Advances in Zoology and Botany 12(1): 63-75, 2024
DOI: 10.13189/azb.2024.120107

Toxic Effect of Green Seaweeds on the Larval Instars of Vector Mosquitoes

Vaithiyanathan Selvi[1], Subramanian Arivoli[2], Samuel Tennyson[3,*]

[1]Department of Microbiology, Sourashtra College, India
[2]Department of Zoology, Thiruvalluvar University, India
[3]Department of Zoology, Madras Christian College, India

Received December 28, 2022; Revised March 22, 2023; Accepted November 14, 2023

Cite This Paper in the Following Citation Styles

(a): *[1] Vaithiyanathan Selvi, Subramanian Arivoli, Samuel Tennyson , "Toxic Effect of Green Seaweeds on the Larval Instars of Vector Mosquitoes," Advances in Zoology and Botany, Vol. 12, No. 1, pp. 63 - 75, 2024. DOI: 10.13189/azb.2024.120107.*

(b): *Vaithiyanathan Selvi, Subramanian Arivoli, Samuel Tennyson (2024). Toxic Effect of Green Seaweeds on the Larval Instars of Vector Mosquitoes. Advances in Zoology and Botany, 12(1), 63 - 75. DOI: 10.13189/azb.2024.120107.*

Abstract Seaweed species have been reported for their toxic effects on mosquito larvae. In the present study, the petroleum ether, chloroform, acetone and methanol extracts of two green seaweeds, *Caulerpa racemosa* and *Ulva fasciata* were tested for toxicity against the second and third instar of *Aedes aegypti* and *Culex quinquefasciatus* as per the guidelines of World Health Organization at concentrations of 100, 200, 300, 400 and 500 mg/L for 24 hours. *Caulerpa racemosa* extracts recorded 100% mortality at the highest concentration on the second and third instar of *Aedes aegypti* and *Culex quinquefasciatus*, and maximum larvicidal activity was exhibited by the chloroform extract, and their respective LC_{50} values were 140.49 and 144.554 mg/L, and 153.704 and 158.313 mg/L. In the case of *Ulva fasciata*, the chloroform extract exhibited 100% mortality at the highest concentration on the second and third instar of *Aedes aegypti* and *Culex quinquefasciatus*, and also the maximum larvicidal activity with LC_{50} values of 158.358 and 166.025 mg/L; and 154.156 and 187.435 mg/L against the second and third instar larvae, respectively. Overall results indicated that amongst the two green seaweeds tested, *Caulerpa racemosa* exhibited more activity when compared to *Ulva fasciata*, and with reference to solvent extracts, the chloroform extract exhibited maximum activity against the larval instars of the vector mosquito species tested. With regard to the vector mosquito species tested, *Aedes aegypti* was more susceptible than *Culex quinquefasciatus*, and in the case of instars, second instar larvae were more susceptible than the third instar. In conclusion, the bioassay result of the present study indicated the larvicidal property of the chloroform extract of both the green seaweeds against the larval instars of vector mosquitoes, which encourages further investigation on its bioactive compounds that might own virtuous larvicidal properties when isolated in pure form.

Keywords Green **S**eaweeds, *Caulerpa racemosa*, *Ulva fasciata*, Solvent Extracts, Larvicidal Activity, *Aedes aegypti*, *Culex quinquefasciatus*

1. Introduction

Mosquitoes are ravaging humans and other animals for generations. The mosquito vector-borne diseases, malaria, dengue, chikungunya, filariasis, and Japanese encephalitis comprehend the global disease incidence as the control of these disease transmitting vectors are challengeable globally [1]. The immense usage of many synthetic/chemical aerial, terrestrial and aquatic insecticides offers logistic problems on the environment and causes resurgence of different mosquito-borne diseases, and has stimulated investigations for environmentally safe, bio-degradable and target specific insecticides against mosquitoes [2]. This situation has focused more attention on discovering novel beneficial natural products, and has immensely contributed to stimulating the increasing interest in unconventional and unexplored sources of

natural products. In this context, seaweeds have attracted much attention over the past four decades [3]. Marine macroalgae popularly known as seaweeds are autotrophic groups of ecologically important vegetation of oceanic ecosystem that contain secondary metabolites [4], with economically potential renewable and extraordinary sustainable resources [5]. Researchers have found that the seaweeds possess good mosquitocidal properties [6], like bio-insecticides derived from that of terrestrial plants [7-9]. The idea of using marine macroalgae to combat mosquito larvae is not new [10,11]. Certain species of green macroalgae kill larvae primarily because they are indigestible, while blue-green algae offer possibilities for delivery as larvicides since they act as neuro and hepatotoxins to mosquito larvae [12,13]. The long history of seaweed based products in insecticide research on discovering new active agents in seaweeds in growing, and on top of that, many reports have revealed seaweeds' profound insecticidal properties on mosquitoes [6]. Considering the biodiversity of seaweeds in tropical regions, there is a need to study their larvicidal potential, since active metabolites of seaweeds possess larvicidal properties [6,14]. Therefore in the present study, the crude extracts of green seaweeds, viz., *Caulerpa racemosa* and *Ulva fasciata* were tested for their toxicity on the larval instars of *Aedes aegypti* and *Culex quinquefasciatus*, the principal vectors for dengue and filarial fever, respectively.

2. Materials and Methods

2.1. Seaweed Collection

Green seaweed species, *viz*., *Caulerpa racemosa* (Forsskål) J.Agardh (Caulerpaceae) commonly called sea grapes and *Ulva fasciata* Delile (Ulvaceae) popularly known as sea lettuce were collected by hand picking from the intertidal zone of Rameswaram, Tamil Nadu, India (8° 46 N, 78° 9 E and 9° 14 N, 79° 14°E), rinsed in water to remove sand and other particles, and transferred to laboratory in sterilized ziplock bags for further studies. Taxonomical identification and confirmation of the two collected seaweeds was done at the Marine Algal Research Station Mandapam, Tamil Nadu, India with the help of morphological key characters and identification manual [15-17].

2.2. Preparation of Seaweed Extracts

The two green seaweeds were shade dried at room temperature for a week, and were powdered with the aid of a mixer grinder. The powdered sample of each seaweed species (250g) was sequentially suspended in a selective solvent system ranging from non-polar to polar (petroleum ether, chloroform, acetone and methanol) for 72 hours, (750 mL for each solvent), and then soxhlated for eight hours to obtain crude extracts [18]. Thereafter, each extracted sample was filtered using Whatman No.1 filter paper, and the filtered sample was individually centrifuged at 5000 rpm for 10 minutes at 4 ℃, and the supernatant was collected in a separate flask. Each extract was then concentrated using a rotary vacuum evaporator (Puchi RII, Switzerland). The final concentrated crude solvent extract of each seaweed obtained was individually stored in sterile air tight bottles and kept in a refrigerator until further use. Prior to this, the percentage of yield of extraction of the crude extracts was calculated.

