Journal of the American Mosquito Control Association, 34(3):217–223, 2018

INSECTICIDAL EFFECT OF *SOLENOSTEMMA ARGEL* EXTRACTS AGAINST *CULEX PIPIENS*

FAHD A. AL-MEKHLAFI, NAEL ABUTAHA, MUHAMMAD FAROOQ AND MUHAMMAD AL-WADAAN

Bioproducts Research Chair, Department of Zoology, College of Science, King Saud University, Riyadh, Saudi Arabia

ABSTRACT. Of the various plant extracts from 10 plant species tested against larvae of *Culex pipiens* in the laboratory, only extracts from *Solenostemma argel* exhibited larvicidal activity. A chloroform leaf extract of *S. argel* exhibited relatively high activity with a lethal concentration causing 50% mortality (LC_{50}) of 15.89 ppm, while chloroform and ethyl acetate extracts of *S. argel* fruits were 19.70 and 19.52 ppm, respectively. The chloroform fruit extract at 10 ppm reduced the hatchability of *Cx. pipiens* eggs by 20%, whereas the chloroform leaf extract was found to be less effective (5% reduction). At 10 ppm, adult emergence was reduced by 84% and 75% for chloroform and ethyl acetate extracts of fruits, respectively. Metamorphosis of larvae exposed to chloroform fruit extract (10 ppm) was extended to 15 days, as compared to 10 days for control larvae. It took 12 days at 1 ppm, and 15 days at 6 and 10 ppm for chloroform fruit extract–treated embryos to develop into adult mosquito while it took 10 days in the control treatment. However, 100% toxicity was observed in the embryos of zebrafish, *Danio rerio*, treated with the ethyl acetate fruit extracts (LC_{50} of 20 ppm and LC_{100} of 40 ppm) and chloroform leaf extract (LC_{50} of 30 ppm and LC_{100} of 60 ppm). These findings emphasize the need to further isolate the bioactive molecules in *S. argel* crude extracts that may still be mosquitocidal but produce no, or minimal, adverse effects on nontarget organisms such as zebrafish.

KEY WORDS Adult emergence, *Culex pipiens*, hatchability, *Solenostemma argel*, zebrafish

INTRODUCTION

Culex pipiens L., or the common house mosquito, is one of the most widely distributed species around the world. *Culex pipiens* is the primary vector of several arboviruses, such as West Nile virus, Japanese encephalitis virus, Rift Valley fever virus, St. Louis encephalitis virus, and Sindbis virus, as well as worms responsible for lymphatic filariasis (Diaz-Badillo et al. 2009) and the World Health Organization has declared *Cx. pipiens* as "public enemy number one" (WHO 1996).

Among the widely used methods to control mosquitoes is the use of synthetic insecticides (WHO 2017). However, the extensive use of these chemicals in urban and semiurban environments has resulted in the development of resistance among the mosquito populations to these insecticides. Several studies have reported the development of resistance in *Cx. pipiens* against temephos (Cheikh and Pasteur 1993), pyrethroids (Scott et al. 2015), *Bacillus sphaericus* toxins (Nielsen-Leroux et al. 1997, Paul et al. 2005, Darboux et al. 2007), deltamethrin, lambda-cyhalothrin, beta-cyfluthrin, and bifenthrin (Al-Sarar 2010). Recently, *Cx. pipiens* has developed resistance against diflubenzuron, one of the most effective mosquito larvicides used in Europe and some other countries (Grigoraki et al. 2017).

In addition to resistance, some mosquitocides may be associated with diverse environmental hazards, such as accumulation of non-biodegradable residue, biomagnification in the food chain, and toxicity to human and animal health (Rawani et al. 2009). Therefore, there is a need for novel insecticides that are effective, cheap, and ecologically friendly.

Plants comprise a rich pool of phytochemical compounds that can be used to replace synthetic chemical insecticides (Ghosh et al. 2012). Kishore et al. (2011) reported the efficacy of phytochemicals against mosquito larvae and described the mosquito larvicidal potential of compounds such as alkynes, alkenes, alkanes, fatty acids, essential oils, terpenes, steroids, lactones, isoflavonoids, pterocarpans. alkaloids, and lignans. They also reported the isolation of several bioactive insecticides from various plants against different mosquito species such as octacosane, geranial, azadirachtin, alpha-terpinene, marmesin, and pipermonaline.

