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Mosquito larvicidal and pupicidal activity of seaweed extracts against *Aedes aegypti, Anopheles stephensi* and *Culex quinquefasciatus*

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Abstract
Mosquito problem has become acute in recent years and many programmes have been launched to control these vectors. The use of biologically active plant materials with antilarval properties has attracted considerable interest of scientists all over the world. In this background, this study was undertaken to investigate the larvicidal and pupicidal potential of the seaweeds of *Turbinaria conoides* against the mosquito species *Aedes aegypti, Anopheles stephensi* and *Culex quinquefasciatus*. By using three different solvent seaweed extracts (aqueous, acetone and ethanol), LC_{50} and LC_{90} values were found out and the pupae mortality was also analyzed. The results showed that the aqueous extract of seaweed exhibited high degree of blocking the development by induction of great mortality of larvae and pupae. These findings may help in developing a prospective alternative source to control the mosquitoes

Keywords: Larvicidal activity, Pupicidal activity, Seaweeds, *Anopheles stephensi, Aedes aegypti, Culex quinquefasciatus.*

1. Introduction
Mosquitoes serve as a vector of several diseases, causing serious health problems to human; they transmit diseases *viz*., yellow fever, human lymphatic filariasis and malaria (Abdel-Hameed, *et al*., 1994) [2]. Mosquito problem has become acute in recent years and many programmes have been launched to control these vectors. Synthetic insecticides are well recognized for their speedy action but a major drawback in their application is that they are non-selective and could be harmful to other beneficial organisms, animals and human beings (Abdel-Hameed, 1994) [2]. Besides their adverse environmental effects, pests and mosquito disease vector have become physiologically resistant to many of the synthetic pesticides (Rao *et al*., 1995) [30].

All these restrictions on the usage of synthetic pesticides have stimulated investigations for an environmentally safe, degradable and target specific insecticides against mosquitoes. Ultimately phytochemicals with antilarval properties, derived from various botanical sources are focused. Quite a lot of work has been carried out in higher plants on their biologically active material with antilarval properties (Saxena & Sumithra, 1985) [31]. Few attentions were focused on the larvicidal properties of the marine seaweeds (Semakov & Sirenko, 1985) [32]. Seaweeds are the extraordinary sustainable resources in the marine ecosystem which have been used as a source of food, feed and medicine. It was estimated that about 90% of the species of marine plant are algae and about 50% of the global photosynthesis is contributed from algae.

The extract of seaweeds and essential oil of certain plants have been investigated, and it showed toxic effect against some public health pests (Hadjiakhoondi *et al*., 2006 and Vatandoost *et al*., 2004) [17, 39]. As early as in 1917, the inhibitory substances biosynthesized by the seaweeds were reported (Harder and Oppermann, 1953) [18]. Pratt *et al*., 1944 firstly reported antibiotic activities of seaweeds. Recent findings evidenced that seaweeds contained antibacterial (Tuney *et al*., 2006) [38], antiviral (Serkedjieva, 2004 and Garg *et al*., 1992) [33], antifungal (Tang *et al*., 2002; Aliya and Shamaeel, 1999) [36, 5], cytotoxic (Tang *et al*., 2002) [36] larvicidal and pupicidal potentials (Thangam and Kathiresan, 1991) [37]. Large numbers of plant samples have been screened for their insecticidal and/or repellent activities and a few of them have been found to be promising and their products are commercially available. Thangam and Kathiresan have investigated for the first time seaweeds, sea grasses and

mangrove plants for their larvicidal, skin and smoke repellent activities against mosquito species. Some of them were effective in killing the larvae or repelling adult female mosquito potentials (Thangam and Kathiresan, 1991) [37]. In this background, the present study was initiated to explore the larvicidal and pupicidal potential in different solvent extracts of seaweed *Turbinaria conoides*.

2. Materials and Methods

Collection of Seaweeds

The seaweed *Turbinaria conoides was* collected from the shore of Mandapam, nearby Rameshwaram, Gulf of Mannar, Tamil Nadu, India.

