

Efficacy of *Aedes aegypti* and *Culex quinquefasciatus* against *Sargassum wightii* and *Ulva fasciata*

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Abstract

This study investigated the mosquitocidal activities of marine algae collected from Gulf of Mannar. The larvae *Aedes aegypti* and *Culex quinquefasciatus* tested against *sargassum wightii* and *Ulva fasciata* were extracted using different solvents like Chloroform, Methanol, Petroleum ether and Acetone. The two different seaweed extracts using four different solvents were proved statistically significant ($p < 0.05$). The seaweed extract used for the LC50 assay against II and III instar larval mortality was recorded. The overall results was observed all the solvent extract against *sargassum wightii* especially more toxic in acetone and chloroform of *Culex quinquefasciatus* (II & III -100%) followed by chloroform in *Aedes aegypti* (II & III -83%). Likewise the similar species of mosquito larvae (*Aedes aegypti*) against *Ulva fasciata* extract in Chloroform, acetone and methanol showed the high mortality was noted. These findings may help in developing a prospective alternative source to control the mosquitoes.

Keywords: Larvicidal activity, *Aedes aegypti*, *Culex quinquefasciatus*, *sargassum wightii*, *Ulva fasciata*

Introduction

Mosquitoes are responsible for the spread of more diseases. Mosquito borne diseases still remain a major health problem in both human and veterinary sectors. Mosquitoes, under the order Diptera are ravaging the humans and other animals for generations. There are nearly 2,500 mosquito species in the world but few of them transmit an array of pathogens including viruses (e.g., arboviruses), protozoans (e.g., malaria) and nematode worms (e.g., lymphatic filariasis). According to Taubes (1997), annually more than 700 million people suffer from mosquito borne diseases. Synthetic insecticides have

been widely used to control mosquito vectors of disease in various parts of the world. Plants are a source of bioactive compounds that have insecticidal properties and therefore may be suitable for mosquito control (Prophiro, 1762). The indiscriminate use of synthetic insecticides is creating multifarious problems such as environmental pollution, insecticide resistance and toxic hazardous to human beings (Shalan, 2012). Larvicidal properties of marine water algae (*P. pavonica* and *Zonaria* sp.) were found to be most effective seaweeds (Hamlyn-Harris, 1928; Griffin, 1956; Dhillon and Mulla, 1981; Semakov and Sirenko, 1985;

Pucazhendi *et al.* 1995). The frequent use of systemic insecticides to manage insect pests leads to a destabilisation of ecosystem and enhanced resistance to insecticides in pests (Kranthi *et al.*, 2001; Mohan and Gujar 2003), suggesting a clear need for alternatives. Plant products have been used by traditionally by the human communities in different parts of the world against the vectors and species of insects. The phytochemicals derived from plant sources can act as larvicides and insect growth regulators and have deterrent activities observed by many researchers. Although most of the algae are nutritious food for mosquito larvae, some species kill the larvae when ingested in large quantities (Marten, 1987 and 2007) while blue-green algae toxins may offer possibilities for delivery as larvicides. Certain species of green algae kill larvae primarily because they are indigestible. The algae's metabolites have also been shown in several studies which possess larvicidal activities (Poonguzhali and Nisha, 2012 & Elbanna and Hegazi, 2011). many studies on plant extracts against mosquito larvae have been conducted around the world. In fact, many researchers have reported on the effectiveness of plant extracts or essential oils against mosquito larvae (Sharma *et al.*, 2006; Amer and Mehlhorn 2006a, b). The *Culex* mosquito, better known as the common house mosquito, is one of the three major type of mosquitoes inhabiting the planet. Essential oils from plants and algal extracts may be the rich alternative sources of mosquito larval control agents, as they constitute a rich source of bioactive compounds that are biodegradable into nontoxic products and potentially suitable for use in control of mosquito larvae. Hence in the present study was made as an attempt to find out the mosquito larvicidal efficacy of four solvent extracts of two seaweeds against *Aedes aegypti* (*Ae. aegypti*), *Culex quinquefasciatus* (*Cx. quinquefasciatus*).