Unlike synthetic insecticides, the plant-derived products do not appear to produce resistance in mosquitoes. For example, resistance development to neem extract has not been reported for mosquitoes (Schmutterer 1990, Su and Mulla 1999). The lack of resistance development in response to neem extracts might be attributed to variation in the chemical composition and different modes of action.

Insecticides can be harmful to humans as well as animals, including aquatic species. Various animal models are used to evaluate the risk of insecticides to human and animal health. One of the models used in environmental toxicology is the zebrafish, *Danio rerio* (Hamilton), which has a high degree of homology with humans (Zhang et al. 2003).

The present study was conducted to evaluate the larvicidal and adult emergence inhibition properties

Table 1. Ten xerophytic plants tested against 3rd to 4th instars of *Culex pipiens* in preliminary screening.

Species	Family	Plant part and mass (g)	Mass of extract (g) (% yield)		
			Chloroform	Ethyl acetate	Methanol
Spergularia diandra	Caryophyllaceae	whole plant (39)	0.62 (1.58)	0.150 (0.38)	3 (7.70)
Ochradenus baccatus	Resedaceae	leaves (50)	0.61 (1.22)	0.171 (0.34)	2.98 (5.96)
		stem (50)	0.233 (0.467)	0.078 (0.156)	3.3 (6.66)
Bassia eriophora	Chenopodioideae	stem (31)	0.023 (0.07)	0 (0)	1.48 (4.77)
Cleome amblyocarpa	Capparaceae	leaves (50)	0.644 (1.3)	0.1697 (0.34)	6.66 (13.33)
		stem (50)	0.155 (0.31)	0.121 (0.24)	2.78 (5.56)
Solenostemma argel	Asclepiadaceae	leaves (50)	1.69 (3.4)	0.341 (0.682)	5.341 (10.7)
		stem (50.6)	0.179 (0.35)	0.056 (0.11)	1.764 (3.5)
		fruit (28.7)	2.124 (7.4)	0.242 (0. 84)	2.659 (9.26)
		seeds (7)	0.204 (2.9)	0.064 (0.9)	0.363 (5.2)
Bassia erioplora	cheropodiaceae	stem (18.5)	0.05 (0.27)	0.007 (0.03)	0.646 (3.5)
Aizoon canariense	Aizoaceae	whole plant(50)	0.177 (0.35)	0.073 (0.14)	1.9 (3.8)
Iphiona scabra	Compositae	whole plant(5.3)	0.09 (1.8)	0.071 (1.34)	0.684 (12.9)
Cordia mixa	Boraginaceae	leaves (50)	0.09 (0.2)	0.071 (0.14)	0.684 (1.36)
		fruit (34)	0.21 (0.6)	0.162 (0.47)	0.747 (2.2)
Retama raetam	Leguminosae	stem (51)	0.55 (1)	1.493 (2.9)	5.55 (10.88)
		fruit (50)	0.27 (0.54)	0.218 (0.44)	12.9 (25.8)
		seed (50.6)	0.82 (1.62)	0.149 (0.3)	4.47 (8.8)

of extracts of 10 indigenous plant species against *Cx. pipiens* in the laboratory.

MATERIALS AND METHODS

Plant collection

Spergularia diandra (Guss.) Boiss, *Ochradenus baccatus* Delile, *Bassia eriophora* (Schrad.) Asch., *Cleome amblyocarpa* Barratte and Murb, *Solenostemma argel* (Delile) Hayne, *Aizoon canariense* L., *Iphiona scabra* DC. ex Decne., *Cordia myxa* L., and *Retama raetam* (Forssk.) were collected from the desert around Riyadh, Saudi Arabia in July 2017 (Al-Dir'iya 24°45′3.37″N, 46°32′7.91″E and Rawdat Khuraim 25°23′20.39″N, 47°17′37.33″E). Samples were identified and authenticated, and a voucher specimen of each species was deposited in the Department of Botany and Microbiology, College of Science, King Saud University, Saudi Arabia.

