International Journal of Entomology Research
www.entomologyjournals.com
ISSN: 2455-4758
Received: 26-04-2021, Accepted: 11-05-2021, Published: 28-05-2021
Volume 6, Issue 3, 2021, Page No. 81-83

Larvicidal activity of chosen sponges against *Anopheles stephensi*: A Potential Mosquito Vector transmitting malarial parasites

Priya L[1], S Jawahar[2], Huxley VAJ[1*]

[1] PG and Research Department of Zoology, Thiru. Vi. Ka. Govt. Arts College, Thiruvarur, Tamil Nadu, India.

[2] PG and Research Department of Biotechnology, Bharat College of Science and Management, Tanjavore, Tamil Nadu, India

Abstract

The Anopheles mosquito is also capable of transmitting filarial worms, various arboviruses, onyong-nyong, tataguine, equine encephalitis, as well as other viruses, but malaria is unquestionably the most threatening disease. It has also resulted in the development of resistance, undesirable effects on non-target organisms and fostered environmental and human health concern, which initiated a search for alternative control measures. The bioinsecticides are generally pest-specific, readily biodegradable and usually lack toxicity to higher animals. This study was undertaken to investigate the larvicidal potential of the two different sponges *Callyspongia sp.* and *Sigmadocia carnosa* against the medically important species of malaria vector Anopheles. Of the two sponge screened *S. carnosa* was found to effective against the larva A.stephensi. It is concluded that the sponges such as *Callyspongia sp* and *S. carnosa* serves as an excellent biopotent, which can be exploited for larvicidal property and can be cultivated in the coastal areas of the South East Coast of India.

Keywords: larvicidal activity, sponges, anopheles stephensi, malaria

Introduction

Sponges are the rich source of bioactive potentials (Husain et al., 2020) their products are greatly preferred because of their less harmful nature to non-target organisms and due to their innate biodegradability. There are a number of factors, which have economic impact, including loss in commercial and labour outputs. In countries, particularly with tropical and subtropical climates, mosquito borne diseases is one among them the most important single group of insects in terms of public health importance, which transmit a number of diseases, such as malaria, filariasis, dengue, Japanese encephalitis, etc. causing millions ofdeaths every year. The major disease burden in India is malaria and other vector-borne disease. With approximately 460 different species living on the planet, the mosquito known as the Anopheles has been evolving since it came into existence over 150 million years ago. Despite their very short live span, certain species of the Anopheles mosquito are highly devastating to the human population.

Out of the 460 species of Anopheles, about 60 have been documented as having transmitted malaria to humans. In certain areas of the world, specific species of Anopheles are prevalent. Some dangerous ones include the A. freeborni in North America, A.gambiae in Africa, and approximately 45 different species have been reported in India.

The Anopheles is well known for spreading illnesses to humans, the most dangerous one being malaria which has killed hundreds ofmillion people worldwide, and continues to kill over one million individuals each year. Malaria is transmitted to humans by the female mosquito which requires a blood meal to provide nourishment to her eggs after mating. Once the female bites an infected human, she will then transmit the malarial parasites to the next person she feeds on.

The Anopheles mosquito is also capable of transmitting filarial worms, various arboviruses, onyong-nyong, tataguine, equine encephalitis, as well as other viruses, but malaria is unquestionably the most threatening disease.

To prevent mosquito-borne diseases and improve public health, it is necessary to control them. However, in recent years, mosquito control programmes have failed because of the ever increasing insecticide resistance (WHO, 1992) [18]. It has also resulted in the development of resistance, undesirable effects on non-target organisms and fostered environmental and human health concern (Brown, 1986) [6], which initiated a search for alternative control measures. The bio insecticides are generally pest-specific, readily biodegradable and usually lack toxicity to higher animals (Bowers, 1992) [5]. This study was undertaken to investigate the larvicidal potential of the two different sponges *Callyspongia sp.* and *Sigmadocia carnosa* against the medically important species of malaria vector *Anopheles stephensi*.

