Biocidal activity of *Sygium aromaticum* (Willd) against on the larval mosquito of *Culex quinquefasciatus* (Say.) (Diptera: Culicidae)

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Abstract

To determine the larvicidal, ovicidal and repellent activities crude Ethylene glycol and acetone extract of *Syzium aromaticum* against *Culex quinquefasciatus.* Thirty third, fourth and fifth instarlarvae was selected and it was exposed to various concentrations (100, 150 and-200 ppm) in the laboratory condition. The repellent efficacy was determined against selected mosquitoes at three concentrations *viz.*, 100, 150 and 200ppm under the laboratory conditions. Furthermore, maximum 150 and 200ppmconcentration of acetone extract remarkably showed hundred percentage of repellent effect has been noticed on third fourth and fifth instar larvae of the mosquito except 100ppmin third instar larva. Similarly another extract of ethylene glycol experimental extract was also shown the following repellent effect such as 97.06, 92.57 and 91.34 percentage been noticed on 3rd, 4th and 5th instar larvae respectively. The LC_{50} and LC_{90} values of acetone extract of *S. aromaticum* against the *C. quinquefasciatus* larvae in 24 h were as follows7.31, 8.4 and 9.27 represent the third, fourth and fifth instar larvae. Similarly ethylene glycol extract also denoted 9.67. 11.12 And 12.94ppm, respectively. In addition bioactive compounds were analysed through a GCMS technique. Totally eight compounds were identified in the *S. aromaticum* acetone extracts, representing the 99% of the crude extract, while two compounds such as α,α'humulene and 3,4-di- acetate Eugenol were identified as a peak compoundalong with its retention time and peak abundance 11.64mts 52.53; 17.54mts 62.45% respectively. Meanwhile, 1, 2-dihydroxy eugenol compound observed as a least compound along with fifth and eigth compounds are trace and unknown compounds. Moreover, 2, 2' dihydroxy copene and 1,2-dihydroxy eugenol two compounds are appeared as an optimum level in the experimental extract. From the results it can be concluded the *S. aromaticum* acetone extract was an excellent potential for controlling three life stages of *C. quinquefasciatus* mosquitoes larvae because the mosquitocidal bioactive compound was present in the same experimental plant. Hence the present study was clearly showed that the *S. aromaticum* acetone extract act as a probable mosquitocidal agent.

Keywords: Larvicidal activity, Ovicidal activity, Repellent activity, and *Culex quinquefasciatus*, *Sygium aromaticum*.

Introduction

Mosquitoes can transmit more diseases than any other group of arthropods and affect million of people throughout the world. WHO has declared the mosquitoes as "public enemy number one" (WHO, 1996). Mosquito borne diseases are prevalent in more than 100 countries across the world, infecting over 700,000,000 people every year globally and 40,000,000 of the Indian population proposed by Anupam *et al*. (2012).Mosquitoes are nuisance pests and a major vector for the transmission of several life threatening diseases such asmalaria, dengue fever, yellow fever, filariasis, schistosomiasis, Japanese encephalitis, etc., It is a major causative agent for millions of deaths every year in the world (Brown, 1986). Mosquitoes also cause allergic responses in humans that include local skin and systemic reactions such as angioedema (Simas *et al*., 2004; Russell et al., 2009). *C. quinquefasciatus*acts as a vector for filariasis in India. Human filariasis is a major public health hazard and remains a challenging socioeconomic, problem in many of the tropical countries Bhatt and Khanal, 2009). Mosquito control has been becoming increasingly difficult because of the indiscriminate uses of synthetic chemical insecticides which have an adverse impact on theenvironment and disturb ecological balance. Majority of the chemical pesticides are harmful to man and animals, some of which are not easily degradable and spreading toxic effects (Peng *et al*., 1999). Plants may be alternative sources of mosquito larval control agents, since they constitute a rich source of bioactive compounds that are biodegradable into nontoxic products and potentially suitable for use in control of mosquito larvae (Murugan and Jeyabalan, 1999; Subramaniam *et al*., 2012). Phytochemicals are advantageous due to their eco-safety, target-specificity, non-development of resistance, reduced number of applications, higher acceptability, and suitability for rural areas (Subramaniam *et al*., 2012). Botanicals can be used as alternative to synthetic insecticides or along with other insecticides under integrated vector control programs (Ghosh, 1991). Plants may be a source of alternative agents for control of mosquitoes because they are rich in bioactive chemicals (Harborne, 1998) are active against a limited number of species including specific target insects, and are biodegradable. Recently many researchers are increased interest in developing plant origin insecticides as an alternative to chemical insecticide in the world (Simas *et al*., 2004; Sengottayan *et al*., 2005; Elumalai *et al*., 2012; Kovendan *et al*., 2014). So far, no other study

