Appl Biol Chem (2017) 60(2):113–117
DOI 10.1007/s13765-017-0256-1

Online ISSN 2468-0842
Print ISSN 2468-0834

NOTE

Mosquito larvicidal activities of naturally occurring compounds derived from *Piper* species

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Received: 28 December 2016 / Accepted: 26 January 2017 / Published online: 28 February 2017

Abstract Mosquitos transmit human diseases including malaria, dengue fever, yellow fever, and encephalitis. Methylenedioxy compounds are considered to be safe synergists that enhance the activity of active ingredients to control mosquito populations. Seven naturally occurring compounds from *Piper nigrum* and *P. longum* were used to determine their larvicidal activities against larvae of *Culex pipiens pallens*. Among the tested compounds, myristicin and dodecanol showed potent larvicidal activity. Several modes of larvicidal action have been suggested for natural compounds, and in this study, their larvicidal effects on the surface water tension were considered and determined using a tensiometer in order to understand how lowering water surface tension was associated with mortality. In conclusion, lowering water surface tension was related to the larvicidal activities of myristicin and dodecanol.

Keywords Mosquito larvae · Myristicin · Dodecanol · Surface tension · Larvicidal activity

Introduction

Dengue fever, caused by an arbovirus, has spread in the tropical countries of the Americas and Asia–Pacific and has been transmitted by the *Aedes* mosquito in the past decades [1]. This disease has affected about 390 million people, and recently, outbreaks of this fever have been found in Malaysia, where 234 deaths have occurred out of 85,488 cases [2]. Malaria is also a mosquito-borne disease, and 128 million people were infected by the disease in 2013 [3]. Japanese encephalitis has been reported in many Asian countries with more than 68,000 clinical cases transmitted by mosquitoes with a fatality rate of up to 30% [4].

Chemical control of mosquitoes is widely used throughout the world; however, mosquitoes are becoming resistant to insecticides [5–8]. As Rodriguez et al. [7] noted, pyrethroid resistance in *Aedes aegypti* (L.) is a serious problem, and the control of *A. aegypti* by pyrethroid insecticides is becoming difficult. Those authors showed that esterases and cytochrome P-450 monooxygenases play an important role in deltamethrin resistance in a field population of *A. aegypti* larvae belonging to the SAN-F14 strain, which were subjected to 14 serial generations of selection for deltamethrin (91.25×). Therefore, development of inhibitors on cytochrome P450 activity is important for the control of mosquito larvae [9].

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Another form of chemical control of mosquito vectors is the use of repellents that protect humans from mosquito bites. *N,N*-diethyl-*m*-methylbenzamide (DEET) is a widely used mosquito repellent [10]. In addition to its repellent action, DEET has been shown to have a neurotoxic effect on mosquitoes and is able to block sodium and potassium channels within micromolar dose ranges [11]. However, some toxic effects of DEET have been reported after topical treatment in humans, particularly in children [12, 13]. DEET has also been reported to penetrate the placenta [14]. Therefore, there is an increasing need to develop alternatives to DEET and to other currently available insecticides.

Mosquito larvicidal and insecticidal activities of naturally occurring compounds have been considered as potent alternatives to currently used treatments [15–19]. Lee [16] found that a piperidine amide of *Piper longum*, pipernonaline, possessed mosquito larvicidal activity for *Cx. pipiens pallens* with an LC_{50} value of 0.21 ppm. Perumalsamy et al. [19] also found that karanjin and oleic acid displayed potent toxicity against *C. pipiens pallens* larvae, with a value of 24 h LC_{50} within the range 14.61–28.22 ppm. They demonstrated that the larvicidal activity of these compounds might arise from acetylcholinesterase inhibition in mosquito larvae.

Herein, we report several natural compounds that show mosquito larvicidal activities toward *Cx pipiens pallens* and measured their LC_{50} values. In addition, we identified the possible mechanism of mosquito larvicidal action of some of these natural products by measuring surface tension.

Materials and methods

Chemicals

Piperonal and dodecanol were previously isolated from *P. nigrum* in this laboratory. 1,3-Benzodioxole, myristicin, piperanine, sesamin, and sesamol were purchased from Sigma-Aldrich Co. (St. Louis, MO). All other chemicals used in this study were of the highest purity.