Materials and Methods

Collection of sponge and extract preparation

Two different types of sponges were collected off from Muttom and Poovar coast by netting process. In this process, the local fisher folks were arranged to operate a purse seine on the sponge abundant rocky substratum so that the nets were entangled with sponges. When the net was pulled back with force, the sponges, which get entangled in the nets, were dislodged and get accumulated in the net. Immediately after collection, they were immersed in methanol for extraction.

The collected sponges in the methanol containers were squeezed/minced in a tissue homogenizer, depending up on the nature of sponge species, which was used for extraction. The extract was collected as such from the (with 2% methanol, acetone and benzene) container and filtered through a Whatman no.1 filter paper fitted with a Buchner funnel using suction. They were extracted thrice and the

combined extract was concentrated in a rotary vacuum evaporator at room temperature. The concentrated crude extract was collected in airtight plastic containers and kept in the refrigerator. From this stock solution dilutions were made to prepare different concentrations Such as 100, 200, 300, 400 and 500 mg/L, respectively, including positive and negative controls (larvae exposed to dechlorinated water without methanol, acetone and benzene).

Test mosquito larvae

Larvae of Anopheles stephensi were collected from rice field and stagnant water areas of Thiruvarur. It was maintained at 27 ± 2 °C, 75–85% relative humidity and 14L: 10D photoperiod cycles. The larvae were fed with dog biscuits and yeast at 3:1 ratio.

Bioassay

The larvicidal bioassay followed the World Health Organization (WHO) standard protocols (World Health Organization, 1981) [17]. Instructions for determining the susceptibility or resistance of mosquito larvae to insecticides. WHO/VBC, 81:807.) with slight modifications. Bioassay were conducted with larvae collected with a Pasteur pipette, placed on filter paper for removal of excess water and transferred (25 per test) with a tiny brush into beakers containing different concentrations of algal extracts (100, 200, 300, 400 and 500 mg/L) with 1000 ml of tap water each. Larvae were exposed to the samples at room temperature for 48 hours and the mortality/survival was registered after the first 24 hrs. Each test was run in triplicate

The persistence of larvicidal activity of the algal extract was tested by running bioassays with the same samples after 15, 30 and 60 days. Data analysis

The larval mortality in each concentration and control was recorded after 24 hours of exposure. Percentage mortalities were corrected for the natural mortality observed in the negative controls using Abbots (1925) formula; P = PI – C / 1 – C, where PI denotes the observed mortality rate and C means the natural mortality. The median lethal concentration or dose (LC50 and LD90) was calculated using 'Probit' analysis (Finney, 1971) [8] that has been recommended by OECD guideline as appropriate statistical method for toxicity data analysis. After linearization of response curve by logarithmic transformation of concentrations, 95% confidence limits and slope function were calculated to provide a consistent presentation of the toxicity data.

Table 1: Effect of methanolic, acetone and benzene extracts of *Callyspongia sp* against mosquito larvae A. stephensi

Extract	LC50	95%Confidence	Limits	LC90	95%Confidence	Limits
	(mg/L)	LCL	UCL	(mg/L)	LCL	UCL
Methanol	498.91	462.49	543.49	763.78	698.50	852.22
Acetone	537.79	479.50	621.46	875.58	761.77	1058.18
Benzene	491.58	443.80	554.77	866.98	765.55	1019.12

LC50 = lethal concentration to cause 50% mortality in population; LC90 = lethal concentration to cause 90% mortality in population.

LCL: 95% of Lower Confidence Limit; UCL: 95% of Upper Confidence Limit.

Table 2: Effect of methanolic, acetone and benzene extracts of *S. carnosa* against mosquito larvae A. stephensi

Extract	LC50	95%Confidence	Limits	LC90	95%Confidence	Limits
	(mg/L)	LCL	UCL	(mg/L)	LCL	UCL
Methanol	189.69	142.55	225.42	497.55 `	444.03	580.29
Acetone	145.38	112.01	171.21	330.70	300.31	373.28
Benzene	297.40	261.83	333.04	633.79	569.49	725.52

LC50 = lethal concentration to cause 50% mortality in population; LC90 = lethal concentration to cause 90% mortality in population.