2.3. Test Vector Mosquitoes

The eggs of *Aedes aegypti* and egg rafts of *Culex quinquefasciatus* were procured from Centre for Research in Medical Entomology (CRME), Indian Council of Medical Research (ICMR), Madurai, Tamil Nadu, India. Larvae of each test vector mosquito species were reared separately in larval enamel trays containing dechlorinated water, and were fed with a finely powdered mixture of dog biscuits and dry yeast in a ratio 3:1.

2.4. Larvicidal Bioassay

According to the guidelines of the World Health Organization [19] with minor modifications, bioassays were performed on healthy F_1 generation of laboratory colonized larvae of *Aedes aegypti* and *Culex quinquefasciatus*. Serial dilution of 1.0% stock solution of each crude solvent seaweed extract yielded requisite test concentrations (100, 200, 300, 400, and 500 mg/L) and amount of test solution. Bioassays in triplicates with an overall of three trials were performed on the early second and third instar of the two vector mosquitoes numbering twenty each added separately into glass beakers (250mL) holding distilled water and test concentration for each replicate apiece trial. In parallel, control tests were performed with distilled water (250mL) as a positive control, and Tween 80 (1.0mL) dissolved in distilled water served as a negative control. Larvae were fed with larval feed during the experiment. Larval mortality was observed 24 hours after treatment and larvae were scored dead when they displayed no signs of movement when probed by a needle at their respiratory siphon. The activity level of seaweed extracts based on the average percent larval mortality were construed as highly active (>75%), moderately active (50–75%), weakly active (25–50%), and inactive (<25%) [20].

2.5. Data Analyses

Larval mortality in percentage was calculated, and Abbott's formula [21] was applied when larval mortality of control ranged between 5% and 20%. Statistical analysis was run in IBM SPSS version 27 statistics software [22]. Statistical analysis of all mortality data of larvicidal activity

was subjected to probit, chi-square and regression analysis. One-way analysis of variance and Duncan's multiple comparison significant difference post hoc tests were used to significantly determine whether mortality in treated bioassays differed from controls and at which concentration in particular; and also whether significant differences in response between solvents of the extract group existed. The differences were considered as significant at $P \leq 0.05$ level with significance set at 95% confidence.

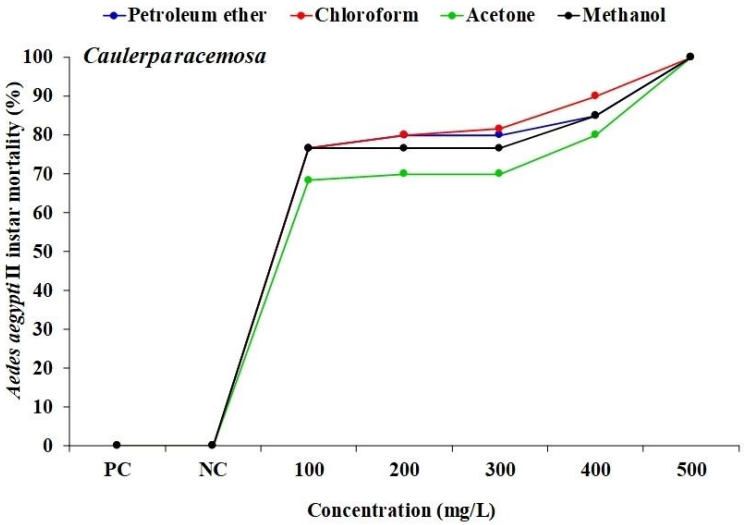
3. Results

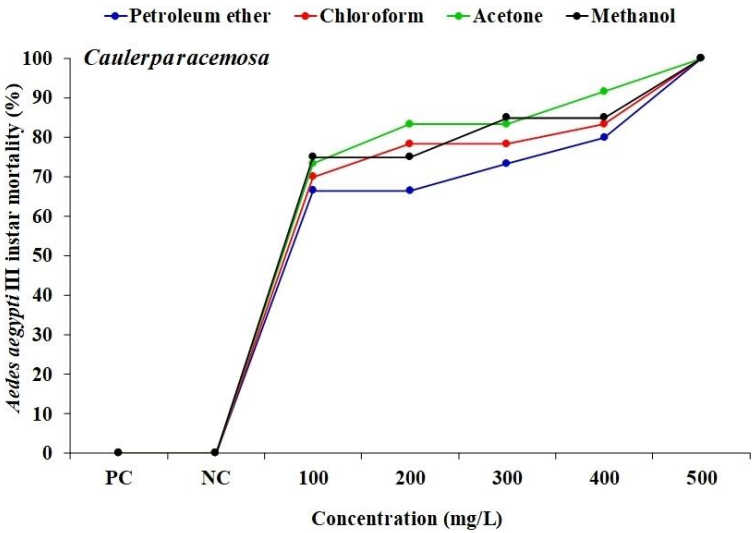
The percentage yield of solvent (petroleum ether, chloroform, acetone and methanol) extracts of *Caulerpa racemosa* was 0.98, 3.32, 0.98 and 1.98, and for *Ulva fasciata* it was 1.98, 2.48, 3.19 and 2.93 respectively. *Caulerpa racemosa* extracts recorded 100% mortality at the highest concentration on the second and third instar of *Aedes aegypti*. *Caulerpa racemosa* petroleum ether, chloroform and methanol extracts were highly active as they exhibited >75% larvicidal activity against the second instar of *Aedes aegypti* at the lowest concentration of 100 mg/L, and against the third instar it was the methanol extract which exhibited 75% larvicidal activity (Table 1; Figure 1). Maximum larvicidal activity was exhibited by the chloroform extract at 500 mg/L against the second and third instars of *Aedes aegypti*, and their respective LC_{50} values were 140.409 and 144.554 mg/L (Table 2). In the case of *Ulva fasciata*, its chloroform extract exhibited 100% mortality at the highest concentration on the second and third instar of *Aedes aegypti*, and was highly active against the second instar of *Aedes aegypti* at the lowest concentration (Table 1; Figure 1). The maximum larvicidal activity was exhibited again by the chloroform extract at 500 mg/L, and the LC_{50} value was 158.358 and 166.025 mg/L, against the second and third instar larvae of *Aedes aegypti*, respectively (Table 2). Against the larval instars of *Culex quinquefasciatus*, all extracts of *Caulerpa racemosa* and the chloroform extract of *Ulva fasciata* displayed 100% mortality at 500 mg/L (Table 3; Figure 1). However, one of the extracts of both the green seaweeds was highly active against the larval instars of *Culex quinquefasciatus* at the lowest concentration. Maximum larvicidal activity was again exhibited by the chloroform extract of both the green seaweed species, and their LC_{50} values were 153.704 and 158.313 mg/L; and 154.156 and 187.435 mg/L against the second and third instars of *Aedes aegypti* and *Culex quinquefasciatus* respectively (Table 4).

