captured significantly more mosquitoes (16.00 ± 3.61 vs. 7.00 ± 1.00 per resting box per night, $p<0.05$). Attraction efficacy did not increase progressively with tested amount for most extracts. These findings indicate that *L. edodes* extract can be used as an inexpensive, non-toxic, and locally sourced attractant to increase the efficacy of mosquito control.

INTRODUCTION

Diseases carried by mosquitoes are responsible for about a million deaths per year worldwide (Tolle, 2009). The World Health Organization (2016) estimates that nearly half of the world's population lives within high-risk zones for mosquito-borne diseases, mainly tropical and subtropical areas. In Thailand, mosquito-borne diseases such as malaria, dengue fever, Japanese encephalitis, chikungunya, and filariasis (Chareonviriyaphap *et al.*, 2013; Chaiphongpachara *et al.*, 2017; Chaiphongpachara & Moolrat, 2017) remain important public health problems (Brusich *et al.*, 2015). Most mosquito-borne infectious diseases are caused by nocturnal mosquitoes, such as *Culex* and *Anopheles* spp. (Service, 1996). Reducing the number of mosquitoes in an endemic area using traps with specific attractant characteristics is one effective method to control disease transmission (Sriwichai *et al.*, 2015).

Numerous efforts have been made to develop mosquito traps and baits to suit different areas and attract disease-carrying mosquito species. Mosquito traps are designed based on certain behaviours, such as egg laying, resting, and biting (Mathew *et al.*, 2013; Kreppel *et al.*, 2015), and known attractant qualities (e.g., color, scent). The

Ovitrap, a device used to control mosquitoes based on egg laying, is inexpensive, easy to use, and especially effective against *Aedes* mosquito populations (Lenhart *et al.*, 2005). Resting traps are designed to capture blood-engorged mosquitoes based on the principle that female mosquitoes must find a suitable area to rest after blood feeding for digestion (Panella *et al.*, 2011). Additionally, both female and male nocturnal mosquitoes hide during the day to avoid sunlight. Several studies have investigated adding a lure to further increase the attractant efficacy of these traps. For instance, Kweka *et al.* (2010) used wet black cotton cloth moistened with fresh cattle urine in a resting box while Logita *et al.* (2016) used odour bait in a sticky trap to capture *Anopheles arabiensis*. Both studies demonstrated that odours can enhance capture efficacy. Host attractants, such as expired CO_2, and biting behaviour of vectors are the primary factors used in the design of the Centers for Diseases Control (CDC) miniature light trap, the Mosquito Magnet® Patriot Mosquito Trap, and the BG-Sentinel Trap. Generally, female mosquitoes show blood-sucking behaviour for egg production (Ryelandt *et al.*, 2011). Female mosquitoes will choose a bait for several reasons, including the scent of constituents such as carbon dioxide, lactic acid, and 1-octen-3-ol (octenol), which are important guides for insect sensory perception (Spitzen *et al.*, 2008). Octenol is a volatile organic compound found in human breath (Bernier *et al.*, 1999) that attracts mosquitoes to humans (Hung *et al.*, 2014; Xu *et al.*, 2015). Octenol is also found in edible mushrooms (Dijkstra, 1976; Hung *et al.*, 2014), suggesting that these fungi may be used as an inexpensive and environmentally sustainable odour bait to enhance trap efficiency.

In the current study, we selected five edible and commercially available mushroom species grown in Thailand that are reported to contain octenol: *Pleurotus ostreatus* (Jacq.) P. Kumm. (Dijkstra, 1976; Beltran-Garcia *et al.*, 1997), *Volvariella volvacea* (Bull.) Singer (Mau *et al.*, 1997), *Pleurotus sajor-caju* (Fr.) Singer (*Gogavekar et al.*, 2014) and *Lentinus edodes* (Berk.) Singer (Akakabe *et al.*, 2005) (Figure 1). *Thaeogyroporus porentosus* (Berk. & Broome) McNabb has been used in other experiments as it has a pungent odour and is widely available in Thailand (although it has not been used specifically for the study of octenol as an attractant). In the present study, ground mushrooms were incubated in ethanol to obtain crude extracts for resting box bait, and used at 50, 100, and 200 mg/mL per box in field tests. Each extract was tested in the same type of resting box constructed from black aluminium and lined internally with black cloth to reduce reflected light (Figure 2). Samut Songkhram Province in coastal Thailand was chosen as the study area due to the presence of many mosquito species that act as disease vectors, including *Culex sitiens* and *Anopheles epiroticus* (Chaiphongpachara & Sumruayphol, 2017). The results of this study may prove useful for the development of more effective baits to control mosquito vector.