Preparation of extracts

Whole plants or parts (stem, leaf, fruit, and seed) from each species were air dried in shade at room temperature (25°C) and ground to a fine powder. The powder (40–50 g) was extracted with chloroform, (450 ml), ethyl acetate (450 ml), and methanol (450 ml) in a Soxhlet apparatus for 24 h. Extracts were then centrifuged at 2,061 × *g* for 10 min (Sigma, Osterode, Germany). The solvent from each extract was evaporated using a rotary evaporator (Heidolph, Schwabach, Germany) at 45°C. Extracts were weighed and then stored at –80°C. All the extracts were reconstituted in methanol for further analysis. Extract yield was calculated as follows: Extract yield (%) = ([mass of the extract]/[mass of the sample]) × 100.

Mosquito colonies and rearing conditions

Laboratory colonies of insecticide-susceptible *Cx. pipiens* larvae were procured from an insectary at King Saud University, Riyadh, and maintained at 28 ± 2°C, with a 12-h light and 12-h dark photoperiod. Adult mosquitoes were maintained in a plastic cage (25 × 35 × 6 cm) and fed 10% sucrose solution (Loba Chemie, India). Female mosquitoes were allowed to feed on mice blood (Swiss albino). Each cage was provided with a 90-mm petri dish containing 100 ml of tap water to collect the eggs after 2–3 days. Larvae were fed ground TetraMin® fish meal (Tetra GmbH, Germany)

Dose-response bioassay

Each plant extract was individually applied to 12 sterile well plates (well dimensions: 22 mm diam × 18 mm deep; 1.52-mm thickness; total volume per well: 6.0 ml) (Corning Inc., NY) and allowed to evaporate and was reconstituted in tap water to prepare concentrations of 10, 20, 40, 60, and 80 ppm (total volume, 3 ml) to test against the 3rd and 4th instars of *Cx. pipiens*. The assay was repeated thrice with 10 larvae per concentration. Methanol (Fisher Scientific, UK) was used as a solvent control. The larvae were fed ground fish meal during the treatment. Percentage of larval mortality was calculated 24, 48, and 72 h posttreatment, and expressed as 50% and 90% lethal doses (LC_{50} and LD_{90}) values. Larval mortality was corrected when mortality in control was ≥5% (Abbott 1925). All dose-response assays were repeated in triplicate.

Sublethal bioassay

The standard World Health Organization methods (WHO 1996) and the method of Su and Mulla (1999)

Table 2. Probit analysis of larvicidal activity of different concentrations of *Solenostemma argel* fruits extracts against 3rd and 4th instars of *Culex pipiens* after 24, 48, and 72 h of exposure.

Extract type	Time (h)	% Mortality					LC_{50} (ppm) (95% LCL–UCL)[1]	LC_{90} (ppm) (95% LCL–UCL)	χ^2	Slope
		Concentration (ppm)								
		5	10	20	40	60				
Chloroform	24	0 ± 00	23.33 ± 1.80	73.33 ± 3.33	100 ± 00	100 ± 00	19.7 (14.19–27.5)	52.9 (38.0–73.8)	0.26	3.0
	48	6.67 ± 2.05	53.33 ± 5.77	93.33 ± 5.77	100 ± 00	100 ± 00	9.87 (7.5–12.83)	17.84 (13.72–23.2)	0.889	4.98
	72	33.33 ± 3.33	76.33 ± 6.67	100 ± 00	100 ± 00	100 ± 00	6.47 (4.6–9.0)	14.18 (10.18–19.7	0.97	3.76
Ethyl acetate	24	0 ± 00	13.33 ± 3.33	60 ± 5.77	83.33 ± 3.33	100 ± 00	19.52 (14.3–26.5)	46.14 (33 .96–62.68)	0.339	3.45
	48	30 ± 6.67	40 ± 00	93.33 ± 2.05	100 ± 00	100 ± 00	8.48 (6.05–11.88)	20.93 (14.95–29.31)	0.227	3.36
	72	43.33 ± 5.77	53.33 ± 2.05	100 ± 00	100 ± 00	100 ± 00	6.24 (4.7–9.62)	16.8 (11.76–23.9)	3.32	0.332

