International Journal of Mosquito Research

ISSN: **2348-5906**
CODEN: **IJMRK2**
IJMR 2019; 6(1): 26-33

Received: 11-11-2018
Accepted: 15-12-2018

Mamta Goyal
Research Scholar, Applied Parasitology Research laboratory, Department of Zoology, JES College, Jalna, Maharashtra, India

Laxmikant Shinde
Associate Professor, Applied Parasitology Research laboratory, Department of Zoology, JES College, Jalna, Maharashtra, India

Ranjit Bayas
Research Scholar, Applied Parasitology Research laboratory, Department of Zoology, JES College, Jalna, Maharashtra, India

Correspondence
Laxmikant Shinde
Associate Professor, Applied Parasitology Research laboratory, Department of Zoology, JES College, Jalna, Maharashtra, India

Study of chemical composition and larvicidal efficacy of secondary metabolites from aromatic phytoextracts against dengue vector: *Aedes aegypti* (Linn) (Diptera: Culicidae)

Mamta Goyal, Laxmikant Shinde and Ranjit Bayas

Abstract
Mosquitoes have not only threatened human health but also adversely affect human and wild life as they act as vector for many deadly diseases. Among vector mosquito *Aedes aegypti* plays a key vector role to transmit viral diseases viz. Dengue, Zika, Yellow Fever and Chikunguniya. The present scenario for vector control strategies exposes the fact of rise in resistance index of mosquitoes against various available synthetic insecticides and also nonresponsive to bacterial larvicides. To overcome these circumstances, more emphasis should be given to bio-control strategies using secondary metabolites from plant extracts; since, they are enriched in bioactive components with larvicidal activity. Present study analyzed chemical composition and larvicidal efficacy of flowers of *Gliricidia sepium* and seeds of *Annona* squamosa in methanolic and hexane extracts. The bioassay for larvicidal activity was carried out using II, III, IV instar larvae of *Aedes aegypti*. It was observed that early instar stages showed more sensitivity to EOs as compared to later immature developing stages. *The lowest LC_{50} values shown by second, third, fourth instar larvae in methanol extract of Gliricidia sepium as 38.01ppm, 45.65 ppm, 60.25 ppm respectively whereas highest LC_{50} values against* II, III, IV instar larvae of *Aedes aegypti were 77.62 ppm, 95.40 ppm, 128.82 ppm respectively in hexane extract of Annona squamosa. EOs of Gliricidia sepium* can be used as an effective biolarvicide to control mosquitoes as environment friendly solution.

Keywords: *Aedes aegypti*, secondary metabolite, *Gliricidia sepium*, *Annona squamosa*, LC_{50}

1. Introduction
Mosquitoes have threatened not only human health but also adversely affect life as they act as vector for many dreadful diseases. Among mosquito species, *Aedes aegypti* plays a key vector role to transmit viral diseases viz. Dengue, Zika, Chikunguniya, Yellow fever. The vector-borne diseases results in high morbidity and mortality rate along with rise in cosmopolitan economic burden of diseases with every passing decade [1]. Synthetic pesticides are used to control mosquito larval species since from last four decades. It has resulted in development of resistance in mosquitoes against Temephos and other insecticides [2].Various drawbacks of chemical insecticide include hazardous effect on human health and it also adversely affect the environment. Hence, it becomes mandatory to search out new bio-larvicidal agent [4]. Biolarvicides of microbial origin such as *Bacillus thuringinesis var israelensis (Bti)* have very good efficacy to control mosquitoes as broad spectrum of activity. On the contrary *B.sphaericus* is effective against *culex* species and certain *anopheline species* but almost ineffective against *Aedes aegypti* species [5]. Thus, the effort to control mosquitoes is in continuous process so as to eliminate the spread of mosquito borne diseases. These problems trigger the researchers to find out alternative bio-pesticides from herbal extracts of flora diversity. In this respect, many researchers have evaluated the efficacy of bioactive component from botanical sources which are enriched with mosquitocidal toxins to control mosquitoes. These compounds are secondary metabolites extracted in essential oils from various parts of medicinal plants [13, 20]. Literature study shows that these secondary metabolites have excellent efficacy as larvicidal, ovicidal, pupicidal, adulticidal, repellant, oviposition deterrant and growth inhibiting activity [1, 7].