was made the similar kind of plant extracts but very few research has been done in essential oil from clove, so the present study was aimed to design the the following objectives based upon this experimental plant of *S.aromaticum* . Hence the present study was undertaken to assess the larvicidal, ovicidal, and repellent potential of *S.aromaticum* against the medically important mosquito larvae of *C. quinquefasciatus.* Then identified the bioactive compounds from the effective extract of acetone in the same experimental plant by GCMS analysis.

Plants collection and solvent extraction

The dried *S.aromaticum* were collected during the July2016- and brought to the laboratory. The sample was shade-dried, and finely ground with the help of electrical blender. The finely ground plant leaf powder (1.0 kg) was loaded and extracted in Soxhlet apparatus. The solvents from the extracts were removed using a rotary vacuum evaporator (Rota vapour, Systronics India Ltd., Chennai,India) to collect the crude extract. Standard stock solutions were prepared at 1% by dissolving the residues in acetone. From this stock solution, different concentrations were prepared and these solutions were used for larvicidal, ovicidal, and repellent bioassays.

Mosquito Rearing

Third, four and fifth instar *Culex quinquefasciatus*, mosquito larvae were reared in the Department of Biotechnology, Malankara Catholic College, Mariagiri. Mosquitoes were held at (28 ± 2^0C), 70%-85% relative humidity (RH), with a photo period of 14 hr light, 10 hr dark.

Larvicidal activity

The larvicidal activity of plant crude extract was assessed by using the standard method as prescribed by World HealthOrganization (2005). From the stock solution, four different test concentrations (50, 100, 150 and 200 ppm) were prepared and they were tested against the third four and fifth instar larvae of *C.quinquefasciatus.* The larvae of test species (30) were introduced in 500-ml plastic cups containing 250 ml of aqueous medium (249 ml of dechlorinated water+1ml of emulsifier; DMSO) and the required amount of *S. aromaticum* extract of ethylene

glycol and acetone were added and testes separately. The larval mortality was observed and recorded after 24 h of post treatment. For each experiment, five replicates were maintained at a time. The LC_{50} and LC_{90}values were calculated by using probit analysis (Finney, 1979).

Ovicidal activity

The method of Su and Mulla (1998) was slightly modified and used to test the ovicidal activity. The various concentrations (100, 150 and 200 ppm) as stated in the previous experiments were prepared from the stock solution. Before treatment, the eggs/eggs raft of *C. quinquefasciatus* was counted individually with the help of hand lens. Freshly hatched eggs (100) were exposed to each concentration of leaf extract until they hatchedor died. Eggs were exposed to DMSO in water served as control. After treatment, the eggs from each concentration wereindividually transferred to distilled water cups for hatching assessment after counting the eggs under a microscope. Each test was replicated five times. The hatchability was assessed 48 h post treatment.

Repellent activity

The repellent study was following the methods of World Health Organization (2009). Third, fourth and fifth instar freshly moulted larvae from *C. quinquefasciatus* mosquitoes (100) were kept in a 1litre plastic containers. The volunteer had no contact with lotions, perfumes or perfumed soaps on the day of the assay. The arms of the test person were cleaned with isopropanol. After air drying the arm only 25 cm2 of the dorsal side of the skin on each arm was exposed, the remaining area being covered by rubber gloves. The *S. aromaticum* extract was dissolved in acetone and this was served as control. The control and treated arms were introduced simultaneously into the cage. The numbers of bites were counted over 5 min every 40 min and the experiment were conducted five times. It was observed that there was no skin irritation from this experimental plant extract.