[1] LC_{50}, lethal concentration causing 50% mortality; LC_{90}, lethal concentration causing 90% mortality; LCL, lower confidence limits; UCL, upper confidence limits.

were adopted with some modifications as follows. Twenty *Cx. pipiens* eggs (1–12 h old) were placed in plastic containers containing 50 ml of tap water. Percentage of hatchability, larval and pupal mortality, adult emergence, and growth index were recorded along with the total duration (days) of larval and pupal stages. In addition, the effects of sublethal concentrations (1, 2, 6, and 10 ppm) of *S. argel* extracts on the developmental period of *Cx. pipiens* were evaluated. All assays were repeated in triplicate.

Zebrafish toxicity assays

Wild-type AB strain zebrafish embryos were obtained from the Zebrafish International Resource Center, University of Oregon, Eugene, OR, USA. Fish were maintained in fish tanks (10-liter plastic tanks; Tecniplast, Exton, PA) at 28.5°C and pH 7.5 and fed live brine shrimp and Zeigler-dried flake food daily (Zeiglers Bros., Inc., Gardeners, PA). Photoperiod was maintained at 14 h light and 10 h dark; spawning and fertilization were stimulated during the light period. Fertilized zebrafish embryos were collected from the bottom of the tank. Embryos were placed in petri dishes, and allowed to develop in an incubator at 28.5°C for further assays.

Toxicity assay of zebrafish embryos was performed in 5-cm petri plates using 10 embryos per plate in Danieau's medium. Two concentrations of plant extracts, 50% lethal concentration (LC_{50}) and 100% lethal concentration (LC_{100}) were selected for the treatments from the earlier dose-response mosquito assays. These included ethyl acetate extracts (LC_{50} of 20 ppm and LC_{100} of 40 ppm) and chloroform leaf extract (LC_{50} of 30 ppm and LC_{100} of 60 ppm). Methanol was used as a control. The assay was performed in triplicate and percentage of mortality recorded at 24 h.

Statistical analysis

Mean larval mortalities were transformed into probit analyses. The LC_{50}, LC_{90}, upper confidence limit and lower confidence limit values, chi-square test, mean and standard deviation were calculated using Microsoft Excel (Finney 1952).

RESULTS

Larvicidal activity of plant extracts

The percentage of yield of all plant extracts is listed in Table 1. All extracts exhibited toxicity against the 3rd- and 4th-stage *Cx. pipiens* larvae in a dose-dependent manner; however, most extracts, except for the fruit and leaf extracts, failed to exhibit significant larvicidal activity, even at a relatively high concentration (500 ppm) (Tables 2 and 3). Larvicidal activity was found to be time dependent for all extracts, with greater toxicity recorded at 72 h compared with that at 24 and 48 h. Chloroform leaf

Table 3. Probit analysis of larvicidal activity of different concentrations of *Solenostemma argel* leaves extracts against 3rd to 4th instars of *Culex pipiens* at 24, 48, and 72 h postexposure.

Extract type	Time (h)	% Mortality					LC_{50} (ppm) (95% LCL–UCL)[1]	LC_{90} (ppm) (95% LCL–UCL)	χ^2	Slope
		Concentration (ppm)								
		10	20	40	60	80				
Chloroform	24	3.33 ± 6.67	20 ± 00	53.33 ± 2.67	96.67 ± 2.67	100 ± 00	15.89 (11.9–21.2)	33.9 (25.4–45.26)	0.82	3.96
	48	20 ± 3.33	40 ± 3.33	83.33 ± 1.55	100 ± 00	100 ± 00	10.32 (7.2–14.6)	27.7 (19.53–39.3)	0.055	3
	72	33.33 ± 5.67	70 ± 2.05	96.67 ± 3.33	100 ± 00	100 ± 00	14.8 (10.6–20.5)	6.75 (4.8–9.37)	0.82	3.76
Ethyl acetate	24	0 ± 00	0 ± 00	6.67 ± 3.33	10 ± 00	10 ± 00	ND	ND	ND	ND
	48	0 ± 00	0 ± 00	13.33 ± 5.67	13.33 ± 5.67	30 ± 3.33	ND	ND	ND	ND
	72	10 ± 2.05	23.33 ± 2.67	30 ± 00	36.67 ± 6.67	40 ± 3.33	ND	ND	ND	ND

[1] LC_{50}, lethal concentration causing 50% mortality; LC_{90}, lethal concentration causing 90% mortality; LCL, lower confidence limits; UCL, upper confidence limits; ND, not determined.

and chloroform and ethyl acetate extracts of *S. argel* were selected for further development- and growth-related assays.