The essential oils have gained considerable attention. The bioactive components have antiviral, antibacterial, larvicidal activities. Furthermore, as the action of secondary metabolites is more complex, it decreases the probability of rise in resistance index of mosquitoes. The identification and eventual use of medicinal plants may acts as a key element for control of mosquito borne diseases in developing country like India. Larvicidal activities have been evaluated in the extracts of *Neem* [15], *Ipomoea carnea* [18], *Calophyllum inophylum* [17], *lemongrass* [22], *Syzygium aromaticum* [23] *and Ocimum basilicum* [21].The phytochemicals present in the extracts exhibit excellent detrimental actions on mosquitoes and larvae [19]. The use of phytoextracts to control propagation of mosquito species is becoming more preferable owing to selective properties like low cost and eco-friendly to our planet and its ecosystem. *Gliricidia sepium (Jacq.) Walp* belongs to the family Fabaceae. The generic name *Gliricidia* literally means Rat Poison. It is a medium size tree about 2-15 m in height found in most of the parts of India. The flowers of *Gliricidia sepium* are usually pink and fading to whitish brown or pale purplish with age. All parts of the tree: bark, leaves, roots have been reported to be beneficial ethno medicinally [8].

Annona squamosa L. commonly known as sugar apple or custard apple belongs to the family Annonaceae, is a fruit tree with many traditional uses. *A. squamosa* is an evergreen tree located in tropical and subtropical regions. A wide range of ethno medicinal uses have been contributed to different parts of plant. Various research studies and findings have inform us about its use as anticancer, anti-oxidant, antidiabetic, antihypertensive, hepatoprotective, antiparasitic, antimalarial, insecticidal, microbicidal and molluscicidal activities [9, 28].

The objective of present study was to investigate the chemical composition and larvicidal efficacy of secondary metabolites from essential oils of flowers of *Gliricidia sepium* and seeds of *Annona squamosa* against *Aedes aegypti*, keeping in mind the aim of finding biolarvicides as supplementary measures for the control of viral diseases. To the best of our knowledge based on literature survey, it is the first report on the larvicidal efficacy of flowers of *G. sepium* and seeds of *A. squamosa* against *Aedes aegypti*.

2. Materials and Methods

2.1 Collection of Plant material

2.1.1 Collection of flowers of *Gliricidia sepium (Jacq.) Walp*

The flowers were collected during Jan-Feb 2015 and 2016 from Forest area of Jalna, Maharashtra. The flowers were shade dried at room temperature for about 6-7 days till it becomes crunchy crushable, ground to powder using electric grinder with stainless steel blade and sieved through wheat mesh to get fine powder from which essential oils were extracted.

2.1.2 Collection of seeds of *Annona squamosa*

The seeds of *A. squamosa* were obtained by collecting fruits of custard apple in the month of Oct-Nov.2015 from our campus garden of Jalna Education Society College, Jalna. The black coat of seeds were removed mechanically and ground as above to get fine powder. Both these plant samples were identified and authenticated by Department of Botany, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad. The herbarium was submitted in Botany Department. The accession number assigned to plants as 0664 for *Annona squamosa L.* and 0665 to *Gliricidia sepium (Jacq.)*.

2.2 Extraction of Essential Oils

The plant materials were extracted separately using Soxhlet extraction method. For this 40gm of powder was packed in thimble (Whatman filter paper no.1) and placed in Soxhlet extractor (Make: Borosil, Glass and glassware). For the extraction, hexane and methanol (Merck 99.9 %) were used as solvents sequentially at 55^0C for 7-8 hours till solvent becomes decolourised each time [10].