Material and methods

Collection and identification of seaweeds

The present study area Gulf of Mannar (GoM) is a transitional zone between the Arabian Sea and the Indian Ocean. Palk Strait connects GoM with the Bay of Bengal. The GoM has a chain of 21 islands located between 8° 46' N, 78° 9' E and 9° 14' N, 79° 14' E on the southeast coast of India. It has been estimated that 302 seaweed species exist in GoM in particular between pudumadam (SS-1) and Pamban (SS-2) Plate-1. Four species of seaweed samples were collected in the early morning from the intertidal zone of Rameswaram, Tamilnadu during low tide on June 2013. The Seaweed *sargassum wightii*, and *Ulva fasciata* were collected by hand picking. The collected macroalgae were immediately rinsed in water to remove all kinds of epiphytes and other impurities (*i.e.*, sand, mollusks, sea grasses etc). The cleaned samples were immediately kept in sterilized ziploc bags and transferred to laboratory.

Preparation of extracts

The cleaned macroalgae were allowed to shade dry (up to 7 days) and all individual samples were made into powder form using mixer grinder. The powdered sample of each species (10 grams) were suspended in selective solvent system (*i.e.*, Chloroform, Methanol, Petroleum ether, Acetone and Butanol) (Merck AR Grade) and kept in a borosilicate soxhlet apparatus for eight hours (WHO, 2005). After that, extracted samples were filtered using Whatman No.1 filter paper. The filtered sample was individually centrifuged at 5000 rpm for 10 min at 4°C. The supernatant was collected in a separate flask. At each centrifugation, the supernatant was pooled and kept separately. Then the extract was concentrated using a rotary vacuum evaporator (Puchi RII, Switzerland) at 40°C. The final concentrated crude extract was individually stored in sterile air tight bottles and kept in a refrigerator until further

use (Celikler *et al.*, 2009). The percentage of extraction was calculated by using the following formula. The extract preparation was done by following the method of Ali *et al.*, (2012).

$$\text{Percentage of extraction} = \frac{\text{Weight of the extract}}{\text{Weight of the plant material}} \times 100$$

Collections and Rearing of Mosquito Larva

The eggs and egg rafts of two mosquito species *Aedes aegypti* and *Culex quinquefasciatus* were procured from CRME (Center for Research in Medical Entomology), Madurai in ICMR (Indian Council of Medical Research), Tamil nadu, India. Larvae of mosquito species *Aedes aegypti* 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 (Govindarajan *et al.*, 2011). Then larvae were observed and monitored for II and III instar stages. The larvae were used for toxicological study.

Larvicidal Bioassay

The seaweed extracts were screened primarily for larvicidal activity. Briefly 10 numbers of II and III instar larvae were exposed with 0.5mg/ml of the chloroform, petroleum ether, acetone and methanol extract and incubated for 24 hrs at 27±2°C and 14:10 light and dark cycles (WHO, 2005). All the experiments were carried out in triplicates and control respective solvents were maintained. The selected extracts were diluted with distilled water to get desired concentrations of 12.5, 25, 50, 100, 200, 400, and 500 ppm per ml of solution. For bioassay test, 25 numbers of II and III instar larvae of *Aedes aegypti* and *Culex quinquefasciatus* were exposed to different concentrations of (12.5, 25, 50, 100, 200, 400, and 500 ppm) of extracts individually. The larvae were starved during the experiment and mortality was

recorded after 24 and 48hrs of post-exposure (WHO 1981). Similarly the control was recorded from the average of three replicates. The mortality percentage was calculated using the Abbott's (1925). The LC₅₀ were calculated using SPSS software using linear regression. Dead larvae were counted after incubation. The larvae failed to move after probing with dropper were considered as dead. The percentage of mortality was calculated using the following formula;

$$\text{Percentage of mortality} = \frac{\text{Number of dead Larvae}}{\text{Number of larvae tested}} \times 100$$

Results

The selected two different seaweeds (*Sargassum wightii*, *Ulva fasciata*, were extracted with organic solvents like chloroform, petroleum ether, acetone and methanol. Sixteen extracts were isolated from the above mentioned marine algae and each of the extracts was weighed and calculated and the levels were statistically significant P<0.05 (Table.2). The results showed that the highest percentage of yield for the seaweeds, *Sargassum wightii* and *Ulva fasciata* were observed with petroleum ether, chloroform, acetone, methanol and butanol respectively. The percentage of *S. wightii* crude extracts ranged from 2.542 to 1.761 % and for *U. fasciata* it was found to be 3.197 to 1.982 %. The differences of the percentage yield among the solvents were statistically significant for all the tested seaweeds at P<0.05.