Analytical conditions of GC-MS

The *S. aromaticum* acetone extract was obtained by hydrodistillation were analyzed by gas chromatography-mass spectrometry (GC-MS) using a ShimadzuQP5050A (Shimadzu Europe, North Rhine-Westphalia,Germany) gas chromatograph equipped with a DB-5 MSfused silica column (30 m x 0.25 mm; film thickness0.25 μm), under the following conditions: helium as the carrier gas at 1.0 mL/min; injector split at 250ºC (splitratio 1/20); detector at 280ºC; column temperature programme of 80ºC for 1.5 min, increase of 4ºC per minto 180ºC, then 10ºC per min to 300ºC, ending with a 10min isothermal at 300ºC. The mass spectra were takenat 70 eV with a scanning speed of 0.85 scan/s from 40 to 550 Da. Peak identification was made on the basisof comparison of their retention indices relative to ann-alkane homologous series obtained by co-injecting theoil sample with a linear hydrocarbon mixture.

Statistical analysis

Statistical analysis of the experimental data was performed using the computer soft wares Stat plus 2007, MS Excel 2003 and SPSS to find out the LC_{50}, values, mean larval mortality, standard error etc.The average larval mortality data were subjected to probit analysis for calculating LC_{50}, and other statistics at 95%confidence limits of upper confidence limit (UCL) and lower confidence limit (LCL), regression value, slope, and chisquare values were calculated using the SPSS17.0 (Statistical Package of Social Sciences) software. Results with $p<0.05$ were considered to be statistically significant.

3. Results

Table: 1- Repellent effect of *S. aromaticum* extracts on the three different larval stage of mosquito

Name of extract	concentration	Life stages of mosquito		
		third	fourth	fifth
Ethylene glycol	100	92.14±2.58	84.02±3.51	81.22±2.40
	150	95.30±5.25	89.14±4.17	85.30±2.11

	200	97.06±4.35	92.57±3.54	91.34±2.85
Acetone	100	98.56±3.31	100±2.53	100±0.0
	150	100±0.00	100±1.86	100±0.0
	200	100±0.00	100±0.0	100±0.0

Table:2- Ovicidal activity of *S. aromaticum* extracts against freshly laid eggs on *c. quiquefasciatus* mosquito

Name of the extract	concentration	Ovicidal effect
Acetone	100	4.21±1.05*
	150	1.08±0.52**
	200	0.00±0
Ethylene glycol	100	24.16±2.38^{in}
	150	10.75±2.74*
	200	2.65±0.43*

*- it shows highly significant at 0.00 5% level

**-it shows significant at0.05% level

In- insignificant

Today, the environmental safety of an insecticide is considered to be of paramount importance. An insecticide must not cause high mortality in target organisms in order to be acceptable many researchers.The current result of the mosquito repellant activity was clearly denoted in table-1. In this table showed that whenever the concentration was increased repellent effect also been increased in all the three larval stages this experimental mosquito. Furthermore, maximum 150 and 200ppmconcentration of acetone extract remarkably showed hundred percentage of repellent effect has been noticed on third fourth and fifth instar larvae of the mosquito except 100ppmin third instar larva. Similarly another extract of ethylene glycol experimental extract was also shown the following repellent effect such as 97.06, 92.57 and 91.34 percentage been noticed on 3rd,4th and 5th instar larvae respectively. The results of the present study clearly have presented in table 2. Data of the larvicidal activity of *S. aromaticum* extracts against selected mosquitoes are presented in (table 3). The LC_{50} and LC_{90} values of acetone extract of *S. aromaticum* against the

C. quinquefasciatus larvae in 24 h were as follows7.31, 8.4 and 9.27 represent the third, fourth and fifth instar larvae. Similarlyethylene glycol extract also denoted 9.67. 11.12 and 12.94ppm, respectively. Furthermore, highest ovicidal activity has been noticed in maximum concentration (200 ppm) of acetone extract of the *S. aromaticum*. From the present results were clearly depicted that 150ppm acetone and ethylene glycol extract possessed highly significant to significant ovicidal activity (1.08±0.52 to 2.65±0.43). Though, interestingly no ovicidal activity has been observed in higher concentration of 200ppm acetone extract. Meanwhile, lowest concentration of 100ppm ethylene glycol extract showed insignificant ovicidal activity. Apart from these results it can be concluded the *S. aromaticum* acetone extract was an excellent potential for controlling three vibrant stages of *C. quinquefasciatus* mosquitoes than ethylene glycol extract.

