JOURNAL OF PURE AND APPLIED MICROBIOLOGY, Sept. 2016. Vol. **10**(3), p. 1949-1956

The Biological Effects of Some Marine Extracts Against *Aedes aegypti* (L.) Mosquito Vector of the Dengue Fever in Jeddah Governorate, Saudi Arabia

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(Received: 26 June 2016; accepted: 15 August 2016)

This study was designed to gain more advanced insights about the efficacy of some marine organisms that thrive along the Saudi Arabian red sea coast against the larvae of *Aedes aegypti* mosquito, the main transmitter of the dengue fever virus in Jeddah Governorate. Ethanolic extracts were prepared from marine organisms of seagrass *Thalassia hemprichii* and sea cucumber *Holothuria atra* in addition to the use of a commercial insecticide from a botanical origin Bug Slug as a kind of a positive control comparison. The preparation of the aqueous measuring solutions of the alcoholic crude extracts was conducted in addition to a series of concentrations for testing their efficacy against the 4th larval stage of *Ae. aegypti* according to the assessment and recommended protocols of the World Health Organization (WHO, 2005). Five replicates for each concentration was prepared with the positive control comparison and the recording of data on the mortality rate was all conducted 24 hours post larval treatment for all the aforementioned series of the concentrations. Our results showed that the percentage mortality of the 4th larval stage of *Ae. Aegypti* mosquito vector post-treatment with the extracts under investigation was positively correlated with the applied concentrations, however the commercial insecticide Bug Slug have produced the highest efficacy of the larval mortality of the mosquito vector with the LC_{50} values (the concentration that kills 50% of the larval population) as 0.0287 ppm and the Lower Confident Limit was (LCL = 0.02) and the Upper Confident Limit (UCL = 0.04 ppm) and this was followed by the efficacy of *H. atra* extract that gave LC_{50} =188.6 ppm (LCL = 181.9 and UCL = 194.9 ppm) then followed by the efficacy of *T. hemprichii* extract with LC_{50} = 201.7 (LCL=181.9 and UCL = 194.9 ppm). Based on the outcome of this investigation it is fair enough to say that the ethanolic extract of the sea cucumber *H. atra* contains promising constituents as insecticides against the larvae of the mosquito vector of dengue fever followed by the seagrass *T. hemprichii*.

Keywords: *Aedes aegypti*, marine extracts, larvicidal activity, commercial insecticide.

The diseases transmitted by the females of the mosquito vectors are considered causes of the urgent hygienic problems in the equatorial and semi –equatorial regions of the world (Samidurai *et al*., 2009). Until now the dengue fever is considered one of the most important viral diseases that is transmitted by the female vectors and is most prevalent and widely spread disease. Contemporary and to date there are no protective vaccines against the infection by the causal agents

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and the culprits of this menace albeit the rational management procedures to ameliorate or halt the progression is through the effective management of the mosquito vector *Ae. aegypti* that belongs to the family *Culucidae* of the order *Diptera* (Autran *et al.,* 2009).

Most of the current mosquito abatement programs are based on the use of larval insecticides at the foci of reproduction whereas adulticides are applied and become highly imperatives in the reduction of the population of adults but for short intervals of time (Andrade et al., 2008).The most currently active endeavors have been directed to the rational logistic natural effective extracts of the botanical origins as potent compounds for mosquito larvae (Silva *et al.,* 2008). Many studies reported that marine organisms contain a rich trove and valuable resources of precious chemical constituents which have biological activities against a variety of viral and fungal diseases (Ravikuma *et al.,*2011; Maye *et al.,* 2009).

The study carried out by Vijayakumar *et al.,* 2014, against the dengue fever mosquito vector showed that the extracts of the roots of *Halodule pinifolia* has led to high larval mortality with the values LC_{50} = 22.0 µL/mL, and LC_{90} =54.2 µL/mL then followed by the leaf extract of *Thalasia testudinum* that gave the values LC_{50} = 44.8 µL/mL and LC_{90} = 81.2 µL/mL, however, the leaf extract of *Cymodocea serrulata* gave the values LC_{50} = 42.9 µL/mL 24 hours post-treatment of the larvae.