Effect of the extracts on hatching and larval development

The effects of the chloroform and ethyl acetate extracts of *S. argel* fruits and leaves on percentage of hatchability were directly proportional to the concentration of extracts. Chloroform fruit extract resulted in 20% ovicidal activity at a concentration of 10 ppm, followed by the ethyl acetate fruit extract with 15% at 10 ppm. The chloroform leaf extract was found to be less effective against the eggs of *Cx. pipiens* (5%) at 10 ppm as compared with the control eggs, which showed 100% hatchability (Table 4).

Effect of the extracts on pupation

The chloroform fruit extract of *S. argel* induced 77% larval mortality (Table 4). Furthermore, 44.4% and 53.3% of the larvae could successfully develop into pupae at 10 ppm of chloroform and ethyl acetate fruit extract, respectively. Larvae in control for chloroform and ethyl acetate fruit extract exhibited successful pupation of 92.8% and 91.6% respectively. The pupal stage was unaffected at relatively low concentrations (1 and 2 ppm) of extracts (Table 4). However, the chloroform and ethyl acetate fruit extracts at 6 and 10 ppm extended the pupal period by 1 day. The chloroform leaf extract did not affect the pupal period.

Effect of the extracts on adult emergence and growth index

The toxicity of *S. argel* extracts reduced the emergence of adults by up to 84% as compared with 92% of adult emergence in controls (Table 4). The ethyl acetate fruit extract reduced adult emergence by 75% at 10 ppm with adult emergence in controls at 90%. Furthermore, larvae developed to adults in 10 days in the control, while it took 12 days at 1 ppm, and 15 days at 6 of the chloroform fruit extract (Table 4). Moreover, the growth indices of larvae exposed to the chloroform fruit extract were 7.4%, 4.3%, 1.3%, and 1.1% at 1, 2, 6, and 10 ppm, respectively, with 9.22% in the control. Similarly, the growth indices of ethyl acetate fruit extract were 7.5%, 5.3%, 4.3%, and 1.7% at 1, 2, 6, and 10 ppm, respectively, and 8.2% in control.

Zebrafish toxicity assay

The extracts of *S. argel* were highly toxic to the zebrafish embryos. The embryos treated with ethyl acetate extracts (20 and 40 ppm) and chloroform leaf extracts (30 and 60 ppm) died after 24 h. The hatching period of zebrafish embryos in controls was 48 h postfertilization. The effect of *S. argel* on the hatchability of zebrafish larvae was not recorded due

Table 4. Effect of *Solenostemma argel* extract on development and growth index of *Culex pipiens*.