Preparation of seaweed extracts

The shade dried seaweed sample was hand crushed, smashed and made to powder with a mortar and pestle, stored in an air tight container until screening for their larvicidal and pupicidal activity. Extracts were prepared using acetone ethanol and distilled water.

One hundred grams of the powder was extracted with 300 mL of the respective solvents for 8 h in a Soxhlet apparatus and the extract was dried in a rotary vacuum evaporator to yield aqueous, acetone and ethanol extracts of *Turbinaria conoides* under reduced pressure 22–26 mmHg at 45 °C the extracts were concentrated. The extracts were collected in plastic vials and stored in the refrigerator for further studies (Aseer *et al.,* 2009). One gram of the residue was dissolved in 100 ml of respective solvents to make a 1% stock solution. Six different concentration of the extracts (100, 200, 300, 400 and 500 mg/L) were prepared from the stock solution for subsequent testing.

Mosquito larvae and pupae

Larvae of the three mosquito species *Aedes aegypti, Anopheles stephensi* and *Culex quinquefasciatus* were reared in enamel trays containing dechlorinated water. The larvae were fed with finely powdered mixture having 3:1 ratio of dog biscuits and dry yeast. The rearing water was changed daily.

The pupae taken up for the study were assembled into two different category for three types of mosquito species (*Aedes aegypti, Anopheles stephensi* and *Culex quinquefasciatus*) as given below.

1.	Larvae that emerged as pupae after larvicidal activity	L .P.
2.	Field pupae that were collected from the stagnate water in the Field	F. P.

Larvicidal bioassay

Preliminary screening of mosquito larvicidal activity was carried out against the above mentioned three different mosquito larvae using the standard protocol of World Health Organization 1981 with minor modifications. In the present bioassay, 25 larvae were taken into six glass beakers (1500 mL capacity containing 1000 mL tap water) using a Pasteur pipette. Five different concentrations of the seaweed extracts *viz*., 100, 200, 300, 400 and 500 mg/L taken up for the study. A control was also run simultaneously which comprised of water only. The experiment was carried out in glass beakers for 24 h at room temperature and after 24 hours the mortality rate was recorded and assessed. The study was undertaken in triplicates for further statistical analysis.

Pupicidal bioassay

Twenty pupae were taken into six glass beakers (1500 mL capacity containing 1000 mL tap water) using a Pasteur pipette. Five different concentrations of the seaweed extracts *viz*., 100, 200, 300, 400 and 500 mg/L taken up for the study. A control was also run simultaneously which comprised of water only. The experiment was carried out in glass beakers for 24 h at room temperature and after 24 hours the mortality rate was recorded and assessed. The pupae mortality in each concentration and control was recorded after 24 hours of exposure from the average of three replicates. The mortality percentage was calculated using the Abbotts (1925).

$$\text{Percentage mortality of pupae} = \frac{\text{Number of dead pupae}}{\text{Number of pupae introduced}} \text{ x100}$$

Statistical analysis

The LC_{50}, LC_{90}, 95% confidence limits of upper confidence limit (UCL) and lower confidence limit (LCL) values were calculated by subjecting the average larvae mortality values to Stat plus 2009 software on the guidelines of OECD recommended 'Probit' analysis (Finney, 1971). Using Abbotts (1925) formula, with the natural mortality observed in the negative controls, percentage mortalities were corrected.

Abbotts formula

$$P = PI – C / 1 – C$$

Where, PI and C denote the observed mortality rate and the natural mortality.

3. Results

Larvicidal activity

The results of biolarvicidal activity of three different extracts (aqueous, acetone and ethanol) of *Turbinaria conoides* against *Aedes aegypti, Culex quinquefasciatus* and *Anopheles stephensi* were tabulated.

The values of larvicidal activity against *Aedes aegypti* were recorded and LC_{50} were found to be 18.74 mg/L of aqueous extract followed by 6427 mg/L of ethanol extract and 100.07 mg/L of acetone extract of *Turbinaria conoides* against *Aedes aegypti*. The LC_{90} values of *Turbinaria conoides* of aqueous and acetone extracts were 164.59 and 269.76 mg/L respectively. The order of hierarchy of biolarvicidal activity of the three different extracts of *Turbinaria conoides* against *Aedes aegypti* was found to be aqueous > ethanol > aectone at the LC_{50} level (Table - 1).