More tests were conducted involving the assessment of the capability of 4 extracts of the marine weeds in combating the larvae of the dengue fever mosquito vector. The results proved that the extract of *Halophila ovalis* has given the highest larval mortality with LC_{50} = 0.067 and LC_{90} = 0.128 mg/mL. Therefore, it could be concluded from this study that the extract of the sea grass *H. ovalis* has a high possibility to be used in combating the dengue fever mosquito vector due to the high larval mortality activities (Ali *et al.,* 2013). Up to date there are many investigations have been underway on the marine life ,sea weeds and the mangrove plants under certain empirical screening and scientific scrutiny because some of these plants have shown an appreciable efficacy in incurring outstanding fatalities of the mosquito larvae. The isolation of these valuable desired constituents from these plants would be more beneficial in the creation of a natural diverse plethora of highly needed insecticides that could be applied predominantly and widely in a variety of situations as a rational complement and component in the integrated management programs of mosquito vectors the vicious transmitters of many human ailments and diseases (Thangam and Kathiresan,1996).

Currently there are many botanical extracts which have efficacies against mosquito larvae because of their rich biologically active eco-friendly chemical constituents (Nazar *et al.,* 2009). The results arising from the studies have shown evident variations in their sensitivity levels for the *Ae. aegypti* mosquito larvae based on the different compounds under investigation. This might be due to differences that emanate from the prevalence of a variety of their basic chemical components of each extract under investigation including phenolic and sugars that have active fatal effect on mosquito larvae (Nazar *et al.,* 2009) which stimulates and embraces the goals and the main objectives of these studies. Moreover, Hemalatha *et al.,* 2015 have reported an appreciable active role on the fatal effects on the mosquito larvae from the commercial botanical extract of *Lantana camara aculeate* against many species of mosquitoes including *Aedes aegypti*, *Anopheles stephensi* and *Culex quafasciatus*. They reported that this might be due to the fact that their leaf extracts contain a rich maze of chemically useful compounds including tannins, alkaloids, flavonoids, anthocyanins, kinenes, trepenoids, saponins and steroids. On the same token Ajaegbu *et al.,* 2016 have suggested that the difference in efficacy may be attributed to the different solvents that might be used during the assessment of the efficacy of the extracts of methanol, hexane, dichloroethane, ethyl acetate of *Spondias mombin* plant leaves against *Ae. aegypti* mosquitoes. Moreover, the extract of dichloromethane has shown the highest efficacy when compared with the other organic extracts. The study concluded that the botanical *Spondias mombin* plant extract are considered an excellent candidate as a natural safe alternative for mosquito abatement programs. Generally speaking the difference in the level of sensitivity of the domestic mosquito larvae for the compounds under investigation and evidently their extracts may be attributed to the differences rooted in their active

constituents and their mode of action on the mosquito larvae which was reported by many research workers from previous studies conducted in different regions of the world (Mahyoub *et al.*, 2014; Gahukar, 2014).

This study was designed to determine the larvicidal activity of marine organisms seagrass *Thalassia hemrichii* and sea cucumber *Holothuria atra* against *Aedes aegypti* mosquito, vector of dengue fever in Jeddah governorate in addition to the use of a commercial insecticide of a botanical origin Bug Slug as a positive comparison control.

MATERIALS AND METHODS

Collection of materials

Seagrass *Thalassia hemprichii* and Sea cucumber *Holothuria atra* were collected from Northern corniche of Jeddah and Southern corniche of Jeddah, respectively (Fig.1). The identification of the samples was authenticated at Marine Biology Department, Faculty of Marine Sciences, King Abdulaziz University, Jeddah. The fresh selected samples were washed with distilled water in order to clean dust and remove the adhering salts and other associated organisms or other particles that might stick to them. The material was kept for drying under shade at room temperature (27±2°C) till they are dried completely. The dried samples were finely powdered using electric blender (Saranya *et al.*, 2013).