2.3 Spectral analysis

Well defined information in qualitative examination can be achieved by GC-MS. (Gas chromatography mass spectroscopy). GC-MS analysis of essential oil was performed by using Shimadzu GCMS coupled to QP 2020 instrument operating in electron impact (EI) mode with MS voltage 0.96 kV with the following specifications of program. Carrier gas; Helium with column flow rate of 0.99 ml/min and inlet flow pressure: 52.7KPa; column oven temperature and injector temp 50^0C and 250^0C respectively. Injection mode: split and split ratio 1:50, purge flow: 3 ml/min. Sample size: 1µl for 1 minute. Column SH-RXI-5silMS (30m x 0.25 mm x 0.25µm) was used. The duration of one complete program was 23 min.MS transfer line temperature: 250^0C with acquisition mode scan type and scan range 35 -500 m/z. The mass spectral survey and identification was performed by using the NIST library of mass spectral search program.

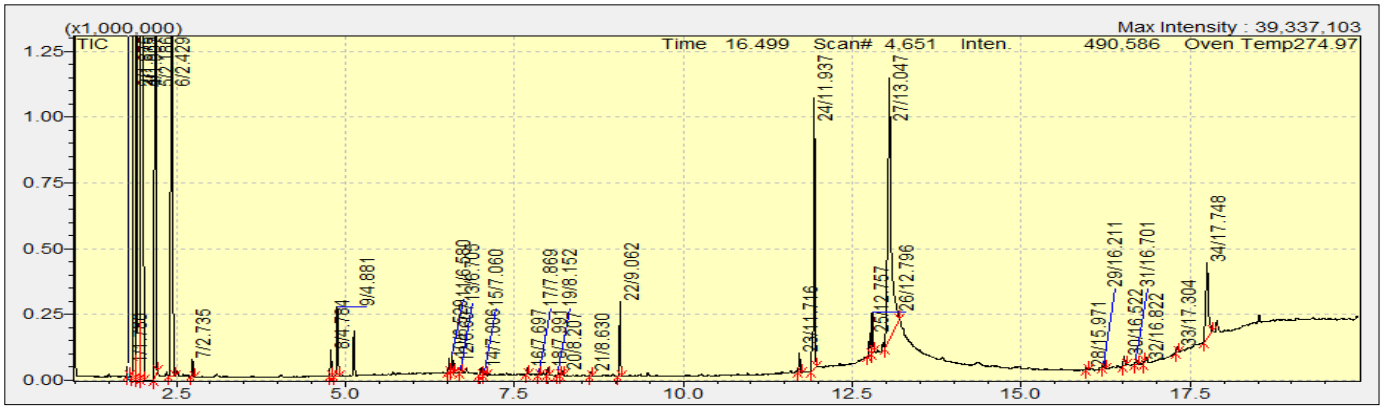

Fig 1: Chromatogram showing GC-MS profile of essential oil obtained from flowers of *Gliricidia sepium (Jacq.)* in hexane

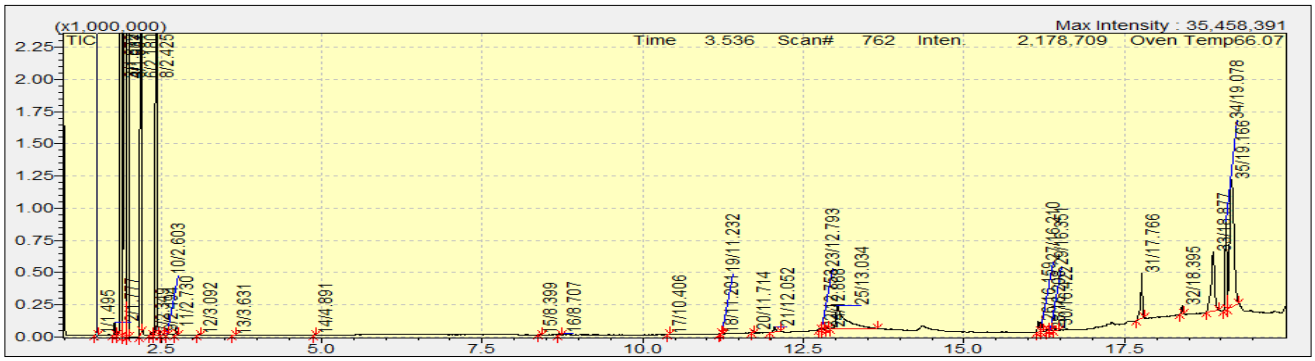

Fig 2: Chromatogram showing GC-MS profile of essential oil obtained from flowers of *Gliricidia sepium (Jacq.)* methanol