Parts used	Solvents	Concentration (g)	Hatchability (%)	Larval mortality (%)	Total larval period in days	Pupal mortality (%)	Total pupal period in days	Adult emergence (%)	Total developmental period (days)	Growth index[1]
Fruit	chloroform	1	98.35 ± 2.89	11.84 ± 2.74	10	9.58 ± 3.22	2	89.37 ± 3.80	12	7.45
		2	88.35 ± 2.89	30.17 ± 2.85	10	37.82 ± 4.21	2	51.78 ± 9.95	12	4.32
		6	83.35 ± 2.89	68.02 ± 3	12	44.44 ± 3.88	3	19.44 ± 7.34	15	1.30
		10	80 ± 00	77.08 ± 3.61	12	55.56 ± 9.63	3	16.67 ± 8.87	15	1.11
		control	100 ± 00	8.33 ± 2.89	10	7.21 ± 2.86	2	92.16 ± 3.39	10	9.22
	ethyl acetate	1	100 ± 00	3.33 ± 2.89	10	13.77 ± 2.84	2	89.95 ± 3.62	12	7.50
		2	98.33 ± 2.89	27.11 ± 2.59	10	40.79 ± 5.52	2	64.35 ± 9.85	12	5.36
		6	91.67 ± 2.89	54.48 ± 4.05	11	43.98 ± 6.26	3	56.67 ± 5.77	13	4.36
		10	85 ± 5	70.52 ± 1.72	11	46.67 ± 11.54	3	25 ± 2.55	14	1.79
		control	100 ± 00	1.67 ± 2.89	10	8.42 ± 2.74	2	90.74 ± 1.85	11	8.25
Leaves	chloroform	1	100 ± 00	5 ± 00	10	5.42 ± 5.50	2	96.57 ± 2.97	12	8.05
		2	96.67 ± 2.89	5.09 ± 5	10	9.67 ± 3.21	2	92.36 ± 3.02	12	8
		6	100 ± 00	16.67 ± 2.89	11	8.30 ± 3.25	2	84.89 ± 4.28	12	7.07
		10	95 ± 2.89	15 ± 00	11	9.8 ± 3.39	2	84.83 ± 4.19	12	7
		control	100 ± 00	1.67 ± 2.89	10	5.33 ± 0.57	2	96.49 ± 3.04	10	9.65

[1] Growth index = adult emergence/total developmental period in days.

to the death of the embryos before they reached the hatching stage.

DISCUSSION

To the best of our knowledge, the present study is the first to report the insecticidal effect and developmental toxicity of *S. argel* extract against *Cx. pipiens* and zebrafish embryos, respectively. *Solenostemma argel* is widely distributed in Algeria, Libya, Mali, Sudan, Chad, Niger, Egypt, and Saudi Arabia. It has been reported to possess antimicrobial activity against pathogenic bacteria (Zain et al. 2012). In addition, this plant species has been used for treating rheumatic pains, loss of appetite, and cough (Zain et al. 2012). The curative properties of *S. argel* include antispasmodic, purgative, antiulcerous, anti-inflammatory, and immunostimulatory properties (Kamel et al. 2000, Hamed 2001). Except for one report on the effect of methanol extracts of *S. argel* aerial parts on the oviposition and egg hatchability of *Cx. pipiens* (Al-Doghairi et al. 2004), other investigations have concentrated mainly on the insecticidal activity of *S. argel* against different mosquito species (El-Kamali 2001, El Tayeb et al. 2009, Edriss et al. 2013).

In our study, the ethyl acetate fruit extract of *S. argel* was the most effective larvicide, followed by the chloroform extracts of fruits and leaves. Analogous results have been obtained previously by El-Kamali (2001) where the‘ LC_{50} value of aqueous extracts of *Cx. quinquefasciatus* Say was reported to be 490 ppm. However, the LC_{50} value of chloroform fruit extract observed in our study was greater than that of the aqueous fruit extract reported by El-Kamali (2001). This variation can be attributed to the use of different solvents. Further, the plant habitat could also play an important role in the quality and quantity of phytochemical compounds.

With differences in the concentration of bioactive compounds in different parts of a plant (Schmutterer 1990), the variation in insecticidal activity of the leaf and fruit extracts of *S. argel* in the present study was consistent with an earlier study (El-Kamali 2001) in which the larvicidal activity of the flower and seed extracts was higher than that of the leaf extract.

We also observed that percentage of egg hatchability of *Cx. pipiens* was affected by the chloroform and ethyl acetate extracts of *S. argel*. This is in agreement with a previous report by Al-Doghairi et al. (2004), reporting a reduction in percentage of hatchability of *Cx. pipiens* eggs by the alcoholic extracts of the aerial parts of *S. argel*. However, the effectiveness of fruit and leaf extracts of *S. argel* on the growth and development of larvae of *Cx. pipiens* has not been investigated until now.

A number of plant derivatives have been evaluated for their growth-regulating activity (Lejczak et al. 1988, Satti et al. 2004). We found that the treatment of chloroform and ethyl acetate extracts of *S. argel* resulted in the prolongation of *Cx. pipiens* larval

development by 5 days. Similar effects have been reported for plant extracts of *Azadirachta indica* Juss and *Pseudocalymma alliaceum* (Lam) against *Cx. pipiens*, *Aedes aegypti* (L.), and *Cx. quinquefasciatus* (Dua et al. 2009, Tom et al. 2009, Granados-Echegoyen et al. 2014).

