Original Article

Larvicidal Effects of Carbon Nanotubes Loaded with Selected Marine 'Sponges' Extracts

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Abstract

Recently, the use of eco-friendly and biodegradable insecticides has gained great attention. The present study was concerned to evaluate the larvicidal potential of the extracts of the Red Sea sponges *Xestospongia testudinaria* and *Amphimedon chloros* and biogenic carbon nanotubes (CNTs) against *Aedes aegypti* (Diptera: Culicidae). The third instar larvae of *Ae. aegypti* was used to test the insecticidal activity of the methanolic extract of *X. testudinaria* and *A. chloros*. The results showed that the tested concentrations (62.5, 125, 250, and 500 ppm) of both extracts possess high and moderate larvicidal effects after 48 h of exposure. The methanolic extract of *A. chloros* with CNTs showed 96 % (LC_{50} = 15.569 ppm) mortality after 24 h of exposure. While the *A. chloros*, extract without CNTs, the larval mortality was 99 % (LC_{50} = 65.77 ppm) after 48 h of exposure. These results suggested that the synthesized biogenic CNTs can be used as an ideal eco-friendly approach for controlling *A. aegypti*.

Keywords: Red-Sea sponges, Organic extracts, Larvicidal activities, Biodegradable

Introduction

Mosquitoes (*Diptera Culicidae*) are the most critical group in blood-sucking arthropods [1]. Mosquitoes not only create a nuisance to humans but also transmit serious diseases [2]. They belong to three prominent families, e.g., Anophelinae, Culicidae, Toxorhyncitinae, which have been further categorized into approximately 3700 species. The hooked beak can recognize the adult mosquito not to penetrate the skin to have a meal of blood. It feeds only on flower nectar [3, 4].

Most genera *Anopheles*, *Culex*, and *Aedes*, transmit different types of infectious diseases, *e.g.*, Japanese Encephalitis, Dengue fever, Yellow fever, Malaria, Filariasis, etc., causing large scale of deaths each year worldwide [1, 5]. Arthropods transmit dengue, a serious viral disease, that occurs worldwide. Therefore, a high diffusion is observed of Dengue Hemorrhagic Fever (DHF) in Asia and Pacific countries [6]. This condition triggers an acute illness that can kill patients more rapidly than Acquired Immunodeficiency Syndrome, an immune disease (AIDS).

Chemical vector control application is a conventional approach; however, it has environmental and human dangers [7]. In recent years, repeated use of synthetic insecticides for mosquito control has weakened natural biological control mechanisms and resulted in resurgences in mosquitoes' population [8]. Repeated use of chemical pesticides led to the development of resistance by mosquitos against such pesticides [9]. It is necessary to find alternatives to control mosquitoes [10]. It is a big challenge to monitor mosquitoes without the effect of producing larvicide-resistant insects successfully. The World Health Organization (WHO) is also promoting the future use of several insecticides with various action modes. This can be accomplished either by using a mixture of insecticides, by cycling through multiple growing seasons, or by a combination of both [11].

Natural products from plant and marine animals showed promising effects to control mosquitoes [9]. Pesticides from

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plant origin are eco-friendly, readily biodegradable, and non-toxic to animals [12].

Marine organisms are considered an essential source for numerous bioactive compounds and secondary metabolites to combat these environmental challenges [9, 13]. Secondary metabolites produced by marine invertebrates like sponges displayed interesting insecticidal activities [9].

Carbon nanotubes exist in two different forms, including the Single-Walled Nanotubes (SWNT) and nanotubes with several walls (MWNT) [14]. The nanoparticles can be synthesized by using fungi, which render the nanoparticles more biocompatible. Therefore, bacteria, yeast, and fungi are potentially useful in preparing metal nanoparticles [14].

This study aimed to investigate the effect of the extracts of two Red Sea sponges, *X. testudinaria* and *A. chloros*, against *Ae. aegypti*, as well as the analysis of resistance extent in the strain and to evaluate the effect of the interaction of Multi-Walled Carbon Nanotubes (MWCNTs) and marine animal extracts against the larvae of mosquitoes *Ae. aegypti.*

Materials and Methods

Collection of Red Sea Sponges

The Red Sea sponges, *X. testudinaria,* and *A. chloros* **(Figure 1)** were collected from the Saudi Red Sea by Hands using SCUBA diving at different depths (-12-25 meters). The samples were kindly identified by Dr. Rob van Soest, The Naturalis, The Netherlands. Samples were kept frozen at -20 until organic extracts were prepared.