Preparation of extracts

The dried and powdered samples (1000 g) were subjected to extraction three times with 70% Ethanol in H_2O (v/v) at room temperature. The extracts were filtered and the solvents were evaporated under reduced pressure at low temperature using a rotavapor until dryness. This powdered extract was used for experiments (Hawas *et al*., 2013).

Mosquito larval culture

For the purpose of having adequate larval population for conducting the experiments, a mosquito colony was established under laboratory conditions in the premises of the mosquito dengue fever research station that belong to the Department of Biological Sciences at King Abdul-Aziz University, Jeddah. The dengue fever mosquito vector egg masses were obtained from the station where filter paper containing the eggs were immersed in small ceramics sizes 30-20cm filled half way with water free from chlore and the rearing of the larvae was continued for a number of generations according to the method by Mahyoub, 2013.

Larvicidal activity

Test the sensitivity of the larvae of *Ae. aegypti* to the alcoholic crude extract for the selected samples was carried out based on the method and special recommended measurements of the World Health Organization (WHO, 2005). To cater for the preparation of the aqueous measuring solutions 0.1 g of each extract was dissolved in 0.5 ml of triton-x 100 and 99.5 mL of distilled water was added. Then a series of concentrations were prepared including 5 concentrations of each extract and each concentration has 5 replicates where each replicate containing 100 ml water +20 late 3rd instar or early 4th instar larvae and the mortality rate of each replicate was calculated for each concentration 24 hours post-treatment. The number of larvae that are considered dead was counted including those that are motionless after being pricked by a needle at the neck region or the siphon and showed no response. The observed malformed or deformed dead larvae were photographed under the microscope with a digital camera connected to a computer stereo dissecting microscope (leica ez4ds/no:56499000).

Statistical analysis

The Idp line software program was applied during the statistical analysis based on the degree of probability Probit analysis and calculating LC_{50} and LC_{90} together with the lower and upper confident limits and the inclination of toxicity line and Chi square according to Finney,1971.

RESULTS AND DISCUSSION

Our results showed that the mortality rate of the 4th larval instars of *Ae. aegypti* mosquito treated with a series of concentrations of the seagrass *T. hemrichii* and sea cucumber *H. atra* were positively correlated with the used concentrations (Figs 2,3) and the effective concentrations were between 150 - 300 ppm, and the corresponding mortality rate of these concentrations between 20 - 96%. Then the sensitivity of the 4th larval instar of *Ae. aegypti*

was assessed for the botanical extract prepared in a commercial manner under the name Bug Slug for comparison with the extracts that were prepared from the local environment. Hence the results showed an evidently clear reduction by the applied concentration when compared with already mentioned formulations where the effective concentrations were between 0.01 - 0.1ppm and the mortality rate corresponding to these concentrations between 20 - 94%. Moreover the results showed a positive correlation between the concentrations under investigation and the mortality rate of the treated larvae (Fig.4).

On the other hand the statistical analysis of data of the laboratory toxicity of the extracts under investigation reflected a clear variation in the level of sensitivity of the mosquito larvae under the effect of the prepared formulations. Moreover from the toxicity lines LC–p line and the recovery of the values of the mortality concentrations for 50% and 90%of the larvae that were subjected to the different concentrations of the extracts under investigation are shown in (Figs. 5,6 & table 1), there was an apparent variation in the larval sensitivity based on the applied botanical extract. The concentrations of sea cucumber *H. atra* extract needed to kill 50% and 90% of the treated larvae 24 hours post –treatment for this extract were 188.59 and 261.28 ppm consecutively, while the extract concentrations of the seagrass *T. hemrichii* that

Table 1. Susceptibility of *Ae. aegypti* larvae to Compound tested by dipping technique following continuous exposure for 24 h

