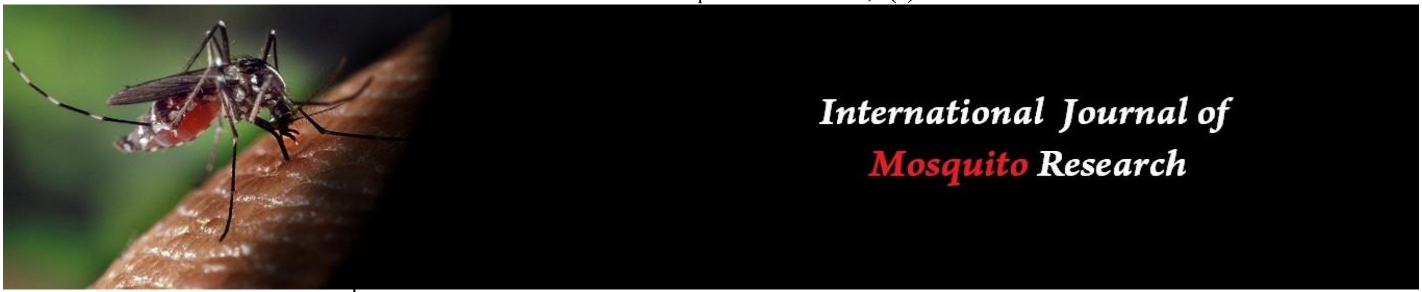

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Mosquito Larvicidal activity of seaweed extracts against *Anopheles d'thali* with reference to its side effects on aquatic nontraget organisms

Jazem A Mahyoub

Abstract
The biological effects of three Seaweed extracts Seagrass *Thalassia hemprichii*, Sea lettuce *Ulva lactuca*, and Gray Mangrove *Avicennia marina* against 4*th* instar mosquito larvae of *Anopheles d'thali* were evaluated. Values of IC_{50} (concentration which to inhibit the emergence of 50% of mosquito adults survived from larval treatments) indicated that *A. marina* (107.3 ppm) proved to be the most effective extract against *An. d'thali*, followed by *T. hemprichii* (197.3 ppm) while the seaweed extract *U. lactuca* (335.3 ppm) was the least effective one. Variations in susceptibility levels of *An. d'thali* mosquito larvae may probably due to differences in toxicity levels among the active components of seaweed extracts. On the other hand, treatments with IC_{90} values of the test extracts *A. marine* (284.7 ppm), *T. hemprichii* (293.8 ppm) and *U. lactuca* (588.3 ppm) did not have any adverse effects towards some aquatic nontarget organisms (Odonata naiads, aquatic hemipterous, aquatic coleopterans) prevailing in mosquito breeding sites.

Keywords: *Anopheles d'thali,* seaweed extracts, larvicidal activity

Introduction

There are nearly 3,500 mosquito species in the world but a more fraction of them transmit several human diseases in different parts of the world [1]. Problems related to the use of chemical insecticides for controlling mosquitoes have not only resulted in development of resistance in mosquito strains but have also caused environmental pollution and the death of nontarget organisms including natural enemies and beneficial organisms such as honeybees and fish [2, 3].

Most of the current mosquito control programs are based on the use of larval insecticides at the breeding sites. 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 [4]. Large numbers of plant species have been screened for their insecticidal activities. The potential of botanical products as reducing and stabilizing agents for the synthesis of nano-pesticides has been also investigated [5].

In recent years, many studies mentioned that marine natural products contain valuable resources, of precious chemical components which have biological activity against a variety of fungal and bacterial diseases [6, 7]. On the other hand, marine extracts of some seaweeds, sponges and algae have been reported for their potential larvicidal action against different mosquito vectors [8, 9, 5]. Such marine extracts may serve as a suitable alternatives to synthetic chemical insecticides in the future as they are relatively safe, biodegradable and are easily available around the world [10, 11].