Mosquitos' Sampling

The mosquitoes, *Ae. Aegypti*, were collected from Jeddah and were reared in Mosquito Research Unit, King Abdulaziz University. The *Xestospongia testudinaria* and *Amphimedon chloros* were collected from the Red Sea coast in Saudi Arabia at depths from 15 to 25 meters by scuba divers. All samples were taken from the substratum and transferred to the laboratory of King Fahd Medical Research Center, Abdulaziz University. After that, it was freeze-dried to minimize problems with foaming and emulsions.

a)

b)

Figure 1. The Red Se sponges *X. testudinaria* (left) and *A. chloros* (right).

Preparation of the Crude Extracts of the Sponges

The samples were freeze-dried before extraction. The freeze-dried sponges were extracted with methanol solvent (absolute%) (2 × 800 mL). The extracts were dried under reduced pressure. The dried sample was resuspended insolvent.

Biosynthesis of Carbon Nanotubes Using Biological Extracts

An MWCNT (Sigma Aldrich, USA) stock solution was prepared according to the manufacturer guideline, using 0.02% Suwannee River Natural Organic Matter (SRNOM) as a dispersant in an ultrasonic bath (Decon FS300) for 2 hours. SWCNTs were synthesized using inductivity coupled plasma mass spectrometry (ICP-MS) and characterized using SEM, TEM, Raman spectroscopy, DLS, and zeta potential. Carbon nanotubes were synthesized in collaboration with the Environmental Protection and Sustainability Department at KAU.

Larvicidal Bioassay

Larvae of third instars were used for larvicidal bioassay. The stock solution was prepared by dissolving 1 g of the crude extracts into 99 mL of distilled water. The extract was kept within the refrigerator (3 °C) in dark glass containers until experiments were conducted. Twenty larvae of the 3rd instars of *A. aegypti* were tested, and five replications were used for each concentration. Four concentrations, including 62.5, 125, 250, and 500 ppm, along with a standard control, were used against larvae. The 'larvae's mortality rate was recorded after extract use, and larval mortality was calculated in each concentration.

Preparation of the Extracts with Carbon Nanotubes

One gram of each of the extracts of *X. testudinaria* or *A. chloros* were added, separately, to 1 mL of carbon nanotubes and 98 mL distilled water in a flask, and the mixtures were kept at room temperature for 24 h until the color changed.

Statistical Analysis

Data were expressed as a mean ± SD (1971). The statistics of mortality was carried out. In addition to 25%, 50%, 75%,

80%, 90%, and 95% of the test material's mortalities, the corresponding concentration Probit (Ldp line) of the 3rd instar larvae were estimated.

Results and Discussion

Bioassay of Carbon Nanotubes

Despite advances in medical research, mosquitoes are responsible for transmitting life-threatening pathogens in nearly all tropical and subtropical countries. The use of pharmacological control agents is, therefore, important. The results **(Table 1)** revealed that the active series of *A. chloros* extract concentrations were 62.5-500 ppm and the mortality rate of larval of *A. aegypti* mosquito of this concentration between 51-99 %, respectively. The results also show the toxicity line of *A. chloros* that needed to kill 50% of treated larvae after 48 h of this extract was 65.77 ppm **(Figures 2 and 3)**. While *A. chloros* was recorded, the confidence limit in the lower and upper of the LC_{50} value was 52.1635 and 77.8909 ppm. However, the concentrations of *A. chloros* that needed to kill 90% of *Ae. Aegypti* 3rd instar larvae were 209.7424, and the confidence limit in both lower and upper of LC_{90} value was 176.1857 and 267.217 ppm, respectively. The value of chi was 0.724.