Compound tested	Conce. (ppm)	Larval Mortality(%) Mean* ± SD	LC	Con. (ppm)	Confidence limit Lower -Upper	Slope	Chi**
T. hemprichii	150	23.00*± 1.50	25	156.1	143.1 - 166.3	6.06	1.45
	180	35.00*± 2.00	50	201.7	192.4 - 210.9		
	210	58.00*± 2.29	75	260.6	246.2 - 281.2		
	240	65.00*± 2.00	90	328.2	300.7 - 372.4		
	300	86.00*± 2.00	95	376.7	338.0 - 441.7		
H. atra	150	20.00*± 1.50	25	158.9	150.1 - 165.9	9.05	2.6
	180	37.00*± 0.89	50	188.6	182.0 - 194.9		
	210	71.00*± 2.00	75	223.9	216.1 - 233.5		
	240	83.00*± 3.00	90	261.3	248.6 - 278.9		
	300	96.00*± 2.00	95	286.6	269.8 - 310.8		
Bug Slug	0.01	20.33*± 0.59	25	0.01	0.01 - 0.02	2.24	6.08
	0.03	43.03*± 0.21	50	0.03	0.02 - 0.04		
	0.05	68.03*± 0.15	75	0.06	0.04 - 0.09		
	0.08	84.03*± 0.60	90	0.11	0.08 - 0.22		
	0.1	88.87*± 1.00	95	0.16	0.12 - 040		

*. The mean difference is significant at the 0.05 level.
** Chi square tabulated = 7.8 (When tabulated (Chi)2 larger than calculated at 0.05 level of significance indicates the homogeneity of results).

Table 2. Comparison Efficiency of Compound tested against larvae of *Ae. aegypti* by Anova

Compound tested	Source of Variances	Degree of Freedom	Mean Square (MS)
T. hemprichii	Between Groups	4	1860.900*
	Within Groups	10	3.900
H. atra	Between Groups	4	3048.900*
	Within Groups	10	4.008
Bug Slug	Between Groups	4	2499.776*
	Within Groups	10	0.355

* Significant at $p \leq 0.05$ level

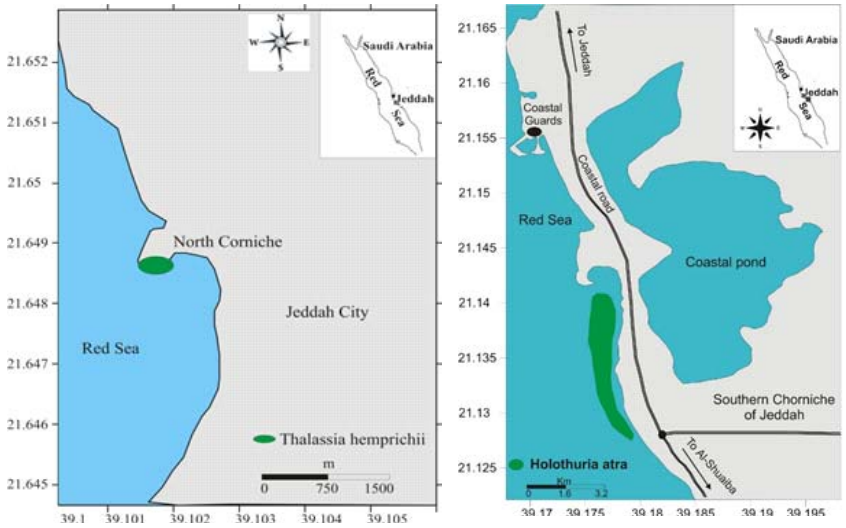

Fig. 1. Locality sites of Sample collection

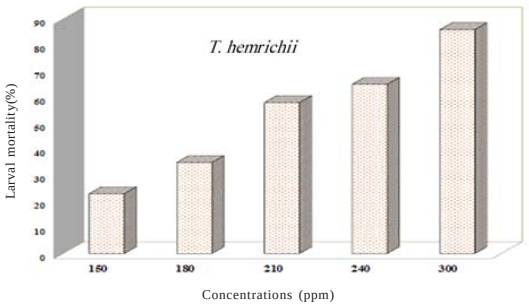

Fig. 2. The relationship between the concentrations of the *T. hemprichii* and the mortality rate of the larvae of the mosquito *Ae.aegypti*