Table 1: Effect of aqueous, acetone and ethanol extracts of the *Turbinaria conoides* against *Aedes aegypti*, *Culex quinquefasciatus* and *Anopheles stephensi* larvae

Mosquito species	Extract	LC_{50} (mg/L)	95% Confidence Limit		LC_{90} (mg/L)	95% Confidence Limit	
			LCL	UCL		LCL	UCL
Aedes aegypti	Aqueous	18.74	134.35	106.54	164.59	67.18	228.95
	Acetone	100.07	20.74	173.42	269.76	201.31	328.41
	Ethanol	64.27	53.83	138.7	508	426.67	645.48
Anopheles stephensi	Aqueous	66.62	50.67	139.41	472.03	400.45	586.34
	Acetone	76.35	25.07	143.85	184.4	110.89	237.87
	Ethanol	88.18	33.67	163.3	212.78	131.06	272.04
Culex quinquefasciatus	Aqueous	82.74	20.05	149.39	393.27	333.56	477.33
	Acetone	62.12	7.12	104.27	167.95	128.65	200.4
	Ethanol	74.45	8.78	124.21	137.09	81.71	180.1

LC_{50} - median lethal concentration; LC_{90} - 90% lethal concentration; LCL – Lower confidence limit; UCL – Upper confidence limit

The LC_{50} values for aqueous extracts of *Turbinaria conoides* against *Anopheles stephensi* was 66.618 mg/L. followed by acetone extract with 76.35 mg/L. The LC_{90} values for effective larvicidal activity were found to be acetone and it was 184.4 mg/L. Successive effects was observed on ethanol extract and it was 212.78 mg/L (Table - 1). In the case of *Culex quinquefasciatus,* acetone extracts was found to be high and the LC_{50} value was 2.12 mg/L followed by 74.45 mg/L and 82.74 mg/L for ethanol and aqueous extracts respectively (Table - 1). The above results inferred that out of the three mosquito larvae tested, exposure of *Aedes aegypti* to aqueous extracts of *Turbinaria conoides* showed the maximum larvicidal efficacy.

Pupicidal activity

The acetone extract of *Turbinaria conoides* showed hundred per cent pupicidal effect at 30 mg/L concentration to the field pupae (F.P.) and aqueous extract showed 90% mortality followed by ethanol extract that 70% pupicidal activity to the field pupae.

Table 2: Mortality percentage of aqueous, acetone and ethanol extracts of the seaweed *Turbinaria conoides* against *Aedes aegypti* pupae

Extract	Concentration of *Turbinaria conoides* mg/L									
	10		20		30		40		50	
	L .P.	F. P.	L .P.	F. P.	L .P.	F. P.	L .P.	F. P.	L .P.	F. P.
Aqueous	55	75	60	85	75	90	85	100	90	100
Ethanol	10	50	10	60	20	70	25	80	35	85
Acetone	20	60	20	85	30	100	35	100	35	100

L .P. - Larvae that emerged as Pupae after larvicidal activity
F. P. - Field Pupae

Interestingly it is noticed that aqueous extract of *Turbinaria conoides* showed 90% pupicidal activity to the L.P. at 50 mg/L against *Aedes aegypti*.

Lethal effect of different extracts of *Turbinaria conoides* against *Anopheles stephensi* showed one hundred per cent mortality in aqueous extract at 50 mg/L concentration to the larval pupae, whereas ethanol and acetone extracts showed a less significant effect. It is evident that 65and 35% mortality were recoded to the L.P whereas 60% and 40% of lethal effects were observed to the F.P. in ethanol and acetone extracts of *Turbinaria conoides* against *Anopheles stephensi* mosquito pupae (Table 3).