Preliminary phytochemical analyse of different biomolecules such as carbohydrates, proteins, flavonoids, phenols, tannins, saponin, steroids and alkaloids were tested in sixteen different seaweed crude extracts (Table-3). Alkaloids and flavonoids did not show any positive result in *Sargassum wightii* and *Ulva fasciata*. The presence or absence of the phytochemicals depends upon the solvent medium used for the extraction. Carbohydrates, proteins, steroids, phenols, tannins, saponin and

alkaloids flavonoids showed the maximum presence in two different extracts of *Sargassum wightii* and *Ulva fasciata*.

The eight different phytochemical compound present in sixteen extracts. The results show some phytochemical compounds are more toxic. Using the two different seaweed extracts the larvicidal activities were noted. It is observed that good larval mortality rate occurred at the highest concentration of 500 mg/ml of methanol extract of *Sargassum wightii* against II instar larvae of *Aedes aegypti* (Table. 4). The other concentrations of 100, 200, 300 and 400 mg/ml showed moderate larval mortality. In comparison with the control, all concentrations of *Sargassum wightii* contributed high larvicidal activity against *Ae. aegypti*. The lowest mortality was observed in petroleum ether extract at 28 % level in 500 mg/ml concentrations. In III instar, larval mortality was observed in acetone extract 92 % at 500 mg/ml concentration. For the *S. wightii* against II and III instar larvae of *Culex quinquefasciatus* 100 percent larval mortality were detected in chloroform and acetone extracts at 500 mg/ml concentration level. Low mortality rate was found in petroleum ether extract (Table. 5). Dead larvae were not observed in the entire control group.

The data obtained from 400 and 500 mg/ml of acetone and 500 mg/ml of chloroform and methanol extract of *Ulva fasciata* gave 100 % mortality and were recorded in II instar larvae of *Ae. aegypti* (Table. 6). The III instar larvae also exhibited 100 % mortality at 400 and 500 mg/ml of chloroform and acetone extracts and 500 mg/ml methanol extract. All the extracts showed concentration dependent mortality. Similar results were obtained in *Cx. quinquefasciatus* II and III instar (Table. 7).

Discussion

Algae synthesize a number of chemically diversified secondary metabolites. Among them, some of the compounds are recognized

as insecticides. The control of adult mosquito is an unsuccessful strategy as the adult stage occurs beside human inhabitation and they can easily overcome remedial measures (Service, 1983 and 1992).

Phytochemicals are naturally present in the seaweeds. phytochemicals in marine algae may reduce the risk of human diseases, possibly due to dietary fibers, polyphenol antioxidants and anti-inflammatory effects (Boonchum *et al.*, 2011; Oumaskour *et al.*, 2012; Abirami *et al.*, 2012). The preliminary phytochemical screening is a part of chemical evaluation of the seaweeds. Seenivasan *et al.* (2012) found that the results with the highest total phenol and flavanoid was in the brown seaweed *S. wightii*. The green seaweed like *Ulva* (Elmegeed *et al.*, 2014) contain saponin, alkaloids, steroids and terpenoids. Tannin and phenolic content shows positive result to all solvent extract from both seaweeds.). The phytochemical component saponins serve as biocontrol agent against vector mosquitoes as reported by Chapagain *et al.* (2008). Phenol compounds are also responsible for the antimicrobial, anti inflammatory, antifeedant, antiviral, anticancer and vasodilatory actions (Aliyu *et al.*, 2009; Valentina *et al.*, 2015 and Gupta, 2011). The tannin containing drugs are used in the treatment of piles, inflammation, burns and as astringent (Kolodziej *et al.*, 2005). In addition to similar evidence of polyphenols it may be associated with various carbohydrates and organic acids (Manach *et al.*, 2004).