MATERIALS AND METHODS

Mushroom samples

The five mushrooms tested, *Pleurotus ostreatus* (Jacq.) P. Kumm, *Thaeogyroporus porentosus* (Berk. & Broome) McNabb, *Volvariella volvacea* (Bull.) Singer, *Pleurotus sajor-caju* (Fr.) Singer, and *Lentinus edodes* (Berk.) Singer, were collected from local markets and mushroom farms in Ayutthaya Province, Thailand, from September to October 2016. Collected specimens were brought to the College of Allied Health Sciences, Suan Sunandha University, Samut Songkhram Province. All specimens were identified morphologically based on macro-characteristics using the mushroom taxonomic keys (Largent & Thiers, 1977; Largent *et al.*, 1977; Stuntz, 1977; Largent, 1986; Largent & Baroni, 1988).

Mushroom extraction

Samples were dried at 50°C in a hot oven and ground to a coarse powder. Each dried powder was macerated with 95% ethanol at room temperature for 48 hours. The filtrate of each extract was further concentrated to dryness under reduced pressure at 50°C. The

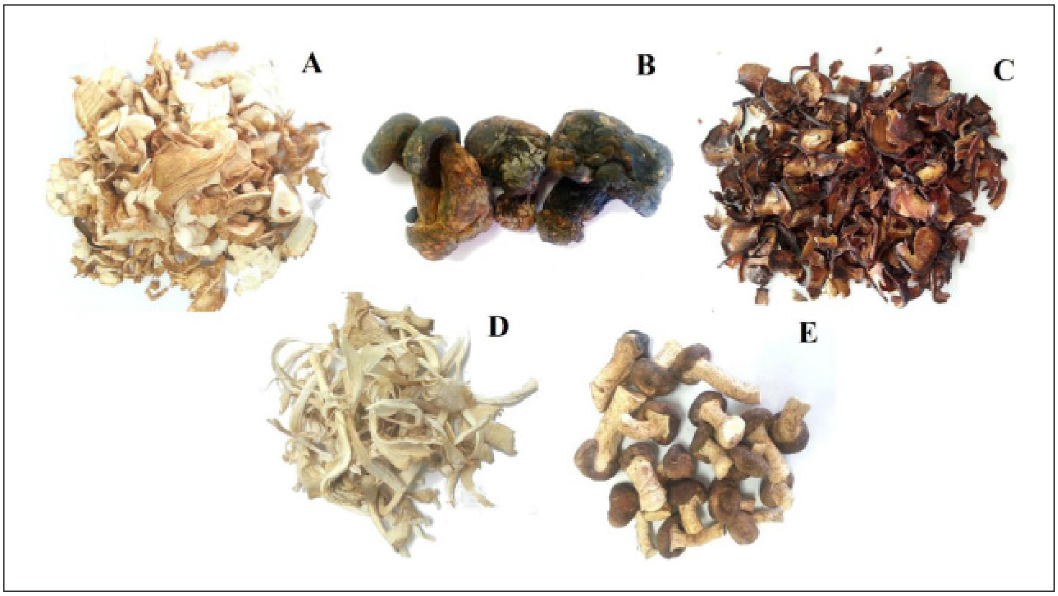

Figure 1. Dried samples of the mushroom species tested as mosquito attractants: *P. ostreatus* (A), *T. porentosus* (B), *V. volvacea* (C), *P. sajor-caju* (D), and *L. edodes* (E).