Bioassays

A total of 1000 *Cx. p. pallens* larvae were acquired from the Korean Food and Drug Administration (Osong, Korea) and adopted in the laboratory. Healthy larvae were selected and used in this study. All bioassays were undertaken at 25 ± 1 °C, and larval exposure testing was performed using at least five serial dilutions of tested compounds. One-hundred milliliters of each test solution containing 1% acetone was placed into a glass beaker along with 30 third instars. Experiments with each concentration and untreated control groups containing a 1% acetone vehicle were replicated three times. Mortality was recorded every 2 h up to 24 h after treatment. The median lethal concentration (LC_{50}) with 95% lethal concentration values was carefully calculated by Probit analysis [20]. Control mortality was accounted for by Abbott's formula [21].

Surface tension measurements of the tested solutions

The surface tension of tested solutions was measured by a surface tensiometer (Surface Tensiomat Model 21, Fisher Scientific, Pittsburgh, PA). A sample of 20 mL of each tested solution was placed in a clean 50-mL glass beaker and placed on the tensiometer platform. After placing the solution on the platform, a platinum wire ring was dipped into the solution. Next, the wire ring was slowly pulled out to meet the liquid–air interface. The surface tension (dyne/cm) was recorded, and the wire ring was rinsed three times with water and acetone. The wire ring was left to dry at room temperature.

Results

1,3-Benzodioxole showed potent larvicidal activity, resulting in 100% mortality at a dose of 100 mg/L, with ~50% mortality observed at 1 mg/L (Table 1). Dodecanol showed strong larvicidal activity, resulting in 100% mortality at 10 mg/L and 40% mortality at 1 mg/L. Myristicin was also found to be a potent natural larvicidal product, exhibiting ~90% mortality at 1 mg/L. Sesamin and sesamol exhibited strong larvicidal activities resulting in 93.3 and 67% mortality, respectively, at concentrations of 10 mg/L (Table 1). Piperonal showed weak larvicidal activity against *Cx. p. pallens* (Table 1). These natural products possess methylenedioxy moieties in their molecular structures.

The LC_{50} and LC_{95} values of seven natural products with larvicidal activity are given in Table 2. Among the tested compounds, myristicin and dodecanol possessed the strongest larvicidal activities with LC_{50} values of 0.56 and 0.65 ppm, respectively. 1,3-Benzodioxole exhibited an LC_{50} value of 3.37 ppm against *Cx. p. pallens* larvae. Dodecanol and sesamin were found to lower surface tension with increasing concentrations as shown in Fig. 1.

Discussion

The presence of methylenedioxy moieties in some molecular structures is important in the control of mosquito larvae in water environments [16]. 1,3-Benzodioxoles and their analogues are present in various plant species and are precursors of methylenedioxy moieties in compounds such

Table 1 Survival rate and surface tension of the tested methylenedioxy-containing compounds

Compound	Concentration (mg/L)	Mortality (mean ± RSD)	Surface tension (dyne/cm) Measured (calculated*)
Control		18.75 ± 15.28	69.4
1,3-Benzodioxole	100	100.00 ± 0.00	65.3
	10	90.00 ± 10.00	66.4
	1	48.18 ± 25.60	64
Dodecanol	100	100.00 ± 0.00	30
	10	100.00 ± 0.00	36.6
	1	40.00 ± 10.00	63.4
Myristicin	100	100.00 ± 0.00	61.1
	10	100.00 ± 0.00	69.3
	1	86.67 ± 23.09	69.2
Piperanine	2	23.33 ± 5.77	48.0*
	0.2	12.22 ± 9.62	
Piperonal	20	28.81 ± 9.85	56.4*
	2	7.04 ± 6.12	
Sesamin	100	90.00 ± 10.00	
	10	93.33 ± 11.55	35.4
	1	40.00 ± 10.00	50.5
Sesamol	100	100.00 ± 0.00	66.5
	10	73.33 ± 20.82	69.7
	1	66.67 ± 15.28	68.4

* ChemSpider supplied by royal society of chemistry was used to calculate the surface tension value

Table 2 Lethal values of larvicidal activities of some natural products against larvae of *Cx. p. pallens*

Natural products	LC_{50} (ppm)	LC_{95} (ppm)
1,3-Benzodioxole	3.37	11.3
Dodecanol	0.65	1.38
Myristicin	0.56	1.23
Piperanine	>20	>20
Piperonal	>20	>20
Sesamin	2.57	8.89
Sesamol	15.5	69.6

as piperine. It has known larvicidal activity against *Cx. p. quinquefasciatus*, and its larvicidal mode of action is related to inhibition of larval development [22]. Liu et al. [23] showed that myristicin, a primary constituent of *Illicium difengpi* essential oil, possessed an LC_{50} of 15.26 μg/mL against *Aedes aegypti*. In our study, myristicin exhibited an LC_{50} of 0.56 μg/mL against *Cx. p. pallens*. This might be the result of differences in mosquito species.