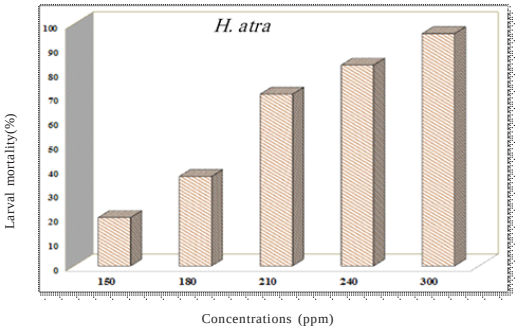

Fig. 3. The relationship between the concentrations of the *H. atra* and the mortality rate of the larvae of the mosquito *Ae.aegypti*

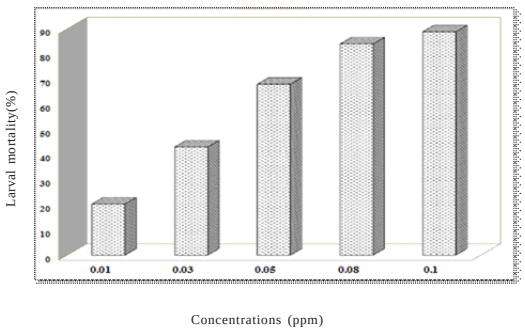

Fig. 4. The relationship between the concentrations of the Bug Slug and the mortality rate of the larvae of the mosquito *Ae.aegypti*

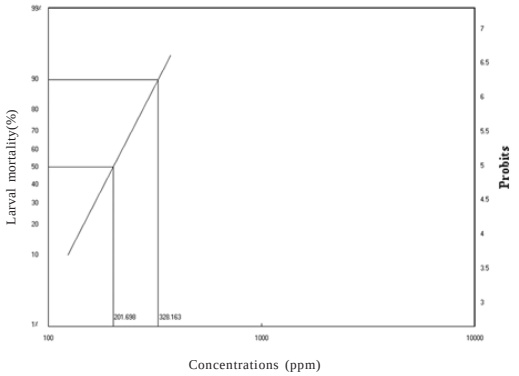

Fig. 5. Laboratory toxicity line of the seagrass *T. hemprichii* extract with the fixed statistics in relation to the determination of the level of efficacy against the larvae of *Ae. aegypti* mosquito

needed to produce a killing effect of 50% and 90% of the larvae post-treatment were 201.69 and 328.16 ppm consecutively. Moreover, in the case of the commercial botanical extract Bug Slug the needed concentration to kill 50% and 90% of the larvae were 0.0287 and 0.1069 ppm after 24 hours post–treatment (Fig.7 & table 1). These results proved that the commercial botanical insecticide extract Bug Slug gave the highest efficacy against the 4th instar larvae of the *Ae. aegypti* mosquito followed by the alcoholic extract of the sea cucumber *H. atra* and the alcoholic extract of the seagrass *T. hemprichii*, gave the lowest effect of these extracts under investigation.

The values of the indicator of resistance ratio (RR) also showed that the larvae of *Ae. aegypti* were more sensitive to the Bug Slug extract than the marine extracts under investigation. The efficacy of the Bug Slug extract was higher than the extract of the sea cucumber *H. atra* and the extract of the seagrass *T. hemprichii* by about 6502.966 and 6955.108 folds consecutively, also the extract of the sea cucumber *H. atra* gave higher efficacy than the seagrass *T. hemprichii* by about 1.0287 fold (Fig. 8 & table 2) .