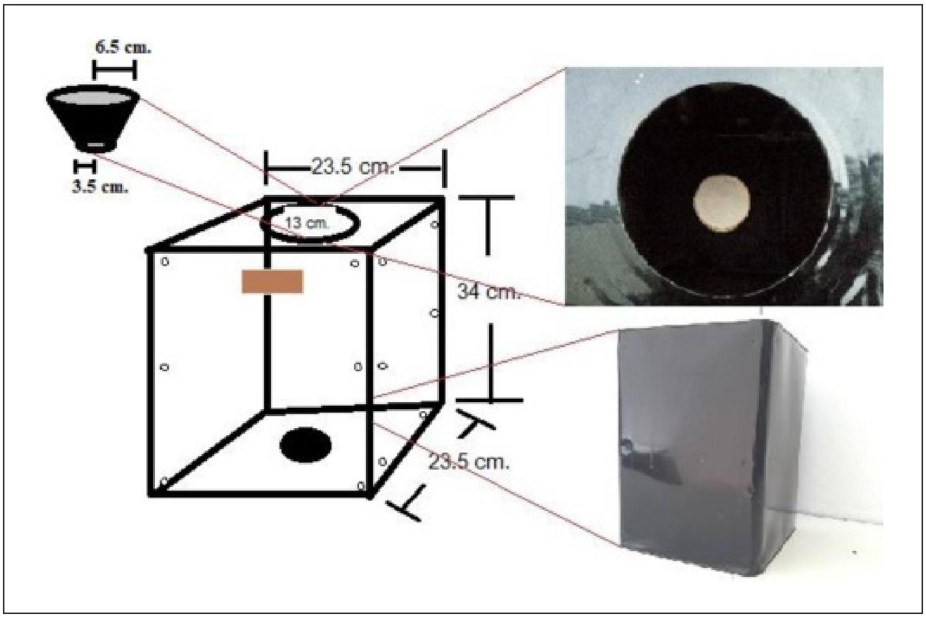

Figure 2. Mosquito resting box.

yields of the different extracts were weighed, recorded and dissolved in methanol (Table 1). The samples were then stored at -20°C before use as bait for mosquito attraction in the field.

Creating an odour-baited resting box

The design of the odor-baited resting box was adapted from previous studies, including Okumu *et al.* (2010), Kweka *et al.* (2009, 2010) and Pombi *et al.* (2014). The design

Table 1. Extract yields from each mushroom species

Species	Used (g)	% Yield (w/w)
Pleurotus ostreatus	50.00	13.11
Thaeogyroporus porentosus	50.00	23.50
Volvariella volvacea	50.00	20.24
Pleurotus sajor-caju	50.00	9.13
Lentinus edodes	50.00	11.96

details are shown in Figure 2. The resting boxes were made from aluminium painted black on the outside. Each was 23.5 cm × 23.5 cm (length × width) and 34 cm from the base to the highest point, with an opening at the top for mosquito entry or exit. In addition, the interior contained a small plate to hold filter paper saturated with mushroom extract. We designed a black cone (6.5 cm in diameter at the top and 3.5 cm at the bottom) to block the entrance for easy storage of specimens. Prior to testing the mushroom extracts as bait, we compared trap yield with and without the interior black cloth.

Field testing of mushroom extracts for mosquito attraction

The odour-baited resting boxes were used at a community dormitory in a populated area of Samut Songkhram Province, Thailand (13°24'32.52"N, 100°0'41.40"E). Each extract was tested at 50, 100, and 200 mg/mL. An unbaited trap (i.e., without extract on the filter paper) was used as a control. All conditions (extract and amount) were tested in triplicate.

The resting boxes were placed in similar environments at each location. Six resting boxes (five baited with one of the five mushroom extracts and an unbaited control) were left for 12 hours from 18:00–06:00, which is the normal blood-feeding time of *Culex* and *Anopheles* mosquitoes. All tests were conducted in November 2016. In the early morning, the mosquito resting boxes were transported to the laboratory and placed into a -80°C freezer for 20 minutes to kill the mosquitoes caught during the previous night. The trapped mosquitoes were then removed, counted, and identified for species and sex. Female mosquitoes were identified using Illustrated keys to the mosquitoes of Thailand (Rattanarithikul *et al.*, 2005) and males using several taxonomic keys, including Norbert (2010), Siverly and Shroyer (1974), Rattanarithikul (1982), and Harbach and Knight (1980, 1982).