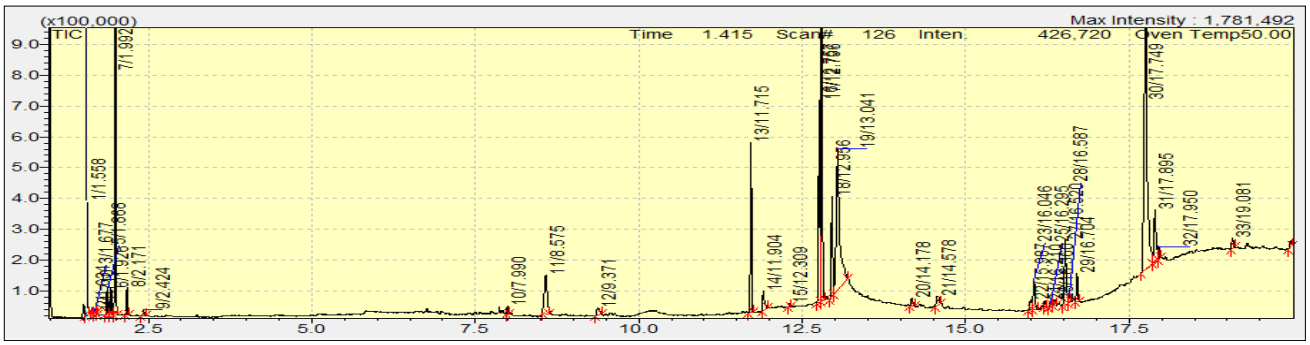

Fig 3: Chromatogram showing GC-MS profile of essential oil obtained from seeds of *Annona squamosa in* hexane

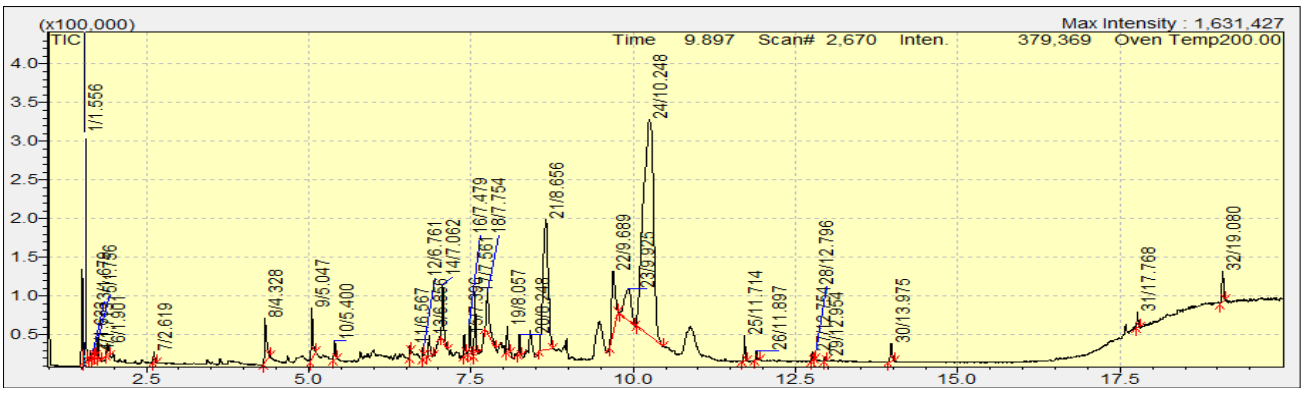

Fig 4: Chromatogram-showing GC-MS profile of essential oil obtained from seeds of *Annona squamosa in* methanol

2.4 Preparation of stock solution

1hour prior to experimental setup for bioassay, varying concentration gradients of the extracts was prepared as per WHO protocol [39]. For achieving 1% (10,000ppm) stock solution 200 mg of test plant extract was first dissolved in 20 ml of solvent used for extraction by vigorous shaking in capped vial. Using this, desired dosages of test concentration were prepared by adding required volumes of aliquots of stock solution to 250 ml of chlorine free tap water [11]. For Control test, 1ml of solvent was added in 250 ml of water.