The present study was planned to evaluate the larvicidal activity of three marine extracts of Seagrass *Thalassia hemprichii*, Sea lettuce *Ulva lactuce* and Gray Mangrove *Avicennia marina* against mosquito larvae of *Anopheles d'thali*, the vector of malaria in Kingdom of Saudi Arabia [12]. The possible side effect of such marine extracts against some aquatic natural enemies was also studied.

Materials and Methods

Mosquito Strain

Tests were performed on a field mosquito strain of *An. d'thali* raised from wild larvae, collected from Jeddah governorate, Saudi Arabia, and had been maintained in the laboratory under controlled conditions of 27±1°C and 70±5% R.H., with a 14: 10 (L: D) photoperiod throughout the research work (January–june,2018). The larvae were reared until pupation and adult emergence took place for maintaining the stock culture.

Collection of Seaweeds

Fresh seaweeds of Seagrass *T. hemprichii*, Sea lettuce *U. lactuca* and Gray Mangrove *A. marina* were collected from southern corniche of Jeddah governorate, Saudi Arabia (Fig. 1). The samples were washed in distilled water to remove the adhering salts and sand and air-dried under shade. The seaweed extracts were prepared by using the standared method of [13]. The dried seaweeds were finely powdered using electric blender. Samples of the seaweed powder each of 40-60 gm were extracted in Soxhlet apparatus for 6 hrs using 200 ml of absolute acetone. The extracts were concentrated using a rotary vacuum evaporator to become semi-dry material. The extracts were kept at–20°C until used.

Bioassay Tests

The stock solution of each seaweed extract was prepared by adding 1gm of it to 99 ml of distilled water containing 0.5% triton X-100 as an emulsifier to ensure complete solubility of the extract in water. Series of concentrations were prepared in distilled water. The susceptibility level of mosquito larvae of *An. d'thali* to the test seaweed extracts of *T. hemprichii*, *U. lactuca* and *A. marina* was conducted following the method of [14]. Larval treatments were carried out by exposing the early 4*th* instar larvae of *An. d'thali* to various concentrations of the tested extracts in groups of glass beakers containing 100 ml of tap water. Five replicates of 20 larvae each per concentration, and so for control trials were set up. The larvae were given the usual food during the experiments.

Mortalities of larvae and pupae were recorded daily. Live pupae were transferred to untreated water in new glass beakers for further observations, i.e. normal emergence, morphological abnormalities or death.

Additional tests were also conducted to study the possible side effects of the above seaweed extracts on some aquatic nontarget organisms associated with mosquito breeding habitats. The test organisms were collected from a pond water known to be mosquito breeding sites. The nontarget organisms were Odonata naiads, aquic bugs (Or: Hemiptera) and diving beetles (Or: Coleoptera). The concentrations corresponding to IC_{90} values were derived from the toxicity lines of the present seaweed extracts and prepared for testing against the aquatic organisms. Treatments were carried out by exposing the individuals to the test concentrations in 500 ml glass beakers with 100 ml of tap water. Three replicates of five individuals each per concentration, and so for control were set up. Individual mortalities were recorded after 24 hr post treatment and compared with control trials.

Statistical analysis

Data of larval mortality were subjected to statistical analysis. The SPSS software package was used for computing all the data including probit analysis and statistical parameters such as IC_{50}, IC_{90}, slope and Chi-square.

Results and Discussion

Susceptibility levels of *An. d'thali* larvae following treatments with different concentrations of seaweed extracts *A. marina, T. hemprichii* and *U. lactuca* are shown in Table 1&2 and illustrated by Fig. 2. In general, 18-71%, 8-78% and 17-88% larval mortalities were obtained when the 4*th* instar larvae were treated with effective concentrations of *A. marina* (50-250 ppm), *T. hemprichii* (150-300 ppm) and *U. lactuca* (200-600 ppm). On the other hand, treatment with the test seaweed extracts produced different biological effects against the present mosquito larvae similar to those induced by other insect growth regulators (Fig. 3). Most 4*th* instar larvae failed to ecdyse to pupae producing larval-pupal intermediates. Dead adults with folded wings in the pupal exuvia were also observed [15]. Therefore, in the present work, cumulative mortalities during larval development to pupae and adults have been taken as a criterion for evaluating the tested seaweed extracts against the target mosquitoes. The records showed that larval treatments with the effective concentrations of the above extracts caused 24-90%, 19-89% and 18-91% inhibition of adult emergence, respectively.