Larval stages of mosquitoes are exclusively aquatic, systematic exposure of algal based larvicides in their breeding habitat is a successful and safer way to interrupt larval stages of vectors rather than the adult stage (Thangam and Kathiresan, 1991). Periodic larvicide is very helpful in favorable conditions (WHO, 1975 and Becker *et al.*, 2003). The marine algae, *C. racemosa* showed minimum LC₅₀ values of *Ae. aegypti*, *Cx. quinquefasciatus* and *An.*

stephensi larvae [LC₅₀=(0.055 6±0.010 3) µg/mL, (0.056 9±0.021 3) µg/mL, (0.066 0±0.007 6) µg/mL] when compared with control respectively; this might due the presence saponin and triterpenoids by Ali et al., 2012, (Syed ali et al., 2013). The extract of *D. dichotoma* showed minimum level of LC₅₀value (0.0683±0.0084 µg/mL) and LC₉₀value was 0.140 1. The regression equations of *D. dichotoma* and *E. intestinalis* for 4th instar larvae were Y=0.333 + 0.684x (R²=0.946) and Y=0.600 + 0.781x (R²=0.812), respectively(Beula,M et al., 2011).

The present study reveals that different solvent extracts of the selected seaweeds were tested for their larvicidal effect on the two mosquito larvae on their two life stages. Based on the results it is evident that all the extracts of the four algal samples were effective against both larvae. The LC₅₀ values showed that ethyl acetate extract of *S. wightii* was found to be more effective against both II and III instar larvae of *Ae. Aegypti* (LC₅₀ 162.23 and 253.78 mg/ml) and *Cx. quinquefasciatus* (LC₅₀142.87 and 180.37 mg/ml). Ethyl acetate extract of *U. fasciata* was found to be more effective to II instar (138.32 mg/ml) and Methanol (197.21 mg/ml) was found to be highly active in III instar of *Ae. aegypti* and II and III of *Cx. quinquefasciatus* (113.32, 200.47 mg/ml). A broad spectrum of algae species like *Caulerpa prolifera*, *Caulerpa serrulata*, *U. lactuca*, *Lobophora variegata*, *Spatoglossum asperum*, *Dictyota dichotoma*, *U. fasciata* and *Grateloupia lithophila* were screened for their high effects on mosquito larvae

(Elbanna and Hegazi, 2011; Ravikumar et al., 2011). The solvent extracts of *U. lactuca* caused significant larvicidal effect against *Cx. pipiens* larvae. Acetone extract was the most potent larvicidal extract with LC₅₀ value of 5.46 mg/ml. On the other hand, marine algae *U. fasciata* and *U. lactuca* have been reported to possess nymphicidal, anti-ovipositional activity, reduced fecundity, hatchability and adult longevity (Abbassy et al., 2014).

Conclusion

It can be concluded from the present study that, the chloroform and methanol extracts of seaweed of *U.faciata* possess more active compounds present in the above mentioned solvent against the larvae of *Aedes aegypti* and *Culex quinquefasciatus*.

Plate 1. Overview image of sample collection site from Google Map



Table: 2. Percentage (%) of different organic solvent extracts of seaweeds

Samples	Chloroform	Petroleum ether	Acetone	Methanol
<i>S.wightii</i>	2.346	1.761	2.487	2.542
<i>U.fasciata</i>	2.482	1.982	3.197	2.931

All the values are denote as percentage.