Table 3: Mortality percentage of aqueous acetone and ethanol extracts of the seaweed *Turbinaria conoides* against *Anopheles stephensi* pupae

Extract	Concentration of *Turbinaria conoides* (mg/L)									
	10		20		30		40		50	
	L .P.	F. P.	L. P.	F. P.	L .P.	F. P.	L .P.	F. P.	L .P.	F. P.
Aqueous	20	20	35	40	60	45	80	50	100	55
Ethanol	15	20	20	20	40	25	55	35	65	60
Acetone	5	10	15	10	20	25	25	30	35	40

L .P. - Larvae that emerged as Pupae after larvicidal activity
F. P. - Field Pupae

Mortality percentage of *Culex. quinquefasciatus* showed hundred per cent pupicidal activity to the field pupae at 50 mg/L concentration in acetone extracts while aqueous and ethanol extract showed insignificant effect to both field pupae and L.P. (Table. 4).

Table 4: Mortality percentage of aqueous acetone and ethanol extracts of the seaweed *Turbinaria conoides* against *C. quinquefasciatus* pupae

Extract	Concentration of T. conoides mg/L									
	10		20		30		40		50	
	L .P.	F. P.	L .P.	F. P.	L .P.	F. P.	L .P.	F. P.	L .P.	F. P.
Aqueous	10	15	15	35	15	40	20	45	25	60
Ethanol	10	20	15	35	20	40	25	50	35	70
Acetone	30	25	40	50	50	70	55	85	65	100

L .P. - Larvae that emerged as Pupae after larvicidal activity: F. P. - Field Pupae

The values of pupicidal activity against *Aedes aegypti*, *A. stephensi* and *C. quinquefasciatus* were recorded and effective LC_{50} were found to be 5.64 mg/L of aqueous extract followed by 9.06 mg/L of acetone extract and 13.83 mg/L of ethanol extract of *Turbinaria conoides* against *A. aegypti* pupa that collected from field (F.P) (Table - 5).

Table 5: The LC_{50} of aqueous, acetone and ethanol extracts of the *Turbinaria conoides* against *Aedes aegypti, Culex quinquefasciatus* and *Anopheles stephensi* pupa that collected from field (F.P)

Extracts	LC50 (mg/L)		
	A. aegypti	***A. stephensi***	***C. quinquefasciatus***
Aqueous	5.64	38.06	40.05
Acetone	9.06	80.8	18.12
Ethanol	13.83	58.5	123.3

Whereas effective LC_{50} values of Pupae that emerged after larvicidal activity (L.P) was found to be 10.16 mg/L of aqueous extract against *A. aegypti* followed by 36.82 mg/L and 38.06 mg/L of ethanol and aqueous extract respectively against *A. stephensi* (Table - 6).

Table 6: The LC_{50} of aqueous, acetone and ethanol extracts of the *Turbinaria conoides* against *Aedes aegypti, Culex quinquefasciatus* and *Anopheles stephensi* pupa that emerged after larvicidal activity (L.P)

Extracts	LC_{50} (mg/L)		
	A. aegypti	***A. stephensi***	***C. quinquefasciatus***
Aqueous	10.16	38.06	40.05
Acetone	151.3	91.01	28.82
Ethanol	115.37	36.82	123.3

4. Discussion

Though synthetic insecticides are effective they create many problems like development of insecticide resistance (Lin *et al*., 2005). Therefore, usage of indigenous plant based products, could provide standardized measure for protection to the human population against various disease caused by mosquito. Many approaches that have been developed to control the mosquito menace. One such approach to prevent mosquito-borne disease is to kill at its larval stage. Many studies made use of plant extracts for mosquito control approach.

Kamaraj *et al*., 2011 reported that plants derived extracts using different solvents crude extracts have potential larvicidal activity. To evaluate the potential larvicidal activity of the plant preliminary screening is a good measure (Ali *et al.* 2012) [4]. Insecticides of botanical origin may serve as suitable alternative biocontrol techniques in the future (Kamaraj *et al*., 2009) [22].