Tacking IC_{50} values (concentration which to inhibit the emergence of 50% of adults) into consideration, *A. marina* (107.3 ppm) proved to be the most effective extract against *An. d'thali*, followed by *T. hemprichii* (197.3 ppm) while the seaweed extract *U. lactuca* (335.3 ppm) was the least effective one. In other words, the results thus indicated that mosquito larvae of *An. d'thali* were more susceptible to *A. marina* than *T. hemprichii* and *U. lactuca* by about 1.8 and 3.1 folds, respectively. It can be concluded that the response of the present mosquito larvae depends entirely on the type of extract and its effective concentrations. The fluctuation in the percentage inhibition of adult survivors obtained for the different concentrations of the test seaweed extracts against the present mosquito larvae support this conclusion. Similar studies in this respect were carried out by several authors [16, 17, 18, 11]. They pointed out that such marine extracts have a high possibility to be used in controlling different mosquito species. This conclusion might be due to the fact that these marine extracts contain effective components (i.e. alkaloids, flavonoids, trepenoids, saponins and steroids) with larvicidal properties against mosquitoes [19, 20, 21]. Table 3 shows the possible side effects of the present seaweed extracts on some nontarget organisms (Odonata naiads, aquatic bugs and aquatic beetles) prevailing in mosquito breeding sites. In general, the records showed that treatment with IC_{90} values (concentration which to inhibit 50% of mosquito adults survived from larval treatments) of seaweed extracts *A. marina* (284.7 ppm), *T. hemprichii* (293.8 ppm) and *U. lactuca* (588.3 ppm) did not exhibit any marked lethal impact toward the above aquatic nontarget organisms. No published data on the effect of other marine extracts on aquatic insects are available for comparison. However, long term follow-up trials were needed to elucidate the possible delayed effects of larval treatments with marine extracts on some biological and behavioural aspects of mosquito adult survivors.

Table 1: The biological effects of seaweed extracts on the developmental stages of *An. d'thali*

Seaweed extracts	Concentrations (ppm)	Larval mortality [a] (%)	Pupae produced (%)	Adults hatched (%)	Inhibition [b] (%)
A. marina	50	18	82	76	24
	100	27	73	61	39
	150	52	48	33	67
	200	60	40	18	82
	250	71	29	10	90
T. hemprichii	150	8	92	81	19
	180	19	81	58	42
	210	36	44	41	59
	240	54	46	22	78
	300	78	22	11	89
U. lactuca	200	17	83	82	18
	300	19	81	64	36
	400	56	44	31	69
	500	74	26	16	84
	600	88	12	9	91

a Five replicates, 20 larvae each.
b Inhibition of adult emergence in control ranged from 2-4%.

Table 2: Statistical parameters calculated from toxicity lines of the tested seaweed extracts against *An. d'thali* mosquito larvae

Seaweed extracts	Statistical parameters			
	IC_{50} (ppm)	IC_{90} (ppm)	Slope	Calculated* $(Chi)^2$
A. marina	107.3 (96.5-117.9)	284.7 (245.8-346.9)	3.0253	6.8648
T. hemprichii	197.3 (189.6-204.9)	293.8 (275.3-321.1)	7.4137	1.9954
U. lactuca	335.3 (315.6-354.5)	588.3 (541.4-655.1)	5.2491	2.8507

* Tabulated $(Chi)^2$ = 7.8 at 5% probability level indicating that the toxicity lines are good fit and the data significantly homogeneous.

Table 3: The effect of seaweed extracts on aquatic nontarget organisms following continuous exposure for 24 hr.