Fig.1:- GC-MS chromatogram view of acetate extract from the flower of *S. aromaticum* (l.)

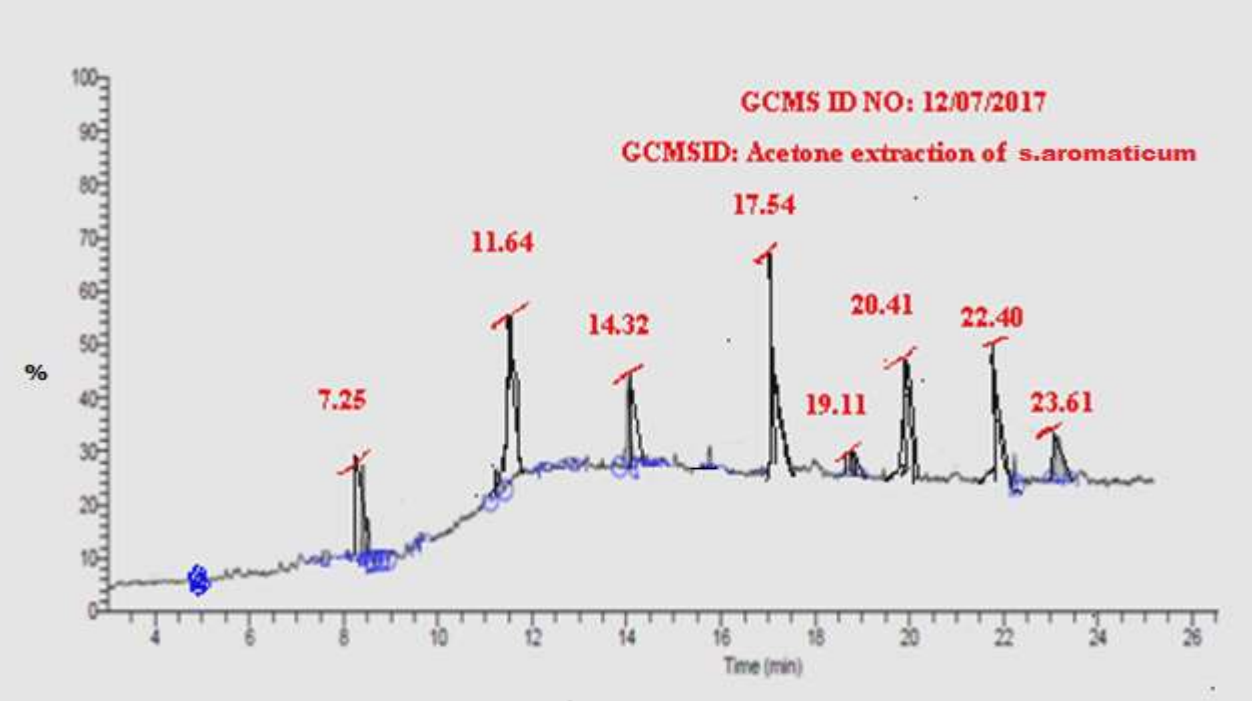

Table: 2- Elucidated bioactive compounds from *S. aromaticum* acetate extract by GC-MS analysis

Name of the analytes	Name of the analytes	Retention time	Peak abundance (%)
1	1,2-dihydroxy eugenol	7.25	26.72
2	3,4-di- acetate Eugenol	11.64	52.53
3	B-caryophylleneoxide	14.32	41.35
4	α,α'humulene	17.54	62.45
5	Trace	19.11	29.02
6	2,2'dihydroxy copene	20.41	42.5
7	-	22.40	42.18
8.	unknown	23.1	30.59

The *S. aromaticum* acetone crude extracts were obtained at 58.64 – 67.950% yield, respectively.Totally eight compounds were identified in the *S. aromaticum* acetone extracts, representing the 99% of the crude extract, while two compounds such as α,α'humulene and 3,4-di- acetate Eugenol were identified as a peak compoundalong with its retention time and peak abundance 11.64mts 52.53; 17.54mts 62.45% respectively. Meanwhile, 1,2-dihydroxy eugenolcompound observed as a least compound along with fifth and eigth compounds are trace and unknown compounds. Moreover, 2,2' dihydroxycopene and 1,2-dihydroxy eugenol two compounds are appeared as an optimum level in the experimental extract (Table-3,Fig-1). From the gcmsresult acetone clove extract clearly showed that the eugenol and it derivatives were act

as a larvicidal compounds so *S. aromaticum* acetone extract possessed verygood potential against the mosquito larvae.