Table: 3. Analysis of phytochemical compounds from different solvent extracts against seaweeds

Name of the Seaweeds	Phytochemicals	Chloroform	Methanol	Petroleum ether	Acetone
<i>Sargassum wightii</i>	Carbohydrates	+	+	-	-
	Proteins	+	-	-	+
	Alkaloids	-	-	-	-
	Steroids	-	+	-	-
	Tannin	+	+	+	+
	Phenol	+	+	+	+
	Saponins	+	-	+	+
	Flavanoids	+	-	+	-
<i>Ulva fasciata</i>	Carbohydrates	+	+	-	-
	Proteins	-	-	-	-
	Alkaloids	-	-	-	-
	Steroids	+	-	-	+
	Tannin	+	+	+	+
	Phenol	+	+	+	+
	Saponins	+	-	+	-
	Flavanoids	-	+	-	-

Table: 4. Mortality percentage of different solvent extracts of the seaweed *Sargassum wightii* against II and III instar larvae of *Aedes aegypti* (n= 100)

Concentrations (mg/ml)	II instar larval mortality (%)			
	Chloroform	Petroleum ether	Acetone	Methanol
100	26 ^c	2 ^{cd}	25 ^c	25 ^c
200	45 ^{bc}	9 ^{cd}	54 ^{bc}	45 ^{bc}
300	66 ^b	13 ^{cd}	71 ^b	59 ^{bc}
400	86 ^{ab}	21 ^c	88 ^a	79 ^{ab}
500	97 ^a	28 ^c	98 ^a	100 ^a
	III instar larval mortality (%)			
100	20 ^{cd}	0 ^d	12 ^{cd}	10 ^{cd}
200	34 ^c	10 ^{cd}	37 ^c	35 ^c
300	45 ^{bc}	15 ^{cd}	68 ^b	60 ^b
400	77 ^{ab}	19 ^{cd}	78 ^{ab}	70 ^b
500	87 ^a	21 ^{cd}	92 ^a	83 ^{ab}
Control	0 ^d	0 ^d	0 ^d	0 ^d

The columns, mean followed by the same letter do not significantly using **Duncan's test** $p \leq 0.05$. d-Reference control

Table:5. Mortality percentage of different solvent extracts of the seaweed *Sargassum wightii* against II and III instar larvae of *Culex quinquefasciatus* (n= 100)

Concentrations (mg/ml)	II instar larval mortality (%)			
	Chloroform	Petroleum ether	Acetone	Methanol
100	22 ^{cd}	10 ^{cd}	31 ^c	23 ^{cd}
200	68 ^b	25 ^c	63 ^{bc}	44 ^{bc}
300	90 ^a	35 ^c	96 ^a	56 ^{bc}
400	98 ^a	38 ^c	99 ^a	77 ^{ab}
500	100 ^a	45 ^{bc}	100 ^a	99 ^a
	III instar larval mortality (%)			
100	23 ^{cd}	2 ^{cd}	23 ^{cd}	10 ^{cd}
200	45 ^{bc}	6 ^{cd}	38 ^c	17 ^{cd}
300	76 ^b	16 ^{cd}	78 ^{ab}	34 ^c
400	90 ^a	25 ^c	87 ^a	60 ^{bc}
500	100 ^a	37 ^c	100 ^a	92 ^a
Control	0 ^d	0 ^d	0 ^d	0 ^d

The columns, mean followed by the same letter do not significantly using **Duncan's test** $p \leq 0.05$. d-Reference control

Table: 6. Mortality percentage of different solvent extracts of the seaweed *Ulva fasciata* against II and III instar larvae of *Aedes aegypti* (n= 100)

Concentrations (mg/ml)	II instar larval mortality (%)			
	Chloroform	Petroleum ether	Acetone	Methanol
100	36 ^c	14 ^{cd}	29 ^c	23 ^{cd}
200	62 ^{bc}	25 ^c	62 ^{bc}	50 ^{bc}
300	93 ^a	33 ^c	93 ^a	75 ^b
400	99 ^a	45 ^{bc}	100 ^a	91 ^a
500	100 ^a	66 ^b	100 ^a	100 ^a
	III instar larval mortality (%)			
100	41 ^c	12 ^{cd}	28 ^c	21 ^{cd}
200	76 ^b	16 ^{cd}	65 ^{bc}	50 ^{bc}
300	95 ^a	33 ^c	90 ^a	73 ^b
400	100 ^a	56 ^{bc}	100 ^a	91 ^a
500	100 ^a	81 ^{ab}	100 ^a	100 ^a
Control	0 ^d	0 ^d	0 ^d	0 ^d