Few studies have reported on the effects of larvicidal plant extracts against nontarget organisms. We found that the zebrafish toxicity assay offers a fast and convenient method to evaluate the toxicity of natural products. In this study, the fruit and leaf extracts of *S. argel* exhibited strong larvicidal effect against *Cx. pipiens*, and produced considerable toxicity against zebrafish embryos. These results suggest that the extract of *S. argel* at the doses tested may prove to be a risk to nontarget organisms in aquatic ecosystems. These findings also emphasize the need to further isolate the bioactive molecules of *S. argel* crude extracts that may still be larvicidal and yet produce no, or minimal, adverse effects on nontarget organisms such as zebrafish.

ACKNOWLEDGMENTS

The authors extend their appreciation to the Deanship of Scientific Research at King Saud University for funding the work through the research group project No. RG-1439-030.

REFERENCES CITED

Abbott WS. 1925. A method for computing the effectiveness of an insecticide. *J Econ Entomol* 18:265–267.

Al-Doghairi M, El-Nadi A, Elhag E, Al-Ayedh H. 2004. Effect of *Solenostemma argel* on oviposition, egg hatchability and viability of *Culex pipiens* L. larvae. *Phytother Res* 18:335–338.

Al-Sarar AS. 2010. Insecticide resistance of *Culex pipiens* (L.) populations (Diptera: Culicidae) from Riyadh city, Saudi Arabia: Status and overcome. *Saudi J Biol Sci* 17:95–100.

Cheikh BH, Pasteur N. 1993. Resistance to temephos, an organophosphorous insecticide, in *Culex pipiens* from Tunisia, North Africa. *J Am Mosq Control Assoc* 9:335–337.

Darboux I, Charles JF, Pauchet Y, Warot S, Pauron D. 2007. Transposon-mediated resistance to *Bacillus sphaericus* in a field-evolved population of *Culex pipiens* (Diptera: Culicidae). *Cell Microbiol* 9:2022–2029.

Diaz-Badillo A, Bolling BG, Perez-Ramirez G, Moore CG, Martinez-Munoz JP, Padilla-Dua VK, Pandey AC, Raghavendra K, Gupta A, Sharma T, Dash AP. 2009. Larvicidal activity of neem oil (*Azadirachta indica*) formulation against mosquitoes. *Malar J* 8:124.

Edriss AE, Satti AA, Alabjar ZA. 2013. Larvicidal properties of two asclepiadaceous plant species against the mosquito *Anopheles arabiensis* Patton (Diptera: Culicidae). *J Saudi Agric Sci* 12:59–66.

El-Kamali HH. 2001. Larvicidal activity of crude aqueous extracts of *Solenostemma argel* against mosquito larvae. *J Herbs Spices Med Plants* 8:83–86.

El Tayeb FMH, Taha AK, Mardi HG, Sid Ahmed OA. 2009. Water extracts of hargal plant (*Solenostemma argel*, Del Hyne), and usher (*Calotropis procera* Ail) leaves as natural insecticides against mosquito larvae. *J Sci Technol* 10:67–76.

Finney, DJ, & Tattersfield, F. (1952). *Probit analysis*. Cambridge, United Kingdom: Cambridge University Press.

Ghosh A, Chowdhury N, Chandra G. 2012. Plant extracts as potential mosquito larvicides. *Indian J Med Res* 135:581–598.

Granados-Echegoyen, Pérez-Pacheco CR, Soto-Hernández M, Ruiz-Vega J, Lagunez Rivera L, Alonso-Hernandez N, Gato-Armas R. 2014. Inhibition of the growth and development of mosquito larvae of *Culex quinquefasciatus* (Diptera: Culicidae) treated with extract from leaves of *Pseudocalymma alliaceum* (Bignonaceae). *Asian Pac J Trop Med* 7:594–601.