The results also showed the presence of morphological deformations on the treated larvae with the sea cucumber extract *H. atra*. These

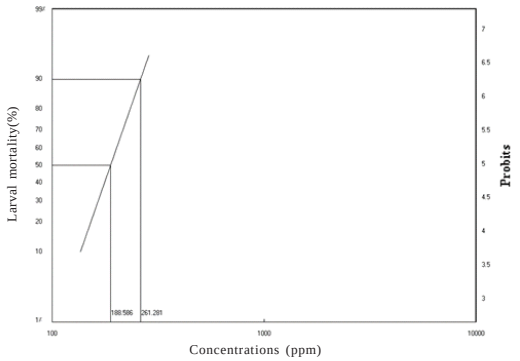

Fig. 6. Laboratory toxicity line of the sea cucumber *H.atra* with some fixed statistics related to the determination of the level of efficacy of the compound against the larvae of *Ae. aegypti* mosquito.

Fig. 8. The morphological deformations on *Ae.aegypti* mosquito larvae after 24 hours post- treatment with the sea cucumber: *H. atra* extract.

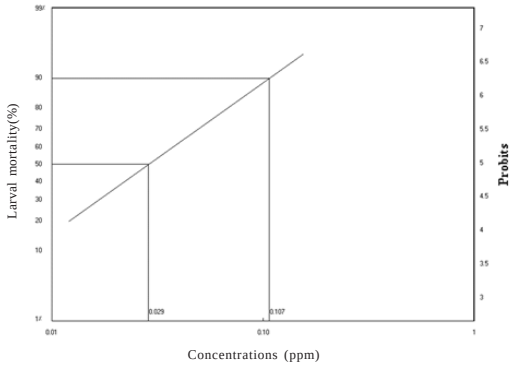

Fig. 7. Laboratory toxicity line of the commercial botanical extract Bug Slug with fixed statistics as related to the determination of the level of efficacy of the compound against the larvae of *Ae. aegypti* mosquito.

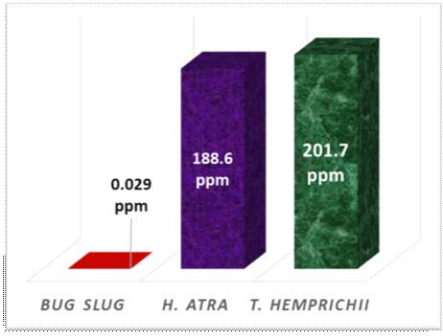

Fig. 9. The values of the fatal concentrations for 50% of *Ae.aegypti* mosquito larvae treated with the immersion technique in the extract .1.Bug Slug, 2. *H.atra* and 3.*T. hemprichii*

deformations manifested as shortening and contraction of body segments specially on the larval abdomens which might be due to an imbalance in the secretion and distribution of the body pigmentation, therefore the affected larvae were totally black or it appeared as black patches and spots as pigmented areas on all body parts with clear evident elongation of the neck region when compared with the untreated larvae (Fig.9).

These observed and reported deformations on the larvae treated with the sea cucumber extract may be due to the fact that it contains many valuable valid active chemical constituents such as saponins and terpenes which might be viewed as playing the role of synthetic analogue of the juvenile hormones or other effective hormone mimics and their involvement and intrusion with the physiological processes during the insect metamorphosis and it might be attributed to the stoppage or inhibition by the hormones that regulate important vital processes that could lead to the imbalance in stimulation or inhibition of the hormones secretion of those chemical constituents. This situation may contradict other hormones or enzymes that are produced from the endocrine glands which spontaneously lead to an imbalance of the growth processes and larval fatalities (Silva and Mendes, 2007; Arivoli and Tennyson, 2011; Mehdi *et al*., 2012 and Grzybowski *et al*.,2013).

ACKNOWLEDGMENTS

This Project was funded by the Deanship of Scientific Research (DSR) at King Abdulaziz University, Jeddah, under grant no. (G-256-130-37) The authors therefore, acknowledge with thanks DSR for technical and financial support. Also our sincere thanks are further extended to the staff and technicians of the research station of the dengue fever laboratory rearing facility and the technical staff of the Department of Biological Sciences, College of Science at King Abdulaziz University, Jeddah for their help and support during this study.

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