Table-3:-Larvicidal effect of *S.aromaticum* acetone extracts on the three different larval stages of *C. quinquefasciatus* mosquito

Tested solvents	Stages of Mosquitoes	LC50 (PPM)	95% Fiducial limit		Slope	Chi-Square
			LCL	UCL		
Acetone	Third	7.31	51.53	104.15	4.32	15.36
	Fourth	8.4	57.15	117.68	4.10	14.67
	Fifth	9.27	61.46	128.75	3.46	13.52
Ethylene glycol	Third	9.67	42.07	135.64	3.22	12.85
	Fourth	11.12	45.68	143.82	3.11	12.34
	Fifth	12.94	49.84	159.43	2.97	11.87

LC_{50}- Lethal Concentration, **LCL-** Lower Confidence Limit, **UCL-** Upper Confidence Limit

Discussion

The present results showed that, the crude ethanol extract of *S. aromaticum* acetone have significant larvicidal activity against the selected human vector mosquitoes *Culex quinquefasciatus.* Theresults are in comparable with an earlier report by Brown, 1986; Karunamoorthi*et al.* 2008; Yadav *et al*., 2002. Previously, several studies were agreed with the similar extract such as essential oil of clove and its bioactive compoundsand its effect on various species of mosquitoes by Chaieb *et al*. (2007) and Simas *et al*. (2004) found that LC_{50} = 44.5 ppm for eugenol, the major compound in *S. aromaticum* essential oil, in *A. aegypti* populations. The larvicidal activity of *S. aromaticum* essential oil has been previously reported by several authors Costa *et al*. (2005) found that LC_{50} = 21.4 ppm, while Fayemiwo *et al*. (2014) found LC_{50} = 92.56 and Barbosa *et al*. (2012) obtained values of 62.3 and 77.0 ppm for field-collected and Rockefeller larvae, respectively. In the previous year Adriana *et al*., 2016 published the similar view has been agreed in larvicidal activity of *S. aromaticum* essential oils are potential candidates for use as auxiliary larvicides to control *Ae. Aegypti*s trains those are resistant to temephos, while their combinations with temephos are not recommended for use as larvicides. Previously Harve and Kamath, (2004); Barbosa *et al*. (2012) published the agreed view of these similar finding was studied on *Aedes aegypti* mosquito because the present experimental plant acetone extract possessed the eugenol compound so *S. aromaticum* remarkably showed the Larvicidal activity against the *C. quinquefasciatus* mosquito. The results suggest that the *S. aromaticum* acetone extract represent an alternative as low-toxicity natural larvicides and a promising approach for control, especially in *C. quinquefasciatus* larvae showing resistance to currently employed larvicides (Yadav *et al*., 2002; Mullai), such as organophosphates. Marques *et al*. (2013) reported the oviposition index (OI), ranging from -1 to +1, to evaluate oviposition-attractant or repellent substances. A positive OI means that the substance has an attractant activity, while a negative value means the substance has a repellent activity. *C. sinensis* and *S. aromaticum* exhibited OIs of -0.2 and -0.93, respectively (Mittal *et al*., 2014). Although *C. sinensis* exhibited a low repellency profile, the essential oils evaluated here exhibit negative OIs and therefore have repellent effects on *C. quinquefasciatus* oviposition. According to Machado and Fernandes (2011); Rahuman and Venkatesan, (2008), essential oils are natural, volatile and complex compounds, characterised by a strong odour, which influence the

ovipositional behaviour of the gravid female. Repellence is an aggravating influence for larvicides because the vector avoids oviposition in treated containers, favouring non-treated water sources for oviposition previously proposed by Su and Mulla, (2010)