The columns, mean followed by the same letter do not significantly using **Duncan's test** $p \leq 0.05$. d-Reference control

Table:7. Mortality percentage of different solvent extracts of the seaweed *Ulva fasciata* against II and III instar larvae of *Culex quinquefasciatus* (n= 100)

Concentrations (mg/ml)	II instar larval mortality (%)			
	Chloroform	Petroleum ether	Acetone	Methanol
100	33 ^c	20 ^{cd}	32 ^c	27 ^c
200	65 ^{bc}	33 ^c	62 ^{bc}	48 ^{bc}
300	90 ^a	40 ^c	88 ^a	69 ^b
400	100 ^a	55 ^{bc}	99 ^a	91 ^a
500	100 ^a	71 ^b	100 ^a	100 ^a
	III instar larval mortality (%)			
100	23 ^{cd}	9 ^{cd}	20 ^{cd}	21 ^{cd}
200	47 ^{bc}	21 ^{cd}	38 ^c	30 ^c
300	75 ^b	24 ^{cd}	70 ^b	52 ^{bc}
400	93 ^a	38 ^c	88 ^{ab}	68 ^b
500	100 ^a	56 ^{bc}	92 ^a	90 ^a
Control	0 ^d	0 ^d	0 ^d	0 ^d

The columns, mean followed by the same letter do not significantly using **Duncan's test** $p \leq 0.05$. d-Reference control

References

- Abbott, W. S. 1925. A method of computing the effectiveness of an insecticides. *Journal of Economic Entomology*. 18: 265-267.
- Abirami, R.G. and Kowsalya, S. 2012. Anticancer Activity of Methanolic and Aqueous Extract of *Ulva fasciata* in albino mice. *International Journal of Pharmacy and Pharmaceutical science*. 4(2): 1681- 684.
- Ali, M.S., Ravikumar, S. and Beula, J.M. 2012. Spatial and temporal distribution of mosquito larvicidal compounds in mangroves. *Asian Pacific Journal of Tropical Disease*. 2: 401-404.
- Ali, M.S., Ravikumar, S. and Beula, J.M. 2012. Bioactivity of seagrass against the dengue fever mosquito *Aedes aegypti* larvae. *Asian Pac J Trop Biomed*. 2(1):S70-S75.
- Aliyu, A.B., Musa, A.M., Sallau, M.S. and Oyewale, A.O. 2009. Proximate composition, mineral elements and anti-nutritional factors of *Anisopus mannii* N.E.Br.(Asclepiadaceae). *Trends in Applied Sciences Research*. 4(1): 68-72.
- Amer, A and Mehlhorn, H. 2006a. Larvicidal effects of various essential oils against *Aedes*, *Anopheles*, and *Culex* larvae (Diptera, Culicidae). *Parasitol Res*. 99: 466– 472.
- Amer, A and Mehlhorn, H. 2006b. Persistence of larvicidal effects of plant oil extracts under different storage conditions. *Parasitol Res*. 99:473–477
- Becker, N.D., Petric, M., Zgomba, C., Boase, C., Dahl, J., Lane, A. and Kaiser. 2003. Mosquitoes and their control. Kluwer Academic/Plenum Publishers, New York, USA: 5-23.
- Boonchum, W., Peerapornpisal, Y., Kanjanapothi, D., Pekkoh, J., Amornlerdpison, D., Pumas, C., Sangpaiboon, P. and Vacharapiyasophon, P. 2011. Antimicrobial and anti-inflammatory properties of various seaweeds from the