On the other hand, *A. chloros* extract with carbon nanotubes showed significant concentrations against 3rd instars larvae of *Ae. aegypti* mosquito at 62.5-500 ppm, respectively, and the mortality rate of the 3rd larval instars of *Ae. aegypti* mosquito of this concentration was between 75-96, respectively **(Tables 1 and 3)**. Therefore, the toxicity line of *A. chloros* with CNTs that needed to kill 50% of treated larvae after 24 h of this extract was 15.569 ppm **(Figures 2 and 3)**. The value of the lower and upper confidence limit of LC_{50} was 2.751 and 32.3797 ppm. However, LC90 value was 408.121, and in a consecutive range, the LC_{90} limit was 173.8269 ppm. In sum, the value of chi was 0.0878, which means the toxicity line of *A. chloros* with CNTs was more effective and toxic without CNTs **(Table 1)**. The results in **Tables 2 and 3** showed the significant concentrations of *X. testudinaria* extracts were 62.5-500 ppm and the mortality rate of the 3rd larval instar of *A. aegypti* mosquito of this concentration between 41-98 ppm, respectively. Our results also showed the toxicity line of *X. testudinaria* that needed to kill 50% of treated larvae after 7 days of this extract was 95.6729 ppm **(Figure 2)**. While *X. testudinaria* was recorded, the confidence limit in lower and upper of LC_{50} value was 78.843 and 111.8294 ppm, and the concentrations of *X. testudinaria* were needed to kill 90% of *Ae. Aegypti* 3rd larvae were 375.6465 ppm, and the confidence limit in both lower and upper of LC_{90} values were 308.6802 and 490.1225 ppm, respectively. The value of chi was 3.4612 **(Table 2)**.

On the other hand, *X. testudinaria* extract with CNTs shows significant concentrations against 3rd instars larvae of *Ae. aegypti* mosquito and was 62.5-500 ppm, respectively, and the mortality rate of the 3rd larval instars of *Ae. aegypti* mosquito of this concentration between 7-97%, respectively **(Tables 2 and 3)**. Therefore, as shown in **Figure 2**, the toxicity line of *X. testudinaria* with CNTs needed to kill 50% of treated larvae after 72 h of this extract was 158.3125 ppm. However, the values ranged from 143.6556 to 174.2906 ppm in both lower and upper confidence limits of the LC_{50}. But in a consecutive 298.1747 and 406.3433 ppm the value was both lower and upper LC_{90} limit value. Finally, the value of chi was 1.7042 **(Table 2)**.

Table 1. Results of the Larvicidal Activities of *A. chloros* Extract with and without CNTs against *Ae. aegypti* Larvae

Extract Tested	Conc. (ppm)	Larval Mortality (%) Mean* ± SD	LC	Con ppm	Confidence Limit Lower – Upper	Slope	Chi**
Amphimedon chloros	62.5	51^{d} ±1.15	25	35.7236	46.0915-24.2809	2.5446	0.7240
	125	74^{C} ±1.17	50	65.77	52.1635 -77.8909		
	250	94^{b} ±1.05	75	121.0879	104.7328-140.8442		
	500	99^{a} ±0.57	90	209.7424	267.217-176.1857		
	Control	3^{e} ± 0.01	95	291.3802	402.573-234.2076		
Amphimedon chloros with CNTs	62.5	75^{d} ±1.12	25	3.6822	11.5333-0.2296	1.0772	0.0878
	125	84^{C}±0.577	50	15.569	2.751-32.3797		
	250	90^{b} ±1.14	75	65.8287	31.2113-96.0083		
	500	96^{a} ±0.48	90	240.9911	408.1217-173.8269		
	Control	2^{e} ± 0.03	95	523.9155	1424.4649-330.8671		

Table 2. Results of the Larvicidal Activities of *X. testudinaria* Extract with and without CNTs against *Ae. Aegypti* Larvae

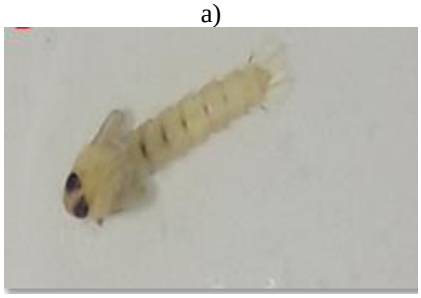
Extract Tested	Conc. (ppm)	Larval Mortality (%) Mean* ± SD	LC	Con ppm	Confidence Limit Lower – Upper	Slope	Chi**
Xestospongia testudinaria	62.5	41^{d} ±1.73	25	46.5774	33.5163 - 58.9324	2.1576	3.4612
	125	55^{C} ±1.15	50	95.6729	78.843 - 111.8294		