Acknowledgement

We would like to sincere thanks Rev. Fr. Jose Bright Correspondent as well the great deepsense of gratitude to respected Principal **Dr. J. Thampi Thanka Kumaran** in Malankara Catholic College, Mariagiri, for given the informative motivation for publication of this manuscript. In addition we acknowledge to several well-wishers and colleagues for them gentle ideas regarding this research work and them technical assistance for this manuscript completion sucessfully

References

Adriana F. O. A., João T. R.-P., Juliana T. de D. Sócrates C. Holanda C., Rogéria S. N.and Péricles B. A., Maria L. G.M. 2016. Larvicidal activity of *Syzygium aromaticum* (L.) Merr and *Citrus sinensis* (L.) Osbeck essential oils and their antagonistic effects with temephos in resistant populations of *Aedes aegypti*, *Mem Inst Oswaldo Cruz, Rio de Janeiro*, 111(7): 443-449.

Anupam G., Nandita C. and Goutam, C. 2012. Plant extracts as potential mosquito larvicides. *Indian J. Med. Res*, 135, pp 581-598.

Barbosa, J.D, Silva V.B, Alves P.B, Gumina G, Santos R.L, and Sousa D.P. 2012. Structure-activity relationships of eugenol derivatives against *Aedes aegypti* (Diptera: Culicidae) larvae. *Pest Manag Sci*. 68(11): 1478-83.

Bhatt, R.P, and Khanal, S.N.2009. Environmental im-pact assessment system in Nepal - an overview of policy, legal instruments and process. *Kathmandu Univ J Sci Enginnering Tech,* 5: 160-70.

Brown A.W. Insecticide resistance in mosquitoes: a pragmatic review. *J Am Mosq Control Assoc* 1986; 2: 123-40.

Chaieb K, Hajlaoui H, Zmantar T, Kahla-Nakbi AB, Rouabhia M. and Mahdouani K, 2007. The chemical composition and biological activity of clove essential oil, *Eugenia caryophyllata* (*Syzigium aromaticum* L. Myrtaceae): A short review. *Phytother Res*.; 21(6): 501-6.

Costa J.G.M., Rodrigues F.F.G., Angélico, E.C., Silva M.R., Mota M.L. and Santos N.K.A. 2005. Chemical-biological study of the essential oils of *Hyptis martiusii*, *Lippias idoides* and *Syzygium aromaticum* against larvae of *Aedes aegypti* and *Culex quinquefasciatus*. *Rev Bras Farmacogn,* 15(4): 304-9.

Elumalai K, Dhanasekaran S, and Krishnappa K. 2012. Larvicidal activity of Saponin isolated from *Gymnema sylvestre* R.~ 34 ~International Journal of Mosquito Research Br. (Asclepiadaceae) against Japanese Encephalitis vector, *Culex tritaeniorhynchus* Giles (Diptera: Culicidae). *European Review for Medical and Pharmacological Sciences*, 17:1404-1410.

Fayemiwo K.A, Adeleke M.A, Okoro O.P, Awojide S.H.and Awoniyi I.O. 2014. Larvicidal efficacies and chemical composition of essential oils of *Pinus sylvestris* and *Syzygium aromaticum* against mosquitoes. *Asian. Pac. J .Trop. Biomed*, 4(1): 30-4.

Finney, D.J. 1979. Probit analysis. Cambridge University Press, London, , 68–72.

Ghosh, G.K. 1991. Biopesticide and integrated pest management. A.P.H. Publishing Corporation, New Delhi, 145–146.

Harborne J.B. 1998. Phytochemical methods, A guide to modern techniques of plant analysis. New York: Chapman and Hall, 3rd edn.

Harve, G, and Kamath, V. 2004. Larvicidal activity of plant extractsused alone and in combination with known synthetic larvicidal agents against *Aedes aegypti*. *Indian Journal of Experimental Biology*, 42(12):1216–1219 730.

Kannathasan K., Senthilkumar A., Chandrasekaran M, and Venkatesalu V. 2007. Differential larvicidal efficacy of four species of *Vitex* against *Culex quinquefasciatus* larvae. *Parasitology Resesearch*, 101(6):1721–1723.

Karunamoorthi K, Ramanujam S, and Rathinasamy R. 2008. Evaluation of leaf extracts of *Vitexnegundo*L (Family:Verbenaceae) against larvae of *Culex tritaeniorhynchus* and repellent activity on adult vector mosquitoes. *Parasitology Research*, 103:545–550.