- Gulf of Thailand. *International Journal of agriculture and biology*. 13: 100-104.
- Celikler, S., Tas, S., Vatan, O., Ziyank-Ayvalik, S., Yildiz, G. and Bilaloglu, R. 2009. Anti hyperglycemic and antigenotoxic potential of *Ulva rigida* ethanolic extract in the experimental diabetes mellitus. *Food Chemistry Toxicology*. 47 (8):1837-1840.
- Chapagain, B.P., Saharan, V. and Wiesman, Z. 2008. Larvicidal activity of saponins from *Balanites aegyptiaca* callus against *Aedes aegypti* mosquito. *Bioresource Technology*. 99(5): 1165-1168.
- Dhillon, M.S. and Mulla, M.S. 1981. Biological activity of the green algae *Chlorella ellipsoidea* against the immature mosquitoes. *Mosquito News*. 41: 368-372.
- Elbanna, S.M. and Hegazi, M.M. 2011. Screening of some seaweed species from South Sinai, Red Sea as potential bioinsecticides against mosquito larvae, *Culex pipiens*. *Egyptian Academic Journal of Biological Science*. 4(2): 21-30.
- Elmegeed, D.F.A., Ghareeb, D.A., Elsayed, M. and ElSaadani, M. 2014. Phytochemical constituents and bioscreening activities of green algae (*Ulva lactuca*). *International Journal of Agricultural Policy and Research*. 2(11): 373-378.
- Govindarajan, M. 2011. Evaluation of *Andrographis paniculata* Burm.f. (Family: Acanthaceae) extracts against *Culex quinquefasciatus* (Say.) and *Aedes aegypti* (Linn.) (Diptera: Culicidae). *Asian Pacific Journal of Tropical Medicine*. 4(3): 176-181.
- Govindarajan, M., Mathivanan, T., Elumalai, K., Krishnappa, K. and Anandan, A. 2011. Mosquito larvicidal, ovicidal, and repellent properties of botanical extracts against *Anopheles stephensi*, *Aedes aegypti*, and *Culex quinquefasciatus* (Diptera: Culicidae). *Parasitology Research*. 109: 353-367.
- Griffin, G. 1956. An investigation of *Anabaena unisporea* Gardner and other cyanobacteria as a possible mosquito factor in Salt Lake County, Utah. M.Sc. thesis, Dept Zoology, University of Utah.
- Hamlyn-Harris, 1928. The relations of certain algae to breeding places of mosquitoes in Queensland. *Bulletin of Entomological Research*. 18: 377-389.
- Kamaraj, C., Bagavan, A., Elango, G., Zahir, A.A., Rajakumar, G. and Marimuthu, S. et al., 2011. Larvicidal activity of medicinal plant extracts against *Anopheles subpictus* and *Culex tritaeniorhynchus*. *Indian J Med Res*. 134: 101-106
- Kolodziej, H. and Kiderlen, A. F. 2005. Antileishmanial activity and immune modulatory effects of tannins and related compounds on *Leishmania* parasitised RAW 264.7 cells. *Phytochemistry*. 66(17): 2056-2071
- Kranthi, K.R., Jadhav, D., Wanjari, R., Kranthi, S and Russell, D. 2001. Pyrethroid resistance and mechanisms of resistance in field strains of *Helicoverpa armigera* (Lepidoptera: Noctuidae). *J Econ Entomol* 94:253– 263.
- Manach, C., Scalbert, A., Morand, C., Remes, C. and Jimenez, L. 2004. Polyphenols: food sources and bioavailability. *American Journal of Clinical Nutrition*. 79: 727-747.
- Margaret Beula, J., Ravikumar, S. and SyedAli, M. 2011. Mosquito larvicidal efficacy of seaweed extracts against dengue vector of *Aedes aegypti*. *Asian Pacific Journal of Tropical Biomedicine*. 1(2): S143-S146.
- Marten, G.G. 1987. The potential of mosquito-indigestible phytoplankton for mosquito control. *Journal of American Mosquito Control Association*. 3:105-106.