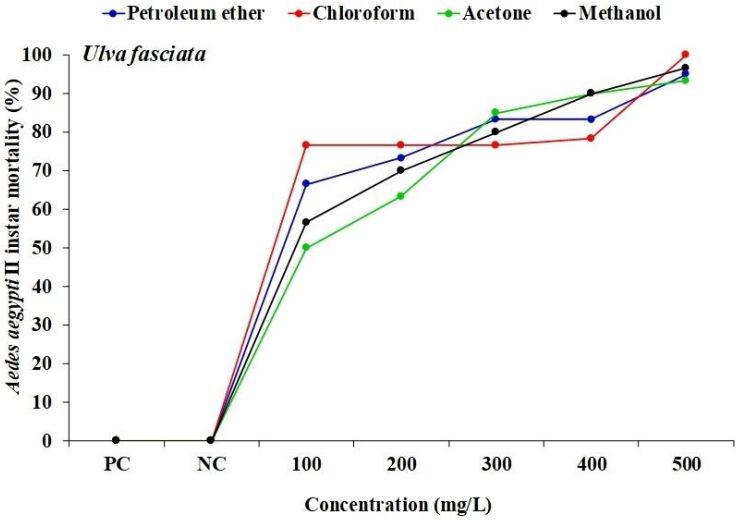
Table 1. Toxicity of green seaweed extracts on the larval instars of *Aedes aegypti*

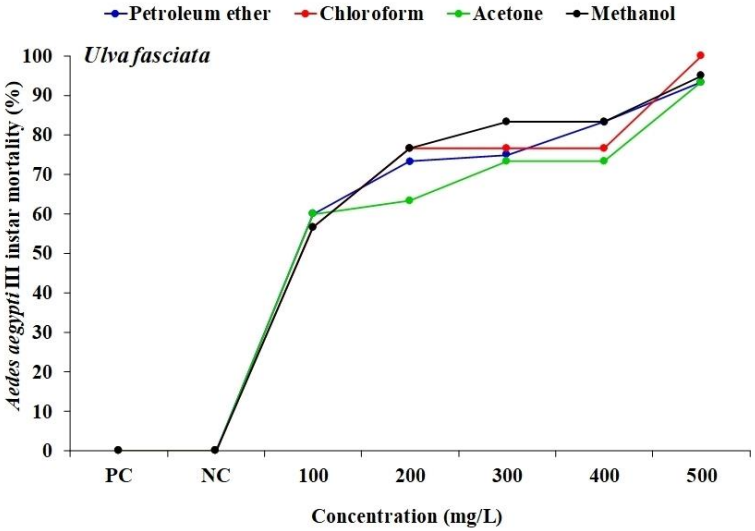
Concentration (mg/L)	II instar				III instar			
	Petroleum ether	**Chloroform**	**Acetone**	**Methanol**	**Petroleum ether**	**Chloroform**	**Acetone**	**Methanol**
				Caulerpa racemosa				
PC	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}
NC	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}
100	15.33 ± 2.51^{b1}	15.33 ± 2.30^{b1}	13.66 ± 0.57^{b1}	15.33 ± 0.57^{b1}	13.33 ± 2.08^{b1}	14.0 ± 2.0^{b1}	14.66 ± 0.57^{b1}	15.0 ± 1.0^{b1}
200	16.0 ± 2.64^{bc1}	16.0 ± 2.0^{bc1}	14.0 ± 2.0^{b1}	15.33 ± 0.57^{b1}	13.33 ± 2.08^{b1}	15.66 ± 2.08^{b12}	16.66 ± 0.57^{bc2}	15.0 ± 1.0^{b12}
300	16.0 ± 2.64^{bc1}	16.33 ± 1.52^{bc1}	14.0 ± 3.0^{b1}	15.33 ± 3.78^{b1}	14.66 ± 2.08^{b1}	15.66 ± 3.05^{b1}	16.66 ± 3.05^{bc1}	17.0 ± 1.0^{c1}
400	17.0 ± 1.0^{bc1}	18.0 ± 1.0^{cd1}	16.0 ± 2.64^{bc1}	17.0 ± 1.0^{bc1}	16.0 ± 1.0^{b1}	16.66 ± 2.08^{b1}	18.33 ± 1.15^{c1}	17.0 ± 1.73^{c1}
500	20.0 ± 0.0^{c}	20.0 ± 0.0^{d}	20.0 ± 0.0^{c}	20.0 ± 0.0^{c}	20.0 ± 0.0^{c}	20.0 ± 0.0^{c}	20.0 ± 0.0^{c}	20.0 ± 0.0^{d}
				Ulva fasciata				
PC	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}
NC	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}
100	13.33 ± 2.88^{b12}	15.33 ± 3.05^{b2}	10.0 ± 2.0^{b1}	11.33 ± 1.15^{b12}	12.0 ± 1.0^{b1}	11.33 ± 2.08^{b1}	12.0 ± 1.0^{b1}	11.33 ± 1.15^{b1}
200	14.66 ± 2.08^{b1}	15.33 ± 2.51^{b1}	12.66 ± 1.52^{c1}	14.0 ± 1.73^{c1}	14.66 ± 2.08^{bc1}	15.33 ± 2.30^{b1}	12.66 ± 1.15^{bc1}	15.33 ± 2.30^{c1}
300	16.66 ± 2.08^{bc1}	15.33 ± 2.51^{b1}	17.0 ± 1.73^{d1}	16.0 ± 2.64^{cd1}	15.0 ± 2.64^{c1}	15.33 ± 3.21^{b1}	14.66 ± 2.08^{c1}	16.66 ± 1.52^{c1}
400	16.66 ± 2.51^{bc1}	15.66 ± 3.78^{b1}	18.0 ± 1.0^{de1}	18.0 ± 2.0^{de1}	16.66 ± 2.08^{c1}	15.33 ± 4.04^{b1}	14.66 ± 2.08^{c1}	16.66 ± 2.08^{c1}
500	19.0 ± 0.0^{c1}	20.0 ± 0.0^{c2}	18.66 ± 0.57^{e1}	19.33 ± 0.57^{e12}	18.66 ± 0.57^{d1}	20.0 ± 0.0^{c2}	18.66 ± 0.57^{d1}	19.0 ± 1.0^{d12}

PC: Positive control; NC: Negative control; Data are mean±standard deviation of larval mortality of three replicates of three trials; Different numerical superscripts in column indicate values significant than respective PC and NC, and different superscript alphabets in rows indicate values significant between the extracts at $p \leq 0.05$ level by one way ANOVA followed by Duncan's multiple comparison post hoc test performed; Similarity in alphabetical and numerical superscripts in rows and columns indicate no significant variation


Petroleum ether
Chloroform
Acetone
Methanol
Caulerpa racemosa
Aedes aegypti II instar mortality (%)
PC
NC
100
200
300
400
500
Concentration (mg/L)


Petroleum ether
Chloroform
Acetone
Methanol
Caulerpa racemosa
Aedes aegypti III instar mortality (%)
PC
NC
100
200
300
400
500
Concentration (mg/L)


Petroleum ether
Chloroform
Acetone
Methanol
Ulva fasciata
Aedes aegypti II instar mortality (%)
PC
NC
100
200
300
400
500
Concentration (mg/L)


Petroleum ether
Chloroform
Acetone
Methanol
Ulva fasciata
Aedes aegypti III instar mortality (%)
PC
NC
100
200
300
400
500
Concentration (mg/L)