Kovendan, K, Shanthakumar S.P, Cheruparambath P, Palanisamy M.K, Kadarkarai M, and Savariar V. 2014. Mosquitocidal properties of *Morinda citrifolia* L. (Noni) (Family: Rubiaceae) leaf extract and *Metarhizium anisopliae* against malaria vector, *Anopheles stephensi* Liston. (Diptera: Culicidae). *Asian Pac J Trop Dis*, 4(1):S173-S180.12.

Mittal M, Gupta N, Parashar P, Mehra V, and Khatri M. 2014. Phytochemical evaluation and pharmacological activity of *Syzygium aromaticum*: a comprehensive review. *Int J Pharm Pharm Sci*, 6(8): 67-72.

Mullai K, Jebanesan A, and Pushpanathan T. 2008. Effect ofbioactive fractions of *Citrullus vulgaris* Schrad. Leaf extract against *Anopheles stephensi* and *Aedes aegypti*. *Parasitology Research*, 102(5):951–955.

Murugan K, and Jeyabalan D. 1999. Effect of certain plant extracts against the mosquito, *Anopheles stephensi* Liston. *Current science*, 76(5):631-633.

Peng, Z., Yang J, Wang H. and Simons F.E.R.1999. Production and characterization of monoclonal antibodies to two new mosquito *Aedes aegypti* salivary proteins. *Insect Biochemical Molecular Biology*, 29:909–914.

Rahuman A.A, and Venkatesan P. 2008. Larvicidal efficacy of fivecucurbitaceous plant leaf extracts against mosquitospecies. *Parasitology Resesearch*, 103(1):133–139.

Russell, T.L, Kay B.H, and Skilleter G,A. 2009. Environmental effects of mosquito insecticides on saltmarsh invertebrate fauna. *Aquat Biol*, 6: 77-90.

Sengottayan S. N, Kandaswamy K, and Kadarkarai M. 2005. Effects of neem limonoids on the malaria vector *Anopheles stephensi* Liston (Diptera: Culicidae). *Acta Tropica,* 96:47–55.

Simas N.K, Lima E.D, Conceição S.D, Kuster R.M, de Oliveira A.M, and Lage C.L.S. 2004. Natural products for dengue transmission control - Larvicidal activity of *Myroxylon balsamum* (red oil) and of terpenoids and phenylpropanoids. *Quim Nova*.; 27(1): 46-9.

Su T, Mulla M.S. 2010. Ovicidal activity of neem products (azadirachtin) against *Culex tarsalis* and *Culex quinquefasciatus* (Diptera: Culicidae). *Journal of American Mosquito Con44trol Association* 14:204–209.

Subramaniam J, Kadarkarai M, Kovendan K. Larvicidaland pupicidal efficacy of *Momordica charantia* leaf extract and bacterial insecticide, *Bacillus thuringiensis* against malarial vector, *Anopheles stephensi* Liston. (Diptera:Culicidae). *Journal of Biopesticides,* 2012; 5:163-169.

Subramaniam J, Kovendan K, Palanisamy M.K, Kadarkarai M, and William W. 2012. Mosquito larvicidal activity of *Aloe vera*(Family: Liliaceae) leaf extract and *Bacillus sphaericus*, against Chikungunya vector, *Aedesaegypti. Saudi Journal of Biological Sciences*, 19(4):503–509.

World Health Organization. WHO. Guidelines for efficacy testing of mosquito repellents for human skins. WHO,Geneva, WHO/ HTM/NTD/WHOPES/2009.4.

World Health Organization. Guidelines for laboratory andfield testing of mosquito larvicides. Communicabledisease control, prevention and eradication, WHO pesticide evaluation scheme. WHO, Geneva,WHO/CDS/WHOPES/GCDPP/1.3. 2005.

World Health Organization. Report of the WHO. 1996. Informal consultation on the evaluation on the testing of insecticides, CTD/WHO PES/IC/96.1. Geneva: WHO; p. 69.

Yadav R, Srivastava VK, Chandra R, and Singh A. 2002. Larvicidal activity of latex and stem bark of *Euphorbia tirucalli* plant on the mosquito *Culex quinquefasciatus*. *Journal of Communicable Disease*; 34(4):264–269 895.