- Marten, G.G. and Reid, J.W. 2007. "Cyclopoid copepods". *Journal of American Mosquito Control Association*. 23(2): 65–92
- Mohamed Yacoob Syed Ali, Sundaram Ravikumar and Johanson Margaret Beula. 2013. Mosquito larvicidal activity of seaweeds extracts against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus*. *Asian Pac J Trop Dis*. 3(3): 196–201.
- Mohan, M and Gujar, G.T. 2003. Local variation in susceptibility of the diamondback moth, *Plutella xylostella* (Linnaeus) to insecticides and role of detoxification enzymes. *Crop Prot*. 22:495–504
- Moustafa Abbassy, A., Entsar Rabea, I., Mamdouh Marzouk. and Amany Abd-Elnabi, D. 2014. Insecticidal and Developmental Inhibitory Properties of Some Plant Extracts on *Culex pipiens* and *Spodoptera littoralis*. *International Journal of Agriculture Innovations and Research*. 2(4): 2252-2262.
- Oumaskour, K., Boujaber, N., Etahiri, S. and Assobhei, O. 2012. Screening of antibacterial and antifungal activities in green and brown algae from the coast of SidiBouزيد (El Jadida, Morocco). *African Journal of Biotechnology*. 11(104): 16831-16837.
- Poonguzhali, T.V. and Nisha, L.J. 2012. Larvicidal activity of two seaweeds, *Ulva fasciata* and *Grateloupia lithophila* against mosquito vector, *Culex quinquefasciatus*. *International Journal of Current Science*. 4: 163-168.
- Prophiro, J.S., Rossi, J.C.N., Pedroso, M.F., Kanis, L.A. and Silva, O.S. 2008. Leaf extracts of *Melia azedarach* Linnaeus (Sapindales: Meliaceae) act as larvicide against *Aedes aegypti* (Linnaeus, 1762) (Diptera: Culicidae). *Rev Soc Bras Med*. 41:560-564
- Pucazhendi, N., Pucazhendi, S., Vaitheeswaran, M., Shanmugasundaram, R. and Lakshmanan, C. 1995. Biologically active saltern and marine algal extracts for control of *Culex quinquefasciatus* larvae. *Pesology*. 19(3): 291-301.
- Ravikumar, S., Ali, M.S. and Beula, J.M. 2011. Mosquito larvicidal efficacy of seaweed extracts against dengue vector of *Aedes aegypti*. *Asian Pacific Journal of Tropical Biomedicine*. 143-146.
- Seenivasan, R., Rekha, M., Indu, H. and Geetha, S. 2012. Antibacterial Activity and Phytochemical Analysis of Selected Seaweeds from Mandapam Coast, India. *Journal of Applied Pharmaceutical Science*. 2(10): 159-169.
- Semakov, V.V. and Sirenko, L.A. 1985. Toxicity of some blue green algae on some insect larvae. *Journal of Hydrobiologia*. 20: 72-75.
- Service, M.W. 1983. Biological control of mosquitoes: Has it a future?. *Mosquito News*. 43: 113-120.
- Service, M.W. 1992. Importance of ecology in *Aedes aegypti* control. *Southeast Asian Journal of Tropical Medicine and Public Health*. 23: 681- 690.
- Shalan, E.A. Predation capacity of *Culiseta longiareolata* mosquito against some mosquitoes species larvae. *J Entoml*. 2012;9(3):183–186
- Sharma, P, Mohan, L and Srivastava, C.N. 2006. Phytoextract induced developmental deformities in malaria vector. *Bioresour Technol*. 97(14): 1599-1604
- Taubes, G. 1997. A mosquito bites back. *New York Times Magazine*. 24: 40-46.
- Thangam. T.S. and Kathiresan, K. 1991. Mosquito larvicidal activity of marine plant extracts with synthetic insecticides. *Boi.Mar*. 34: 537-539.
- Valentina, J., Poonguzhali, T.V. and JosminLaali Nisha, L.L. 2015. Phytochemical Analysis of selected Seaweeds collected from Mandapam coast in Rameshwaram, Tamilnadu,

India. *International Journal of Advanced Research*. 3(7): 972-976.

WHO. 1975. Instructions for determining the susceptibility of resistance mosquito larvae to insecticides. Mimeographed Document WHO/VBC/75 583.

World Health Organization. 1981. Instructions for determining the

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susceptibility or resistance of mosquito larvae to insecticides. WHO/VBC. 81:807-962.

World Health Organization. 2005. Guidelines for laboratory and field testing of mosquito larvicides. WHO/CDS/WHOPES/GCDPP/2005.13. Geneva: WHO, 2005:9.