Data analysis

All data were analysed using the Statistical Package for the Social Sciences (SPSS) version 17 (SPSS Inc. Chicago, IL). The mean numbers caught (± standard deviation) were compared among conditions (extract and amount) by one-way ANOVA followed by post hoc tests. The numbers caught in unbaited traps with or without the internal black cloth lining were compared by independent samples t-test. The Mann-Whitney U test was used to compare sex ratios of the species caught with each mushroom extract. A $p<0.05$ (two-tailed) was considered significant for all tests.

RESULTS

Before examining the efficacy of mushroom extracts, we compared the average number of mosquitoes caught in unbaited resting boxes with or without internal black cloth. While the mean difference did not reach statistical significance (7.33 vs. 4.00 mosquitoes per night per trap with and without black cloth; $p=0.11$; Table 2), the numerical difference (83%) suggested that the black cloth enhances trapping efficiency and so was included in all subsequent tests comparing the extracts.

Efficacy of mushroom extracts for mosquito attraction

There were substantial differences in trapping efficacy among extracts and amounts used (Figure 3). At 50 mg/mL, *T. porentosus* extract attracted the greatest number of mosquitoes (13.33 ± 10.50 per night per resting box) and *V. volvacea* the least (6.67 ± 0.58). At 100 mg/mL, *T. porentosus* extract attracted the most mosquitoes (12.67 ± 4.93) and *L. edodes* the least (5.67 ± 4.73). Finally, at 200 mg/mL, *L. edodes* extract attracted the most mosquitoes (16.00 ± 3.61) and *V. volvacea* the least (5.67 ± 3.79). Statistical

Table 2. Mean numbers of mosquitoes caught in the resting box with and without black cloth internal lining

	Number of mosquitoes caught				
	n	Mean	S.D.	t	*p*
With black cloth	23	7.33	1.73	-2.78	0.11
Without black cloth	12	4.00	0.58		

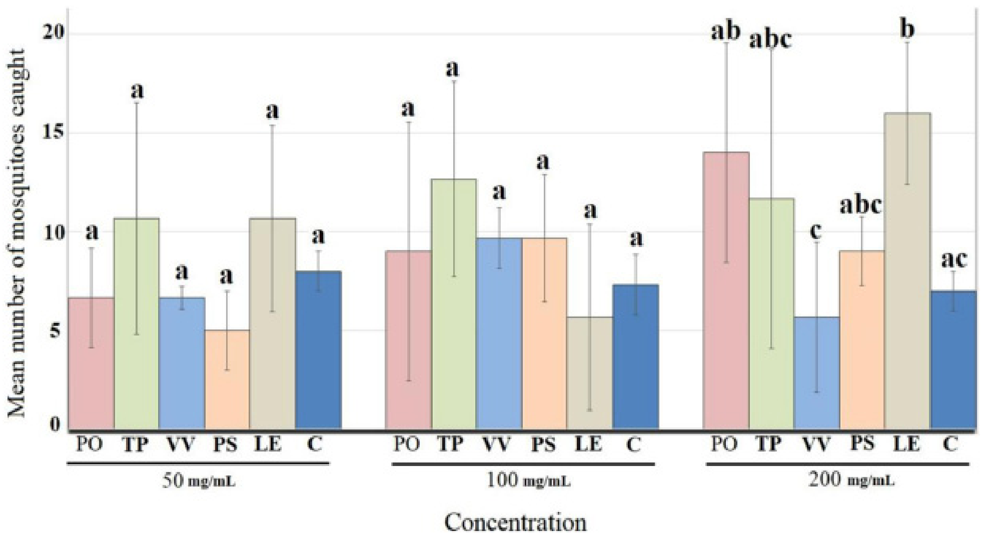

Figure 3. Mean numbers of mosquitoes caught using different amounts of each mushroom extract. The same letter indicates no significant difference at $p<0.05$ (PO = *P. ostreatus*, TP = *T. porentosus*, VV = *V. volvacea*, PS = *P. sajor-caju*, LE = *L. edodes*, and C= Control group).

evaluation revealed that only 200 mg/mL of *L. edodes* captured significantly more mosquitoes than the control unbaited resting box (7.00 ± 1.00). Moreover, the numbers caught using each mushroom extract did not increase progressively with amount used except for *P. ostreatus* (Fig. 3).