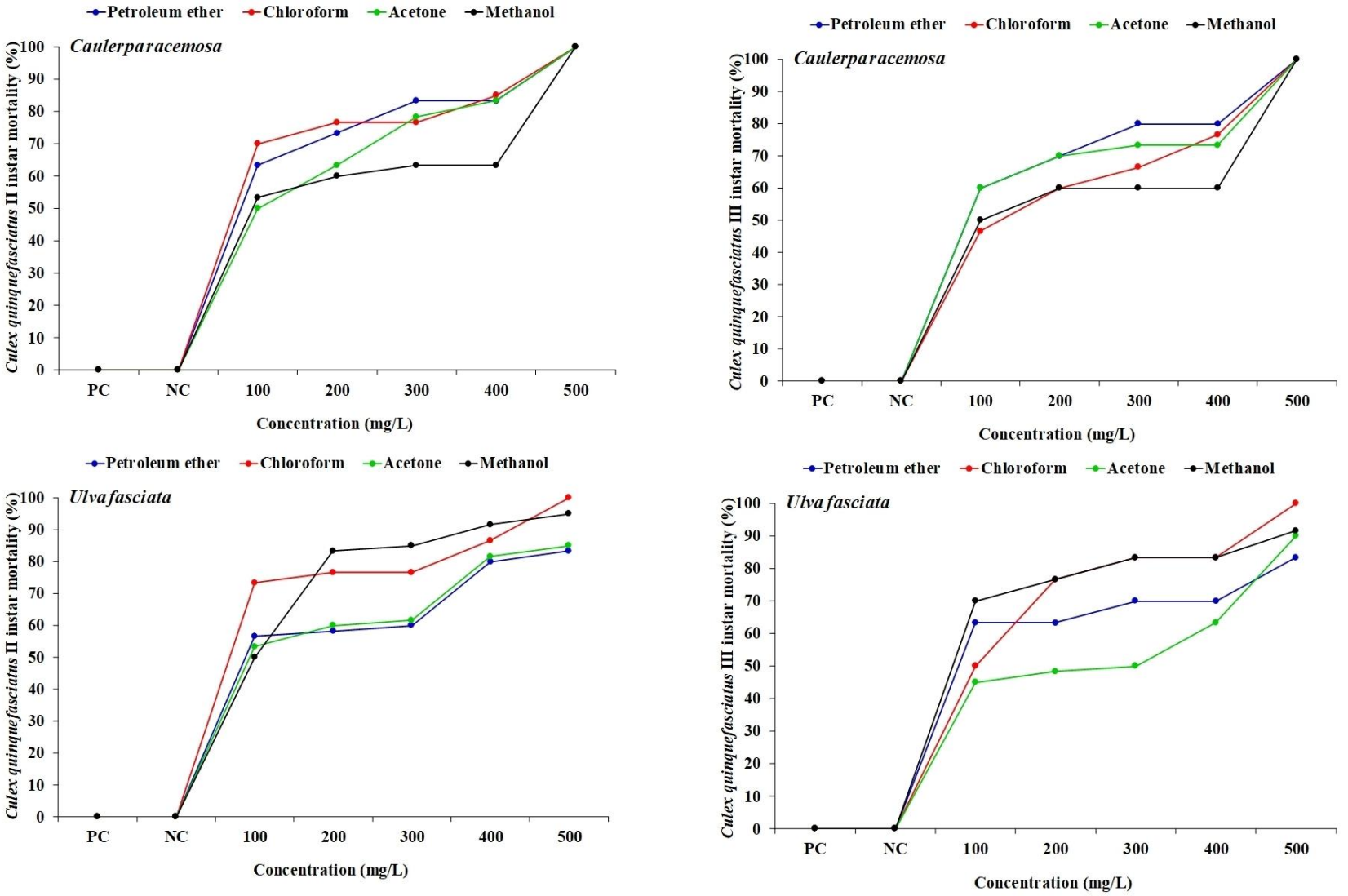


Figure 1. Percent larval mortality of vector mosquitoes on exposure to green seaweed extracts

Table 2. Probit analysis and associated statistical inferences of green seaweed extracts against the larval instars of *Aedes aegypti*

Seaweed extracts	LC_{50} (mg/L)	95% CL (LB-UB)	LC_{90} (mg/L)	95% CL (LB-UB)	Regression equation	R^2	χ^2	P value
II instar								
			Caulerpa racemosa					
Petroleum ether	151.412	83.182-211.038	361.664	288.743-501.453	Y=-0.511+0.112x	0.921	25.890*	0.002*
Chloroform	140.409	85.581-190.989	311.369	250.794-422.510	Y=-35.95+0.152x	0.847	15.875*	0.007*
Acetone	157.082	95.381-213.709	347.764	279.405-477.747	Y=-1.666+0.123x	0.960	24.465*	0.004*
Methanol	153.078	88.242-210.726	357.562	287.122-489.609	Y=-0.411+0.182x	0.949	5.727*	0.767†
			Ulva fasciata					
Petroleum ether	158.425	100.308-212.633	343.430	277.887-464.877	Y=5.633+0.125x	0.987	14.789*	0.001*
Chloroform	158.358	78.039-228.600	367.978	285.557-548.996	Y=8.607+0.222x	0.914	26.470*	0.002*
Acetone	173.850	140.613-207.225	331.918	288.620-396.332	Y=11.42+0.215x	0.915	28.888*	0.001*
Methanol	167.337	125.143-208.963	333.448	281.216-418.732	Y=2.914+0.212x	0.980	52.073*	0.001*
III instar								
			Caulerpa racemosa					
Petroleum ether	184.924	126.719-239.923	413.990	341.498-544.078	Y=-1.366+0.054x	0.930	13.987*	0.123†
Chloroform	144.554	78.085-203.041	338.672	269.158-470.597	Y=-13.53+0.240x	0.960	48.247*	0.001*
Acetone	183.294	118.774-243.480	416.856	338.782-565.168	Y=-0.622+0.088x	0.973	12.087*	0.208†
Methanol	155.575	89.864-214.140	363.192	291.152-500.808	Y=-2.25+0.039x	0.964	11.848*	0.222†
			Ulva fasciata					
Petroleum ether	173.185	122.432-222.015	362.227	300.747-468.599	Y=0.311+0.096x	0.896	21.004*	0.013†
Chloroform	166.025	116.529-213.512	343.472	284.899-443.783	Y=3.522+0.212x	0.967	44.377*	0.001*
Acetone	193.219	138.468-246.815	406.108	336.590-530.362	Y=0.966+0.202x	0.948	25.673*	0.002*
Methanol	178.565	114.652-239.069	378.008	304.827-520.081	Y=0.911+0.170x	0.975	38.319*	0.001*

LC_{50} & LC_{90}: Lethal concentration that kills 50% and 90% of the treated larvae respectively; CL: Confidence limits; LB: Lower bound; UB: Upper bound;

χ^2: Chi-square value; R^2: Coefficient of determination; *Values significant at $p \leq 0.05$ level; †Values not significant at $p \leq 0.05$ level