LCL: 95% of Lower Confidence Limit; UCL: 95% of Upper Confidence Limit.

Results

Results of the larvicidal activity of three different extracts (methanol, acetone, and benzene) of *Callyspongia sp* and G. corticata against the larvae of

A. stephensi was performed under laboratory evaluation. It illustrates that the larval mortality rate of A. stephensi after the treatment of the three different extracts of *Callyspongia sp* and *S. carnosa* at different concentrations (100 - 500 mg/L). In terms of lethal concentration for 50% and 90% mortality (LC50 and LC90) values were represented as follows: LC50 value of the methanol extract of *Callyspongia sp* was 498.91, acetone extract was 537.79 and benzene extract was 491.58 while for *S. carnosa* LC50 value of the methanol extract was 189.69 followed by acetone extract 145.38 benzene extract 297.40, LC90 value of the acetone extract of *Callyspongia sp* was 875.58, followed by benzene extract and methanol extract; while for *S. carnosa* LC90 value of the benzene extract was 633.79 followed by methanol extract and acetone extract was. (Table 1&2). The LC50 values of *Callyspongia sp* revealed that the larvae

A. stephensiCulex was more susceptible to acetone followed by methanol and benzene extract

(Acetone > Methanol > Benzene) whereas the LC90 values shows that there is a mild variation in the lethality of extracts. LC90 values revealed that A. stephensi was more susceptible to both sponges extracted using benzene followed by acetone and methanol extracts (Benzene > Acetone > Methanol).

Discussion

Mosquitoes are important blood sucking insects. They transmit disease agents that cause malaria, dengue, yellow fever, encephalitis, and filariasis. Many studies have been achieved on the screening of biological effects of marine organisms and many active compounds were isolated and characterized (Blunden, 2001) [4]. Red sponge from genus Chondria are known as a producer of cyclic polysulfides, terpenoids, amino acids and amines. Domoic acid derivatives with larvicidal and lowering blood pressure

activity have been identified in Chondriaarmata (Mangala and Solimabi, 2000) [10]. Secondary metabolites with cytotoxic and antitumor activity have been extracted and identified in Sargassum species (Numata et al.,1991; Tang et al., 2002) [12, 16].

The sponges (U.fasciata and H. musciformis) produced 100% larval mortality at 10 mg/mL (Selvin and Lipton, 2004) [15]. There is no previous report on the mosquito larvicidal activity of *Callyspongia sp* and *S. carnosa* from the Kovalam coast (Chennai) of Tamil Nadu. Of the two sponge screened, G. lithophila was found to be effective against A. stephensi larva in all the 3 extracts. Among the two sponges *S. carnosa* showed an LC50 value with minimum concentration when compared with E. flexuosa. This may be due to the presence of polysaccharides (Andrews et al., 2005) [3]. The post coital contraceptive activity from a crude extract in marine sponge Gelidiellaacerosa is due to the presence of various phytochemical components such as alkaloids, flavonoids, phenols, amino acid, steroids, tannins and carbohydrates was demonstrated by Osman et al. (2010) [13]. Chapagain et al. (2008) [7] reported that, saponins serves as natural larvicidal compounds. Previous report of sponges showed that red sponge had high potency than green sponge (Manilal et al., 2011) [11]. The phytochemical component saponins serve as natural larvicidal compound as reported by Chapagain et al. (2008) [7] Extracts of Gracilariacrassa and Hypneavalentia have shown good larvicidal activity with a LC50 of about 52.2 and53.4 mg/L respectively against Aedessp.(Anandhan and Sorna, 2011) [2].

Conclusion

From the present study it is concluded that the sponges such as *Callyspongia sp* and *S. carnosa* serves as an excellent biopotent, which can be exploited for larvicidal property and can be cultivated in the coastal areas of the South East Coast of India. These algal extracts showed the ability they have an effective mosquito control properties and also can act as a low cost eco- friendly, bio-pesticide for further vector control programs.

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