We identified males and females of three mosquito species in the traps, *Culex quinquefasciatus* Say, *Culex sitiens* Weidemann, and *Anopheles epiroticus* Linton & Harbach. The proportions of these mosquito species were similar for each extract. *Culex* mosquitoes were the most common, especially *Cx. quinquefasciatus* (Table 3). Moreover, the sex ratio of these species did not differ among extracts (Table 4).

DISCUSSION

Reducing the number of breeding adults is an effective method for mosquito control in nature. Recent reports have demonstrated the effectiveness of various traps developed based on sex- and species-specific behaviours and known attractants such as certain colours and odours. A trap incorporating several attractants may be even more effective, such as a resting box combined with a dark colour (to reduce repellent light reflection) and a volatile chemical attractant such as octenol. Indeed, lining the interior of the resting box with a black cloth increased the capture rate by >80% (although the difference did not reach

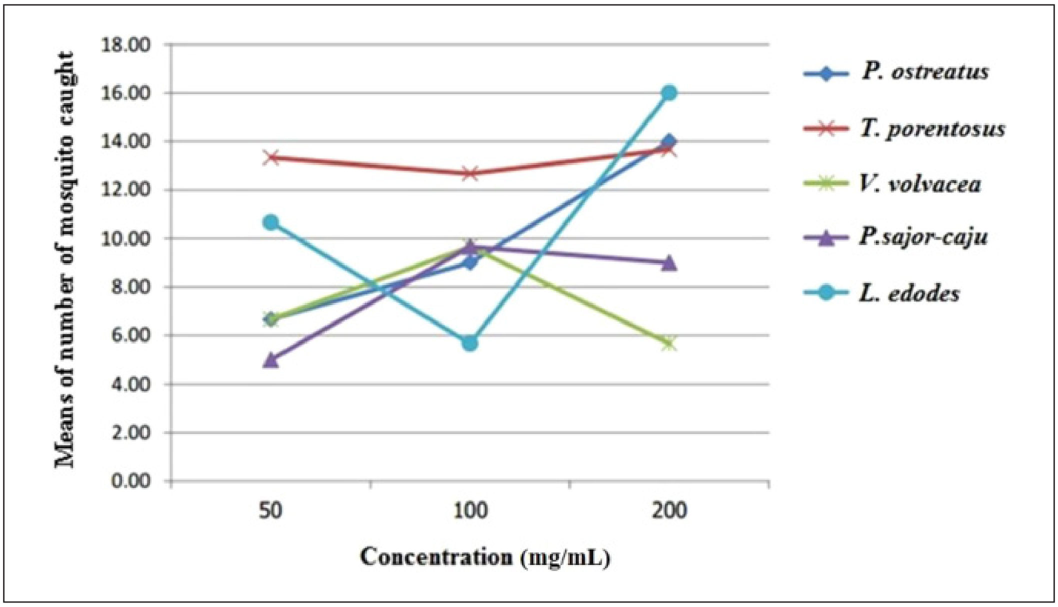

Figure 4. Mean numbers of mosquitoes caught by each mushroom extract.

Table 3. Percentage of each species and sex of mosquito caught by each mushroom extract throughout the experiment

Mosquito spp.	Percentage of each species of mosquito caught				
	P. ostreatus	*T. porentosus*	*V. volvacea*	*P. sajor-caju*	*L. edodes*
Cx. quinquefasciatus female	44.05% (37)	41.18% (49)	51.52% (34)	36.62% (26)	50.52% (49)
Cx. quinquefasciatus male	42.86% (36)	52.10% (62)	37.88% (25)	56.33% (40)	37.11% (36)
Cx. sitiens female	7.14% (6)	4.20% (5)	9.09% (6)	2.82% (2)	9.28% (9)
Cx. sitiens male	0	0	0	0	1.03% (1)
An. epiroticus female	4.76% (4)	2.52% (3)	1.51% (1)	1.41% (1)	2.06% (2)
An. epiroticus male	1.19% (1)	0	0	2.82% (2)	0
Total	100% (84)	100% (119)	100% (66)	100% (71)	100% (97)

statistical significance), while baiting with *L. edodes* (200 mg/mL) further increased the capture rate by more than twofold compared to the control unbaited black cloth-lined resting box.