Table 3. Toxicity of green seaweed extracts on the larval instars of *Culex quinquefasciatus*

Concentration (mg/L)	II instar				III instar			
	Petroleum ether	**Chloroform**	**Acetone**	**Methanol**	**Petroleum ether**	**Chloroform**	**Acetone**	**Methanol**
				Caulerpa racemosa				
PC	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}
NC	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}
100	12.66 ± 1.52^{b1}	14.0 ± 1.73^{b1}	10.0 ± 1.0^{b1}	10.66 ± 4.61^{b1}	12.0 ± 1.0^{b1}	9.33 ± 1.52^{b1}	12.0 ± 1.0^{b1}	10.0 ± 4.35^{b1}
200	14.66 ± 2.08^{bc1}	15.33 ± 1.52^{bc1}	12.66 ± 1.52^{c1}	12.0 ± 5.19^{b1}	14.0 ± 1.73^{bc1}	12.0 ± 1.0^{bc1}	14.0 ± 1.73^{b1}	12.0 ± 4.35^{b1}
300	16.66 ± 1.52^{c1}	15.33 ± 2.30^{bc1}	15.66 ± 0.57^{d1}	12.66 ± 5.50^{b1}	16.0 ± 2.64^{cd1}	13.33 ± 2.08^{cd1}	14.66 ± 2.08^{b1}	12.0 ± 5.19^{b1}
400	16.66 ± 1.52^{c2}	17.0 ± 2.0^{c2}	16.66 ± 1.52^{d2}	12.66 ± 1.51^{b1}	16.0 ± 2.64^{cd1}	15.33 ± 2.51^{de1}	14.66 ± 3.51^{b1}	12.0 ± 1.0^{b1}
500	20.0 ± 0.0^{d}	20.0 ± 0.0^{d}	20.0 ± 0.0^{e}	20.0 ± 0.0^{c}	20.0 ± 0.0^{d1}	20.0 ± 0.0^{e1}	20.0 ± 0.0^{c1}	20.0 ± 0.0^{c1}
				Ulva fasciata				
PC	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}
NC	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}
100	11.33 ± 1.15^{b1}	14.66 ± 2.08^{b2}	10.66 ± 0.57^{b1}	10.0 ± 0.0^{b1}	12.66 ± 1.52^{b23}	10.0 ± 1.0^{b12}	9.0 ± 2.64^{b1}	14.0 ± 1.73^{b3}
200	11.66 ± 2.08^{b1}	15.33 ± 2.30^{b12}	12.0 ± 1.0^{b1}	16.66 ± 2.88^{c2}	12.66 ± 3.05^{b12}	15.33 ± 2.30^{c2}	9.66 ± 3.21^{b1}	15.33 ± 2.30^{b2}
300	12.0 ± 2.0^{b1}	15.33 ± 3.21^{b1}	12.33 ± 2.51^{b}	17.0 ± 2.64^{c1}	14.0 ± 1.73^{b12}	16.66 ± 2.88^{cd2}	10.0 ± 2.0^{b1}	16.66 ± 2.88^{bc2}
400	16.0 ± 2.64^{c1}	17.33 ± 2.51^{bc1}	16.33 ± 2.51^{c1}	18.33 ± 1.52^{cd1}	14.0 ± 4.35^{b1}	16.66 ± 3.05^{cd1}	12.66 ± 4.16^{b1}	16.66 ± 3.05^{bc1}
500	16.66 ± 1.52^{d1}	20.0 ± 0.0^{c3}	17.0 ± 1.73^{d12}	19.0 ± 0.0^{d23}	17.66 ± 2.51^{c1}	20.0 ± 0.0^{d1}	18.0 ± 1.73^{c1}	18.33 ± 1.15^{c1}

PC: Positive control; NC: Negative control; Data are mean±standard deviation of larval mortality of three replicates of three trials; Different numerical superscripts in column indicate values significant than respective PC and NC, and different superscript alphabets in rows indicate values significant between the extracts at $p \leq 0.05$ level by one way ANOVA followed by Duncan's multiple comparison post hoc test performed; Similarity in alphabetical and numerical superscripts in rows and columns indicate no significant variation.

Table 4. Probit analysis and associated statistical inferences of green seaweed extracts against the larval instars of *Culex quinquefasciatus*

Seaweed extract	LC_{50} (mg/L)	95% CL (LB-UB)	LC_{90} (mg/L)	95% CL (LB-UB)	Regression equation	R^2	χ^2	P value
II instar								
				Caulerpa racemosa				
Petroleum ether	180.214	107.808-245.259	426.392	342.941-589.574	Y=-0.877+0.226x	0.910	37.572*	0.001*
Chloroform	153.704	90.580-211.606	340.151	271.166-474.764	Y=-4.313+0.223x	0.946	51.561*	0.001*
Acetone	205.526	153.473-256.434	437.323	368.520-553.190	Y=-13.82+0.202x	0.896	39.344*	0.001*
Methanol	228.341	158.541-301.716	478.571	384.568-677.606	Y=-0.926+0.219x	0.977	67.713*	0.001*
				Ulva fasciata				
Petroleum ether	196.483	128.727-262.523	422.020	339.282-590.159	Y=6.477+0.104x	0.944	16.142*	0.064†
Chloroform	154.156	88.775-213.930	339.277	269.094-478.124	Y=49.75+0.116x	0.914	18.825*	0.002*
Acetone	204.031	158.026-250.652	404.875	343.239-507.564	Y=27.14+0.174x	0.834	26.183*	0.002*
Methanol	155.492	112.760-197.677	303.597	252.979-388.354	Y=11.25+0.194x	0.968	30.425*	0.001*
III instar								
				Caulerpa racemosa				
Petroleum ether	187.748	120.109-250.698	422.870	342.451-577.616	Y=-0.857+0.161x	0.962	86.788*	0.001*
Chloroform	158.313	100.748-211.798	347.138	281.665-467.661	Y=-0.222+0.223x	0.940	94.129*	0.001*
Acetone	223.440	176.480-271.308	455.934	389.068-565.686	Y=-0.188+0.229x	0.891	64.408*	0.001*
Methanol	242.981	174.597-317.775	508.522	409.953-716.744	Y=-5.821+0.179x	0.898	62.415*	0.001*
				Ulva fasciata				
Petroleum ether	205.762	155.654-256.624	414.111	347.267-530.269	Y=0.88+0.104x	0.970	17.494*	0.042*
Chloroform	187.435	126.354-246.117	400.118	326.195-539.914	Y=0.577+0.220x	0.929	38.516*	0.001*
Acetone	257.543	202.033-320.065	498.686	414.948-655.618	Y=0.511+0.215x	0.931	25.234*	0.003*
Methanol	171.893	120.361-221.873	345.222	284.928-451.554	Y=0.088+0.182x	0.990	54.257*	0.001*

LC_{50} & LC_{90}: Lethal concentration that kills 50% and 90% of the treated larvae respectively; CL: Confidence limits; LB: Lower bound; UB: Upper bound;

χ^2: Chi-square value; R^2: Coefficient of determination; *Values significant at $p \leq 0.05$ level; †Values not significant at $p \leq 0.05$ level

4. Discussion

A broad spectrum of seaweed species have been reported for their toxic effects on mosquito larvae [6,23], and in the present study the crude solvent extracts of *Caulerpa racemosa* and *Ulva fasciata* were reported for larvicidal action on *Aedes aegypti* and *Culex quinquefasciatus*. Overall results indicate that amongst the two green seaweeds tested, *Caulerpa racemosa* exhibited more activity when compared to *Ulva fasciata*, and with reference to solvent extracts, the chloroform extract exhibited maximum activity against the larval instars of the vector mosquito species tested. With regard to the vector mosquito species tested, *Aedes aegypti* was more susceptible than *Culex quinquefasciatus*, and among the instars, second instar larvae were more susceptible than the third instar.