In our study, sesamin and sesamol were found to have potent larvicidal activity against *Cx. p. pallens*. Recently, sesamin isolated from *Zanthoxylum heitzii* stem bark was reported to have larvicidal activity against *A. gambiae* with moderate LC_{50} values greater than 150 μg/mL [24]. However, this finding is ques-

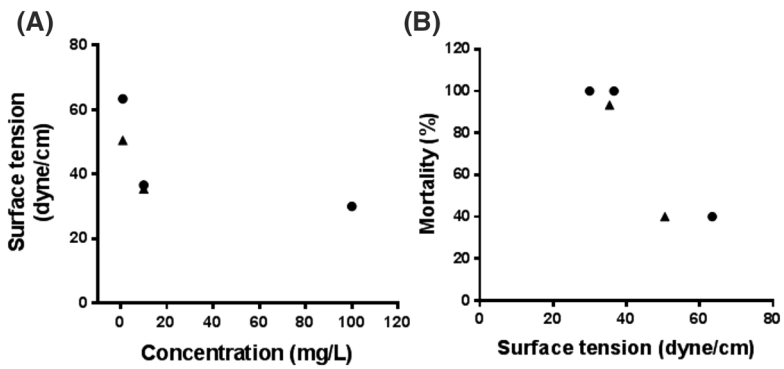

Fig. 1 Relationship between surface tension and concentration of the tested compounds (**A**), mortality (**B**). *Closed circles* indicate dodecanol and *closed triangles* represent sesamin

tionable, because, in the present study, sesamin lowered water surface tension to 35.4 dyne/cm at a concentration of 10 μg/mL (Table 1). Lowering the water surface tension to below 41 dyne/cm is a primary larvicidal route against mosquito larvae and pupae [25]. Therefore, we suspect that the results reported by Moussavi et al. [24] might reflect the use of low-purity sesamin in their study.

Other findings in relation to lower water surface tension provide insight into how mosquito larvae or pupae can be controlled by biosurfactants produced by bacterial biomass. Recently, the bacterium VCRC B483 was shown to lower water surface tension from 72 to 27.7 dyne/cm [26]. One of the most active biosurfactants, surfactin, is able to decrease water surface tension to 27 dyne/cm [27].

Other methylenedioxy-containing compounds such as piperanine and piperonal showed weak larvicidal activity against *Cx. p. pallens* (Table 1). Therefore, the presence of a methylenedioxy moiety may not be necessary for larvicidal activity.

Alkanols, including dodecanol, are stable nonreactive, amphiphilic, head–tail-type compounds. Therefore, they can be used in the control of mosquito larvae [28]. As found for alkanols, dodecanol showed potent larvicidal activity against *Cx. p. pallens* and the mode of action is related to lower water surface tension as shown in Fig. 1. Dodecanol-induced mortality increased with lowering water surface tension and by increasing the concentration of the tested compound (Fig. 1). Hammond and Kubo [29] have suggested that alkanols inhibit mitochondrial respiration as shown using rat livers. This is another important mode of action of dodecanol in the mosquito larvae in our study.

Further studies on the formulation of the active compounds are needed for field application. Therefore, emulsifiable concentrate using tergitol and ethanol has been considered for dodecanol formulation.

In conclusion, the present results indicate that dodecanol and myristicin have potent larvicidal activities against *Cx. p. pallens* larvae and could be considered as safe and effective natural larvicides.

Acknowledgments This study was carried out with the support of the Cooperative Research Program for Agricultural Science and Technology Development (Project No. PJ011983032016), Rural Development Administration, Republic of Korea.

Compliance with ethical standards

Conflict of interest No potential conflict of interest was reported by the authors.

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