	250	81[b] ±1.15	75	196.5184	170.1258- 231.3436		
	500	98[a] ±1.17	90	375.6465	308.6802- 490.1225		
	Control	3[e] ± 0.12	95	553.554	432.8351- 782.416		
	62.5	7[d] ±1.15	25	105.6292	117.6326-92.8279		
Xestospongia testudinaria with CNTs	125	41[C] ±0.67	50	158.3125	143.6556-174.2906		
	250	77[b] ±1.15	75	237.2719	213.6052-268.766	3.8382	1.7042
	500	97[a] ±1.21	90	341.5209	406.3433-298.1747		
	Control	3[e] ± 0.31	95	424.6879	522.9458-362.2603		

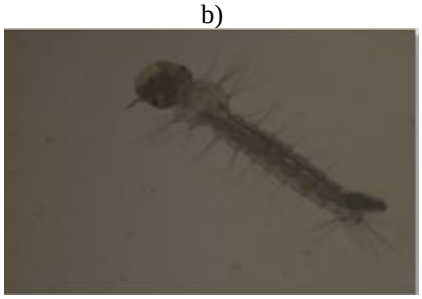
This study in agreement with many scientific studies shows that sponges play an essential role in controlling mosquitoes that transmit many diseases to humans. Investigation of the ethanol extracts of marine sponges *Topsentia ophiraphidites, Amphimedon compressa, Ircinia campana, Agelas sventres,* and *Svenzea zeai* showed that the extract of *Amphimedon compressa* was more effective against *Ae. Aegypti* [15]. The methanol extract of the marine sponge *Cliona celata* (Grantand) showed the highest larvicidal activity against *Ae. aegypti* and *Culex quinquefasciatus* larvae [8]. The methanol extract of the sponge *Acanthella elongate* displayed, among other marine sponge species, the highest larvicidal activity against larvae of *Culex* sp. [16].

The statistical analysis of the extract of the sponge *Amphimedon chloros* with CNTs showed high effectiveness against the *Ae. aegypti* mosquitoes in their larval stage and RR was 4.224 times more effective than *Amphimedon chloros* extract without CNTs **(Table 3)**.

a)



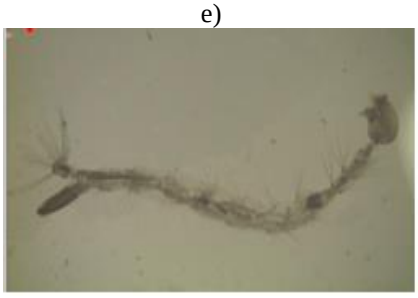
b)



c)

d)

e)



e)

Figure 2. The morphological deformities on *Ae. aegypti* mosquito larvae were treated using *A. chloros* and *X. testudinaria* extracts with CNTs and without CNTs. (a) Adult incompletely emerged and shown the body attached in the pupa exuviae. (b) The intermediate stage between pupa and adult (Pupa winged). (c) Segments of larval body contraction. (d) Albino pupa. (e) Pigmentation and neck elongation. (f) Evident elongation of the neck region & Cells explosion.

Table 3. Susceptibility of 3rd Instar of *A. aegypti* Larvae to the Extracts of *A. chloros* and *X. testudinaria* with and without CNTs followed by Continuous Exposure to the Extracts

Marine invertebrate extracts	Effect concentration (ppm)	Larval mortality (%)	Statistical parameters		
			LC_{50}	RR	Slope
A. chloros with CNTs	62.5-500	75-96	15.569	1	1.077

A. chloros	62.5-500	51-99	65.77	4.224	2.545
X. testudinaria	62.5-500	41-98	95.673	6.145	2.158
X. testudinaria with CNTs	62.5-500	7-97	158.312	10.168	3.838

Conclusion

Mosquitoes are an important group in blood-sucking arthropods. Despite advances in medical investigations, mosquitoes transmit various life-threatening pathogens in almost all tropical and subtropical regions. Recently, the use of biodegradable and eco-friendly insecticides has received much attention. This study evaluated the effect of the extracts of two Red Sea sponges, X. testudinaria and A. chloros, against Ae. aegypti, and analyzed the resistance extent in the strain. Moreover, the effect of the interaction of Multi-Walled Carbon Nanotubes (MWCNTs) and marine animal extracts against the larvae of mosquitoes Ae. aegypti was evaluated. In line with other studies, this study showed that sponges play a crucial role in controlling mosquitoes. The statistical analysis exhibited high effectiveness against Ae. aegypti mosquitoes in their larval stage and RR was 4.224 times more effective than Amphimedon chloros extract without CNTs.

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ETHICS STATEMENT: None

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