Earlier studies of *Caulerpa racemosa* with different solvents tested against mosquito species have been reported. Its petroleum ether-acetone extracts exhibited LC_{50} values <100 mg/L against *Aedes aegypti* and *Culex quinquefasciatus* larvae [24]; ethanol extract showed toxicity against the fourth instar larvae of *Aedes aegypti*, *Culex quinquefasciatus*, *Anopheles stephensi* due to the presence of phytoconstituents like terpenoids, fatty acids, saponins, steroids, alkaloids, tannins, glycosides, carbohydrates, flavonoids, proteins and a compound caulerpin, and their respective LC_{50} values were 0.055, 0.067 and 0.066 µg/mL [25]; methanol extract showed effective activity against *Culex tritaeniorhynchus* as it ruptured the midgut of larvae [26], and reported LC_{50} values of >1000 µg/mL against *Aedes aegypti* and *Aedes albopictus* [23]; hexane, chloroform, ethyl acetate, acetone and methanol extracts showed LC_{50} values of 910.2, 728.4, 579.9, 811.8, 886.0 ppm against *Aedes aegypti* and their activity was due to presence of terpenoids, tannins and phenolics [27]. The results of the present study provided far better results based on LC_{50} values when compared with the above mentioned previous studies. Besides these, *Caulerpa racemosa* exhibited profound larvicidal activity with better LC_{50} values than other species of *Caulerpa*, wherein *Caulerpa scalpelliformis* acetone extract reported LC_{50} value of 53.70 mg/L against *Aedes aegypti* [28], which is an exception when compared to the present study, and LC_{50} value of 338.91 ppm against *Culex pipiens*, larvae and caused >70% larval mortality at 24 hours [29]. Its ethanol extract showed LC_{50} value of 0.07, 0.06 and 0.06 µg/mL against *Aedes aegypti*, *Culex quinquefasciatus* and *Anopheles stephensi* [25], and ethanol extracts of *Caulerpa chemnitzia*, *Caulerpa scalpelliformis* and *Caulerpa taxifolia* against *Aedes aegypti* with LC_{50} values of 2500, 2000 and 1900 ppm respectively [30].

In the case of *Ulva fasciata* too, results of the present study provided pronounced larvicidal effects with LC_{50} values <200 mg/L, which was better when equated with earlier studies with different solvents reported for mosquito larvicidal activity. Its methanol, acetone and benzene extracts reported LC_{50} values of 515.88, 504.47 and 478.66 ppm against *Culex quinquefasciatus* respectively [31]; ethanol extract showed activity against *Aedes aegypti* larvae with LC_{50} value of 1750 ppm [30], and its hexane and ethyl acetate extracts showed activity against the fourth instar of *Anopheles stephensi* [32]. Further, *Ulva fasciata* extracts showed more larvicidal activity when compared with its closely related species, *Ulva lactuca*, whose acetone extract exhibited LC_{50} value of 335.30 ppm against *Anopheles d'thali* [33]; ethanol extract showed activity against *Aedes aegypti* larvae and LC_{50} value was 1400 ppm [30], and 0.08, 0.08 and 0.09 µg/mL against *Aedes aegypti*, *Culex quinquefasciatus* and *Anopheles stephensi* [25]; acetone, chloroform, ethanol, methanol and petroleum ether extracts exhibited LC_{50} values of 5.46, 67.99, 12.82, 27.35, 27.55 mg/mL against the third instar of *Culex pipiens* [34]; methanol extract reported LC_{50} values >1000 µg/mL against *Aedes aegypti* and *Aedes albopictus* [23]; hexane, chloroform, ethyl acetate, acetone and methanol extracts showed LC_{50} values of 950.3, 761.6, 588.1, 831.0, 952.0 ppm against *Aedes aegypti* [27]; acetone, ethanol and petroleum ether extracts exhibited LC_{50} values of 5.00, 11.70 and 31.69 mg/mL against fourth instar of *Culex pipiens* [35].

The toxicity of seaweed depends upon the species of seaweeds, the polarity of solvent, and the mosquito species tested. The chemical composition of the seaweed plays an important role in its bioactivity against mosquito larvae. Green seaweeds are prolific producers of secondary metabolites, and their larvicidal properties might be due to the presence of its effective chemical components like alkaloids, flavonoids, phenolics, saponins, steroids and terpenoids with mosquito larvicidal properties [6,25,29]. *Caulerpa* species are the most effective green seaweeds due to the presence of terpenoids, and a major secondary metabolite compound caulerpenyne involved in the chemical defense of genus *Caulerpa* [14]. Further, alkaloids like caulerpin and caulerpinic acid from *Caulerpa racemosa* act as insecticidal compounds against the second, third and fourth instar of *Culex pipiens* and have reported LC_{50} values of 1.42, 1.81, 1.99 ppm and 3.04, 3.90, 4.89 ppm respectively after 24 hours [36]. These compounds would have been responsible for the larvicidal action in the present study too. On the other hand, genus *Ulva* contains palmitic and octadecanoic acid, and methyl esters [34]. These chemical compounds are known insecticidal compounds, as they affect the metabolism and morphology of mosquito larvae midgut, especially in *Culex quinquefasciatus* [37]. Extracts of nonpolar solvents of green seaweeds showed higher insecticidal activity than extracts of polar solvents [38]. However, in the present study, chloroform, a mid-polar solvent exhibited the maximum activity. Bioactive compounds like alkaloids, saponins, and phenolics are extracted by chloroform. Further, chloroform extract of seaweed, *Codium edule* caused the body of the *Aedes aegypti* larvae to become longer and dark in colour [39]. The same was observed in

the present study also. The susceptibility of different mosquito species is too varied [25]. Manilal *et al*. [40] reported *Aedes aegypti* larvae were more susceptible when compared to *Culex quinquefasciatus* on the basis of low LC_{50} values, and the same was observed in the present study. There was a higher mortality rate for younger larvae compared to older larvae under the same concentration of treatment in the present study. Similar observations were reported by Selvin and Lipton [41] wherein the fourth instar larvae were resistant at the concentration that produced 100% mortality in the second instar exposed to green seaweeds.