Mosquitoes recognize colour by chemical receptor molecules in the eyes, and colour is a significant determinant of attraction (Lima *et al.*, 2014). Black has been reported to be an effective attractant colour (Bidlingmayer & Hem, 1980; Lima *et al.*, 2014). Further, the greater trapping efficiency observed using the black-line trap is consistent with the results using Ifakara odour-baited stations (Okumu *et al.*, 2010).

Octenol is also an attractant for mosquitoes (Grant & Dickens, 2011) and mushrooms contain octenol (Dijkstra, 1976; Hung *et al.*, 2014). The disparate efficacies of the individual extracts at specific concentrations may stem from differences in octenol content. Thongwat *et al.* (2015) screened 143 mushrooms in Thailand for killing effect against mosquito larva and found that only 4 species killed mosquito larvae. In this study, only *L. edodes* extract at 200 mg/mL was significantly more effective than the control unbaited resting box. Cho *et al.* (2003) reported that octenol accounts for 44.04% of all volatile compounds

Table 4. Mean numbers of male and female mosquitoes caught by species

Concentrations	Types of mushroom	No. of release times	Means ± S.D. of each species of mosquito caught					
			Cx. quinquefasciatus		*Cx. sitiens*		*An. epiroticus*	
			Male	Female	Male	Female	Male	Female
50 mg	*P. ostreatus*	3	2.00 ± 1.00	3.33 ± 1.53	0.00 ± 0.00	0.33 ± 0.58	0.00 ± 0.00	0.33 ± 0.58
	T. porentosus	3	8.00 ± 7.21	4.33 ± 3.06	0.00 ± 0.00	1.00 ± 1.00	0.00 ± 0.00	0.00 ± 0.00
	V. volvacea	3	2.00 ± 2.65	4.00 ± 2.00	0.00 ± 0.00	0.33 ± 0.58	0.00 ± 0.00	0.33 ± 0.58
	P. sajor-caju	3	3.00 ± 1.00	1.33 ± 1.53	0.33 ± 0.58	0.00 ± 0.00	0.33 ± 0.58	0.00 ± 0.00
	L. edodes	3	1.00 ± 1.73	7.67 ± 5.51	0.00 ± 0.00	1.33 ± 1.15	0.00 ± 0.00	0.67 ± 0.58
	control group	3	2.00 ± 1.73	3.67 ± 0.58	0.00 ± 0.00	1.67 ± 0.58	0.33 ±	1.00 ± 1.00
100 mg	*P. ostreatus*	3	3.00 ± 3.46	5.33 ± 3.05	0.00 ± 0.00	0.33 ± 0.58	0.00 ± 0.00	0.33 ± 0.58
	T. porentosus	3	5.67 ± 4.73	5.67 ± 2.89	0.00 ± 0.00	0.33 ± 0.58	0.00 ± 0.00	1.00 ± 0.00
	V. volvacea	3	3.00 ± 1.00	5.33 ± 2.52	0.00 ± 0.00	1.33 ± 0.58	0.00 ± 0.00	0.00 ± 0.00
	P. sajor-caju	3	6.33 ± 2.89	3.00 ± 1.00	0.33 ± 0.58	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	L. edodes	3	4.00 ± 4.58	1.00 ± 0.00	0.33 ± 0.58	0.33 ± 0.58	0.00 ± 0.00	0.00 ± 0.00
	control group	3	1.00 ± 0.00	4.00 ± 1.00	0.00 ± 0.00	1.00 ± 0.00	0.00 ± 0.00	1.33 ± 0.58
200 mg	*P. ostreatus*	3	7.00 ± 2.65	5.33 ± 3.21	0.00 ± 0.00	0.67 ± 0.58	0.33 ± 0.58	0.67 ± 1.15
	T. porentosus	3	7.00 ± 3.61	6.33 ± 2.31	0.00 ± 0.00	0.33 ± 0.58	0.00 ± 0.00	0.00 ± 0.00
	V. volvacea	3	3.33 ± 2.31	2.00 ± 1.73	0.00 ± 0.00	0.33 ± 0.58	0.00 ± 0.00	0.00 ± 0.00
	P. sajor-caju	3	4.00 ± 1.73	4.33 ± 1.15	0.00 ± 0.00	0.00 ± 0.00	0.33 ± 0.58	0.33 ± 0.58
	L. edodes	3	7.00 ± 3.61	7.67 ± 1.15	0.00 ± 0.00	1.33 ± 1.15	0.00 ± 0.00	0.00 ± 0.00
	control group	3	1.67 ± 0.58	3.67 ± 0.58	0.00 ± 0.00	0.67 ± 0.58	0.00 ± 0.00	1.00 ± 0.00