Howard *et al.*, 2007 revealed that larval control can be the effective appropriate way in controlling the mosquitoes in breeding habitats, which are man-made. Fahd *et al*., 2013 reported that in early findings the effect of ethanol extract of *Annona squamosa* leaf was effective in larvicidal activity against *C. quinquefasciatus.* The results of the present study also confirm similar results with acetone extracts of *Turbinaria conoides*. Chloroform extract of *Millettia dura*, show higher larvicidal activity against the second instar larvae of *Aedes aegypti* was reported by Yenesew *et al.* (2003) [42]. Rahuman *et al*., 2008 stated that the extracts of *Jatropha. curcas* and *Euphorbia tirucalli* were highly effective against the larvae of *Aedes aegypti*, and the LC_{50} values were 35.39, 256.77, 384.19, 703.76, and 13.14 ppm against *Aedes aegypti.* Chowdhury *et al*, 2008, stated that *Solanum villosum* offers promised as a potential bio control agent against *Aedes aegypti* particularly in its markedly larvicidal effect.

In the present study, larvicidal efficacy of three extracts of *Turbinaria conoides* was assessed. Aqueous extract of *Turbinaria conoides* was found to be more effective in larvicidal activity with a minimum LC_{50} value of 18.47 mg/L against *Aedes aegypti*. A similar effect was also observed by Anandhan and Sorna kumari, (2011) [6] using *Gracilaria crassa* and *Hypnea valentia* in methanolic extract against *Aedes* sp. The LC_{50} values 66.618 mg/L for aqueous extracts of *Turbinaria conoides* was found to be promising against *Anopheles stephensi and* mortality rate of *Culex quinquefasciatus* was found to be high on the acetone extracts of *Turbinaria conoides*, and LC_{50} value was 62.12 mg/L. The results of Mullai and Jebanesan (2007) [25] on larvicidal effect of four different extracts of *Cucumis pubescens* leaf against *Anopheles stephensi, Culex quinquefasciatus*, and *Aedes aegypti* agrees with the relevant results of the present study.

Seaweeds contains rich source of structurally diverse secondary metabolites. It is presumed that, the secondary metabolites offer a defence against mosquito larvae play an effective role in larvicidal activity in the present investigation.

Imaga *et al.* (2010) indicated the presence of alkaloid, flavonoid, saponins and glycosides in the extract of *Carica papaya* leaves and these compounds have been found to possess high larvicidal activities against different species of mosquitoes (Chapagain *et al.*, 2008; Quevedo *et al.*, 2012; Shallan *et al.*, 2005) [10, 29, 34]. A similar study reported the evaluation of the use of *Parthenium hysterphourus* against mosquito *Aedes aegypti* (Muthukrishnan and Pushpalatha, 2001) [26] and combined effect of other phenolic acids *viz*., caffeic acid, vanillic acid, ansic acid, p-ansic acid, chlorogenic acid and parahydroxy benzoic acid may possess larvicidal and pupicidal property on *Aedes aegypti* and *Culex quinquefasciatus.*

Okumu, *et al.*, 2007 the percentage emergence in most cases was less than the percentage pupation, which suggests some pupal mortality. The emergence inhibition (EI) values depicted with the neem oil formulation treatments were much lower than the respective lethal concentration (LC) values, an indication that the growth disruption activity of the neem product extended to pupal stages.

In the present study, acetone extract of *Turbinaria conoides* showed one hundred per cent pupicidal effect at 30 mg/L concentration to the field pupae (F.P.) against *Aedes aegypti*; at 50 mg/L concentration of aqueous extract to the larval pupae against *Anopheles stephensi* and hundred percent pupicidal activity to the field pupae at 50 mg/L concentration in acetone extracts of *Turbinaria conoides* against *Culex quinquefasciatus* were observed. Kumar *et al.*, 2012 reported that *Sargassum wightii* crude extract treatment resulted in higher larval and pupal mortality which might be due to the multiple actions of bioactive compounds present in the seaweed. A similar effect was also observed in the present study.

The present study revealed that treatment of seaweed extracts on *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* contains a toxic chemical leading to remarkable mortality against subsequent developmental stages of mosquito life cycle. Observations showed that seaweed contained chemical that brought out such mortality to the larvae and pupae. Moreover it acts as a regulator of growth in immature larvae to the adult emergence.

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