5. Conclusions

The present study indicated the larvicidal property of the chloroform extract of both the green seaweeds against the vector mosquitoes, which encourages further investigation on its bioactive compounds that might own virtuous larvicidal properties when isolated in pure form may be effective as toxicants against juvenile stages of mosquitoes.

REFERENCES

[1] Manikandan S., Mathivanan A., Bora B., Hemaladkshmi P., Abhisuresh V., Poopathi S., "A review of vector borne disease transmission: Current strategies of mosquito vector control," Indian Journal of Entomology, 2022. DOI: 10.55446/IJE.2022.593.

[2] Milam C. D., Farris J. L., Wilhide J. D., "Evaluating mosquito control pesticides for effect on target and nontarget organisms," Archives of Environmental Contamination and Toxicology, vol. 39, pp. 324-328, 2000. https://doi.org/10.1007/s002440010111

[3] Ana R., Michèle B., Francesco B., Atle B. M., Leonor C. M., Jens C., Maria C. F., Marta C., Jerónimo C., Meltem C. D., Mercedes C., Thanos D., Irem D., Ana D. R., Dragana D., Arita D., Christine E., Hjörleifur E., Ayşegül E., Tufan E. O., David E., Stefano F., Richard F. J., Laura G. M., Susana G. P., Marija G. U., Nadica I. D., Rósa J., Marija K., Katja K., Jonne K., Leila K., Zrinka L., Lada L. B., Manolis M., Alexia M., Inga M., Hanna M., Mohamed M., Laurentius N. S., Lucie N., Donata O., Giuseppe P., Praveen R., Cédine R., Thorsten R., Fernando R., Baruch R., Johan R., Eric R., Vita R., Jerica S., Ivo S., Siret T., Deniz T., Xenia T. S., Olivier T. P., Anna T., Cristina V. G., Marlen V.I., "The essentials of marine biotechnology," Frontiers in Marine Science, vol. 16, pp. 158, 2021. https://doi.org/10.3389/fmars.2021.629629

[4] Mayer A. M. S., Rodriguez A. D., Berlinck R. G. S., Hamann M. T., "Marine pharmacology in 2003-4: marine compounds with anthelmintic antibacterial, anticoagulant, antifungal, anti-inflammatory, antimalarial, antiplatelet, antiprotozoal, antituberculosis, and antiviral activities; affecting the cardiovascular, immune and nervous systems, and other miscellaneous mechanisms of action," Comparative Biochemistry and Physiology: Toxicology & Pharmacology: CBP, vol. 145, no. 4, pp. 553-581, 2007. DOI: 10.1016/j.cbpc.2007.01.015

[5] Dhargalkar V. K., Neelam P., "Seaweed: promising plant of the millennium," Science and Culture, vol. 71, no. 3-4, pp. 60-66, 2005. https://scienceandculture-isna.org/uploads/latestIssue/MARCH-APRIL_2005.pdf

[6] Yu K. X., Jantan I., Ahmad R., Wong C. L., "The major bioactive components of seaweeds and their mosquitocidal potential," Parasitology Research, vol. 113, pp. 3121–3141, 2014. https://doi.org/10.1007/s00436-014-4068-5

[7] Ghosh A., Chowdhury N., Chandra G., "Plant extracts as potential mosquito larvicides," Indian Journal of Medical Research, vol. 135, pp. 581-598, 2012.https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3401688/

[8] Sanjeev K., Sushil K., "Efficacy of phytochemicals against mosquito larvae: An update to Integrated Mosquito Management," International Journal of Zoological Investigations, vol. 8, no. 1, pp. 305-319, 2022. http://ijzi.net/Issue/1625636565IssueMS34.pdf

[9] Shajahan A., Hussian M. I. Z., Kumar S. R., Ajintha K., "A review on plant phytochemicals potential for mosquito control," International Journal of Mosquito Research, vol. 9, no. 6, pp. 47-54, 2022. DOI :10.22271/23487941.2022.v9.i6a.641

[10] Marten G. G., "The potential of mosquito-indigestible phytoplankton for mosquito control," Journal of the American Mosquito Control Association, vol. 3, no. 1, pp. 105-106, 1987. https://pubmed.ncbi.nlm.nih.gov/2904936/

[11] Marten G. G., "Larvicidal algae," Journal of the American Mosquito Control Association, vol. 23, no. sp2, pp. 177-183, 2007. https://doi.org/10.2987/8756-971X(2007)23[177:LA]2.0.CO;2

[12] Kiviranta J., Abdel-Hameed A., Sivonen K., Niemel S.I., Carlberg G., "Toxicity of cyanobacteria to mosquito larvae-screening of active compounds," *Environmental Toxicology and Water Quality, vol.* 8, no. 1, pp. 63-71, 1993. https://doi.org/10.1002/tox.2530080107

[13] Kiviranta J., Abdel-Hameed A., "Toxicity of the blue-green alga *Oscillatoria agardhii* to the mosquito *Aedes aegypti* and the shrimp *Artemia salina*," World Journal of Microbiology and Biotechnology, vol. 10, no. 5, pp. 517-520, 1994. DOI: 10.1007/BF00367656

[14] Elbanna S. M., Hegazi M. M., "Screening of some seaweeds species from South Sinai, Red sea as potential bioinsecticides against mosquito larvae, *Culex pipiens*," Egyptian Academic Journal of Biological Sciences, vol. 4, no. 2, pp. 21-30, 2011. https://www.scopus.com/record/display.uri?eid=2-s2.0- 84888829357&origin=inward&txGid=2b7287073426ea3d8ef992015faff23f

[15] Dhargalkar V. K., Kavlekar D., "Seaweeds - a field manual," (1st edn). National Institute of Oceanography, India, 2004.

[16] Ganesapandian S., Kumaraguru A. K., "Seaweeds resource in the intertidal and subtidal regions of Pudumadam, Gulf of Mannar," Seaweed Research and Utilization, vol. 30, pp. 97-105, 2008.

[17] Rao S. G., "Common seaweeds and seagrasses of India," Herbarium Volume 1, Central Marine Fisheries Research

Institute, Kerala. India, 2012.

[18] Vogel A. L., "Text book of practical organic chemistry," 5th Edition. London: The English Language Book Society and Longman, 1989. https://faculty.ksu.edu.sa/sites/default/files/vogel_-_practical_organic_chemistry_5th_edition.pdf

[19] World Health Organization. "Guidelines for laboratory and field testing of mosquito larvicides," WHO, Geneva, WHO/CDS/WHOPES/GCDPP/13, 2005. WHO_CDS_WHOPES_GCDPP_2005.13.pdf (112.1Kb)

[20] Guedes E. A. C., Carvalho C. M. D., Junior R., Lisboa K. A., Ribeiro L. T. F., de Barros L.D., Sant'Ana G. A. E., "Larvicidal activity against *Aedes aegypti* and molluscicidal activity against *Biomphalaria glabrata* of Brazilian marine algae," Journal of Parasitology Research, no. 501328, 2014. doi: 10.1155/2014/501328

[21] Abbott W. S., "A method of computing the effectiveness of an insecticide," Journal of Economic Entomology, vol. 18, pp. 265-267, 1925. https://pubmed.ncbi.nlm.nih.gov/3333059/

[22] SPSS, "IBM SPSS Statistics for Windows," Version 27.0. Armonk, NY: IBM Corp. 2021.