There was no statistically significant difference in the numbers of male and female mosquitoes ($p>0.05$).

in mature *L. edodes*. Thus, it is possible that among extracts tested, only this large amount of *L. edodes* extract contained sufficient octenol.

The attractant potential of these compounds was not a simple function of the amount used. Indeed, the trapping efficiency increased progressively with the amount used for only one extract (*P. ostreatus*), while the others showed an optimum within the range tested (50–200 mg/mL) with lower efficacy at the highest amount. However, this is consistent with the attractant properties of octenol on mosquitoes as determined under laboratory conditions (Cook *et al.*, 2011; Guha *et al.*, 2014). That is, higher concentrations of octenol may not attract mosquitoes as well as lower concentrations. Thus, the attractant efficacy was different for each extract, likely due to difference in octenol content and specificity of the olfactory receptors of mosquitoes.

The efficacy of this resting box baited with 200 mg/mL of *L. edodes* (16.00 ± 3.61 mosquitoes per night) was lower than several other trap types such as the CDC miniature light trap, Mosquito Magnet® Patriot Mosquito Trap, and BG-Sentinel (Lühken *et al.*, 2014). However, efficacy was not less than in some previous reports testing octenol as a mosquito attractant (Hoel *et al.*, 2007; Beavers *et al.*, 2004).

Three main species of mosquito were caught using the resting box and mushroom *extract*s, *Cx. quinquefasciatus*, *Cx. sitiens*, and *An. epiroticus*. These are the major vectors in costal Thailand. *Anopheles epiroticus* can transmit malaria to humans (Linton *et al.*, 2005), whereas *Cx. quinquefasciatus* and *Cx. sitiens* are vectors for filariasis and Japanese encephalitis (Xu *et al.*, 2005). Most mosquitoes caught in this study were *Cx. quinquefasciatus*, followed by *Cx. sitiens* and *An. epiroticus*. This is consistent with the population densities of these species in the test area (Chaiphongpachara & Sumruayphol, 2017). The density of *Culex* mosquitoes is often higher than *Anopheles* in nature because *Culex* species lay more eggs, and have a quicker growth rate, greater strength, and better adaptive survival abilities (Sumruayphol *et al.*, 2010). Thus, this odor-baited resting box does not appear to selectively attract any particular species (at least of these three). It is surprising, however, that the sex ratio did not differ, including in the 200 mg/mL of *L. edodes* group, given that this design is based on female behavior. However, it is possible that octenol may also drive nectar-seeking by mosquitoes (Syed & Leal, 2007), thereby attracting males as well as females. In addition, Dekel *et al.* (2016) reported that the octenol receptor phenotype of non-blood-feeding *Toxorhynchites* regulates localization of resting sites, oviposition sites, and nectar sources.

We conclude that the *L. edodes* mushroom is a potential source of effective, inexpensive, and environmentally friendly bait products to control mosquitoes, thereby reducing the spread of mosquito-borne diseases.

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Conflict of interest

We have no conflict of interest related to this research.

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