Seaweed extracts	IC_{90} (ppm)[a]	Mortality[b] (%)		
		Aquatic haiads (Or. Odonata)	Aquatic bugs (Or. Hemiptera)	Aquatic beetles (Or. Coleoptera)
A. marina	284.7	0.00	0.00	0.00
T. hemprichii	293.8	0.00	0.00	6.7
U. lactuca	588.3	0.00	0.00	0.00

a Concentration which to inhibit the emergence of 90% of mosquito adults survived from larval treatment.
b 3 replicates, 5 individuals each.

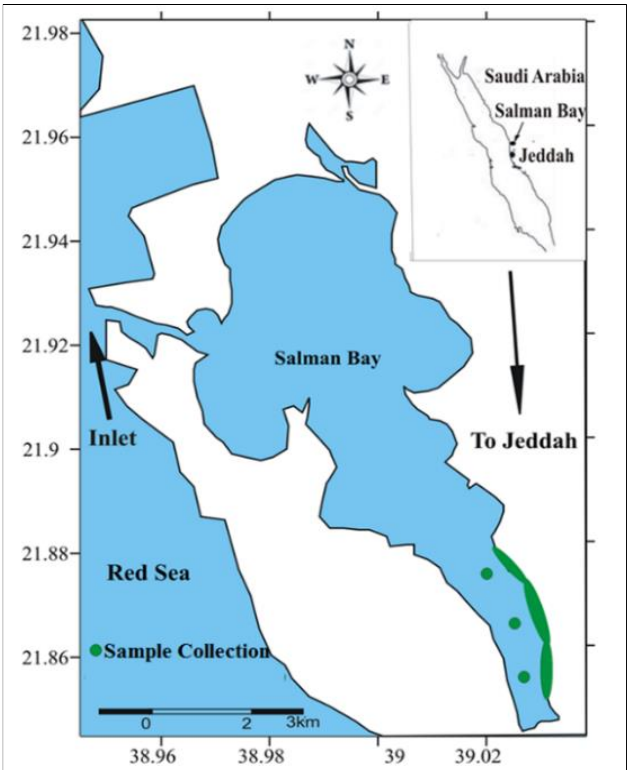
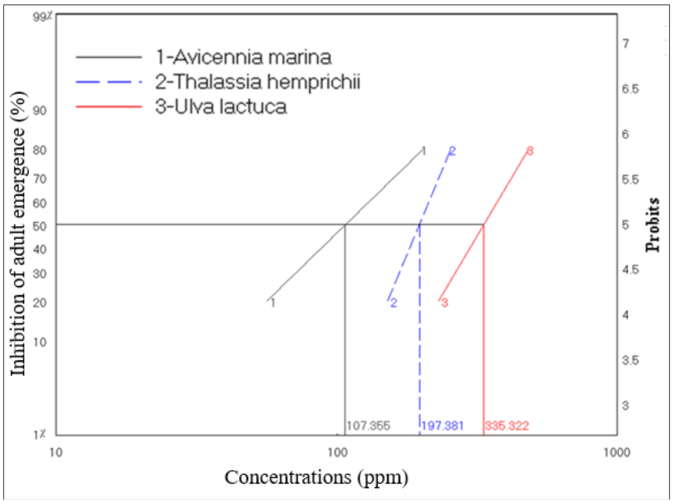


Fig 1: Locality sites of samples collection



Concentrations (ppm)

Fig 2: The relation between concentrations of seaweed extracts and the percentage of inhibition of adult emergence after treatment of 4th instar larvae of *An. d'thali*

Fig 3: Abnormalities in the developmental stages of *An. d'thali* after treatment with some seaweed extracts

Conclusion

It is evident from our results that treatment with seaweed extracts, seagrass *T. heperichii*, Sea lettuce *U. lactuca* and Gray mangrove *A. marina* exhibited promising mosquito larvicidal activity against *An. d'thali* and in the same time have a good margin of safety to aquatic natural enemies.

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