[23] Ahmad R., Yu K., Wong C., Jantan I., "Larvicidal and adulticidal activity of Malaysian seaweeds against *Aedes aegypti* (L.) and *Aedes albopictus* Skuse (Diptera: Culicidae)," Southeast Asian Journal of Tropical Medicine and Public Health, vol. 47, no. 4, pp. 719-730, 2016. https://www.scopus.com/record/display.uri?eid=2-s2.0-85024484349&origin=inward&txGid=68b97082da9beb21f2eae71fb8bb957e

[24] Thangam T. S., Kathiresan K., "Marine plants for mosquito control," In: Wildey KB (ed) Proceedings of the second international conference on urban pests. Edinburgh, Scotland, 1996.

[25] Ali M. Y. S., Ravikumar S., Beula J. M., "Mosquito larvicidal activity of seaweeds extracts against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus*," Asian Pacific Journal of Tropical Disease, vol. 3, no. 3, pp. 196-201, 2013. doi: 10.1016/S2222-1808(13)60040-7

[26] Nagaraj S. R., Osborne J. W., "Bioactive compounds from *Caulerpara racemosa* as a potent larvicidal and antibacterial agent," Frontiers in Biology, vol. 9, pp. 300-305, 2014. https://doi.org/10.1007/s11515-014-1312-4

[27] Raj G. A., Jayaraman M., Krishnamoorthy S., Chandrasekaran M., Venkatesalu V., "Screening of different extracts of marine macro green algae for larvicidal activity against dengue fever mosquito, *Aedes aegypti* (Diptera: Culicidae)," International Letters of Natural Sciences, vol. 62, pp. 44-51, 2017. https://doi.org/10.56431/p-za9vhz

[28] Thangam T. S., Kathiresan K., "Mosquito larvicidal effect of seaweed extracts," Botanica Marina, vol. 34, no. 5, pp. 433-435, 1991. https://doi.org/10.1515/botm.1991.34.5.433

[29] Cetin H., Gokoglu M., Oz E., "Larvicidal activity of the extract of seaweed, *Caulerpa scalpelliformis*, against *Culex pipiens*," Journal of the American Mosquito Control Association, vol. 26, no. 4, pp. 433-435, 2010. https://doi.org/10.2987/10-6046.1

[30] Hira K., Tariq R. M., Sultana V., Ara J., Ehteshamul-Haque S., "Effect of seaweeds occurring at Karachi coast on mosquito larvae and liver function in rats," Pakistan Journal of Pharmaceutical Sciences, vol. 30, no. 2, pp. 387-391, 2017. https://link.gale.com/apps/doc/A494427203/AONE?u=tel_oweb&sid=googleScholar&xid=d36bc00b

[31] Poonguzhali T. V., Nisha L. L., "Larvicidal activity of two seaweeds, *Ulva fasciata* and *Grateloupia lithophila* against mosquito vector, *Culex quinquefasciatus*," International Journal of Current Science, vol. 4, pp. 163-168, 2012. https://www.scopus.com/record/display.uri?eid=2-s2.0-84874007119&origin=inward&txGid=aa6d800abe77f49ea06c5a1c7987eeea

[32] Sowmiya R., Balasubramani G., Deepak P., Aiswarya D., Ravikumar S., Prasannakumar S., Perumal P., "Characterization and screening of *in vitro* antimalarial and larvicidal activities of selected seaweeds from southeast coast of India against *Plasmodium falciparum* and *Anopheles stephensi*," Journal of Coastal Life Medicine, vol. 5, no. 6, pp. 242-248, 2017. https://oaji.net/articles/2017/2154-1497599145.pdf

[33] Mahyoub J. A., "Mosquito larvicidal activity of seaweed extracts against *Anopheles d'thali* with reference to its side effects on aquatic nontarget organisms," International Journal of Mosquito Research, vol. 5, no. 6, pp. 34-38, 2018. https://www.dipterajournal.com/archives/2018/5/6/5-5-24

[34] Abbassy M. A., Marzouk M. A., Rabea E. I., Abd-Elnabi A.D., "Insecticidal and fungicidal activity of *Ulva lactuca* Linnaeus (Chlorophyta) extracts and their fractions," Annual Research and Review in Biology, vol. 4, no. 13, pp. 2252-2262, 2014. DOI: 10.9734/ARRB/2014/9511

[35] Elbanoby M. I., "Larvicidal and inhibition activities of marine algae *Ulva lactuca* extracts *on Culex pipiens* mosquito," Journal of Plant Protection and Pathology, vol. 11, no. 6, pp. 321-325, 2020. https://jppp.journals.ekb.eg/article_111731_e45b9428da3ffae41fd55a9a73e01009.pdf

[36] Alarif W. M., Abou-Elnaga Z. S., Ayyad S. N., Al-lihaibi S.S., "Insecticidal metabolites from the green alga *Caulerpa racemosa*," Clean – Soil, Air, Water, vol. 38, no. 5-6, pp. 548-557, 2010. https://doi.org/10.1002/clen.201000033

[37] de Melo A. R., Garcia I. J. P., Serrão J. E., Santos H. L., dos Santos Lima L.A.R., Alves S.N., "Toxicity of different fatty acids and methyl esters on *Culex quinquefasciatus* larvae," Ecotoxicology and Environmental Safety, vol. 154, pp. 1-5, 2018. https://doi.org/10.1016/j.ecoenv.2018.02.009

[38] Hadear H. A., "*Ulva lactuca* as a cheap and safe biopesticide in fields and its chemical composition (*in vitro*)," Egyptian Journal of Aquatic Biology and Fisheries, vol. 23, no. 5, pp. 415-428, 2019.

[39] Alkuriji M. A., Al-Fageeh M. B., Shaher F. M., Alorf M. S., Almazyad H.F., "Larvicidal effect of seaweed *Codium edule* extracts on *Aedes aegypti* mosquito," Research Journal of Pharmaceutical, Biological and Chemical Sciences, vol. 11, no. 5, pp. 82-89, 2020. https://doi.org/10.33887/rjpbcs/2020.11.5.10

[40] Manilal A., Thajuddin N., Selvin J., Idhayadhulla A., Kumar R.S., Sujith S., "*In vitro* mosquito larvicidal activity of marine algae against the human vectors, *Culex quinquefasciatus* (Say) and *Aedes aegypti* (Linnaeus) (Diptera: Culicidae)," International Journal of Zoological Research, vol. 7, pp. 272-278, 2011. DOI: 10.3923/ijzr.2011.272.278

[41] Selvin J., Lipton A. P., "Biopotentials of *Ulva fasciata* and *Hypnea musciformis* collected from the peninsular coast of India," Journal of Marine Science and Technology, vol. 12, no. 1, pp. 1-6, 2004. https://jmstt.ntou.edu.tw/cgi/viewcontent.cgi?article=2214&context=journal.