Grigoraki L, Puggioli A, Mavridis K, Douris V, Montanari M, Bellini R, Vontas J. 2017. Striking diflubenzuron resistance in *Culex pipiens*, the prime vector of West Nile virus. *Sci Rep* 7:11699.

Hamed AI. 2001. New steroids from *Solenostemma argel* leaves. *Fitotrapia* 72:747–755.

Kamel MS, Mohamed KM, Hassanean HA, Ohtani K, Kasai R, Yamasaki K. 2000. Iridoid and megastigmane glycosides from *Phlomis aurea*. *Phytochemistry* 55:353–357.

Kishore N, Mishra BB, Tiwari VK, Tripathi V. 2011. A review on natural products with mosquitosidal potentials. In: Tiwari VK, ed. *Opportunity, challenge and scope of natural products in medicinal chemistry*. Kerala, India: Res Signpost. pp. 335–365.

Lejczak B, Kafarski P, Gancarz R. 1988. Plant growth regulating properties of 1-methylethylphosphonic acid and its derivatives. *Pest Manag Sci* 22: 263–275.

Marinho RA, Beserra EB, Bezerra-Gusmão MA, Porto VS, Olinda RA, Santos CAD. 2016. Effects of temperature on the life cycle, expansion, and dispersion of *Aedes aegypti* (Diptera: Culicidae) in three cities in Paraiba, Brazil. *J Vector Ecol* 41:1–10.

Nielsen-Leroux C, Pasquier F, Charles JF, Sinègre G, Gaven B, Pasteur N. 1997. Resistance to *Bacillus sphaericus* involves different mechanisms in *Culex pipiens* (Diptera: Culicidae) larvae. *J Med Entomol* 34:321–327.

Paul A, Harrington LC, Zhang L, Scott JG. 2005. Insecticide resistance in *Culex pipiens* from New York. *J Am Mosq Control Assoc* 21:305–309.

Rawani A, Haldar KM, Ghosh A, Chandra G. 2009. Larvicidal activities of three plants against filarial vector *Culex quinquefasciatus* Say (Diptera: Culicidae). *Parasitol Res* 105:1411–1417.

Satti AA, Nasr OE, Bashir NHH. 2004. Technology of natural pesticides: production and uses in Sudan. In: Proceedings of the Second National Pest Management Conference in the Sudan, 2004 December 6–9, Faculty of Agricultural Sciences, University of Gezira, Wad Medani, Sudan.

Schmutterer H. 1990. Properties and potential of natural pesticides from the neem tree. *Annu Rev Entomol* 35:271–297.

Scott JG, Yoshimizu MH, Kasai S. 2015. Pyrethroid resistance in *Culex pipiens* mosquitoes. *Pest Biochem Physiol* 120:68–76.

Su T, Mulla MR. 1999. Oviposition bioassay responses of *Culex tarsalis* and *Culex quinquefasciatus* to neem products containing azadirachtin. *Entomol Exp Appl* 91:337–345.

Tom SH, Pacheco R, Hernandez C, Valverde G, Vega J. 2009. Effects of an aqueous extract of *Azadirachta indica* on the growth of larvae and development of pupae of *Culex quinquefasciatus*. *Afr J Biotechnol* 8:4245–4250.

Viveros AA, Camacho-Nuez M, Diaz-Perez A, Beaty BJ, Munoz ML. 2011. The distribution of potential West Nile virus vectors, *Culex pipiens pipiens* and *Culex pipiens quinquefasciatus* (Diptera: Culicidae), in Mexico City. *Parasit Vectors* 4:70.

WHO [World Health Organization]. 1996. Report of the WHO informal consultation on the evaluation on the testing of insecticides. Geneva, Switzerland: World Health Organization.

WHO [World Health Organization]. 2017. *Dengue control*. [accessed June 6, 2018]. Available from: http://www.who.int/denguecontrol/control_strategies/chemical_control/en/.

Zain ME, Awaad AS, Al-Outhman MR, El-Meligy RM. 2012. Antimicrobial activities of Saudi Arabian desert plants. *Phytopharmacology* 2:106–113.

Zhang C, Willett C, Fremgen, T. 2003. Zebrafish: an animal model for toxicological studies. *Current Protocols in Toxicology* 17.1:1–7.