2.5 Collection and rearing of larvae

The larvae were collected from fresh water domestic reservoirs and pooled in laboratory and identified by microscopic examination of larvae using keys of Theodore *et al.* [26] (2005). Then reared in plastic trays (42cm x28cmx 6.5 cm) having fresh de-chlorinated water covered with nylon mesh until desired larval instar obtained. The larvae were fed with 3% sucrose solution. Every time larvae were collected and followed the same method.

2.6 Larvicidal Assay

Initially, mosquito larvae were exposed to a wide range of test concentration to find out median lethal concentration which causes 50% mortality and finally desired target dosage range was selected for further bioassay. The bioassay experiments were conducted at room temperature to detect its toxicity. Four concentrations were used for each plant extract and experiments were repeated in triplicates. A controlled experiment (blank) was also provided for each set of experiment. 25 larvae (II, III, IV instars) of *A. aegypti* were added to 250 ml of test concentration in glass beaker. The temperature was maintained at 28^0C and humidity 70%, larval mortality was recorded at an interval of 2hrs up to 14 hrs and then after 24 hrs of exposure.

Moribund larvae were also counted and added to dead larvae for calculating mortality percent. Dead larvae were those which failed to respond when probed with a needle and become motionless. During experiment no pupation occurs and if mortality in control set exceeds 15% then that experiment was discarded and repeated again.

2.7 Statistical analysis

Data from replicates were pooled for analysis to evaluate LC_{50} and LC_{90} values by using logit dosage - Probit mortality regression line (Using Microsoft excels software). The percentage of larval mortality rate was calculated after 24 h of exposure time. The % mortality was corrected using Abbott's formula [11, 12].

$$\text{Percent corrected mortality} = \frac{\text{percentage of test mortality} - \text{percentage of control mortality}}{\text{100- percentage of control mortality}} \times 100$$

$$\%\text{Mortality} = \frac{\text{Number of dead larvae}}{\text{number of larvae introduced}} \times 100$$

3. Results

The aim of present article is to study the chemical composition and larvicidal efficacy of secondary metabolites from the plants *Annona squamosa* and *Gliricidia sepium*.

Table 1: Summarizes the nature and color of all the four essential oils

Plant extract	Solvent	Color of Eos
Annona squamosa	Methanol	Light brown and viscous liq.
Annona squamosa	Hexane	Ochre yellow and fluid liq.
Gliricidia sepium	Methanol	Reddish brown and thick fluid
Gliricidia sepium	Hexane	Brown and solid pellets

Table 2: Comparative GC-MS Analysis of different solvent extracts of *Annona squamosa* & *Gliricidia sepium (Gls- Gliricidia sepium,* Asq. *Annona squamosa, Meth-Methanol solvent, Hex-Hexane solvent)*

S. No	Ret. Time	compound name	Gls Meth.	Gls Hex.	Asq.Meth	Asq. Hex
1	1.495	Argon	…	0.02	…	…
2	1.556	Carbonochloridic acid, ethyl ester	7.04	…	…	…
3	1.558	Ethane, 1-chloro-1-fluoro-	…	…	3.91	…
4	1.633	Ethanol	0.17	…	0.12	…
5	1.677	Methyl isocyanide	…	…	0.06	…
6	1.679	Acetonitrile	0.12	..	…	…
7	1.709	Pentane	..	..	0.05	..
8	1.713	Hydrogen azide	0.09	..	..	…
9	1.756	Acetic acid, methyl ester	0.49	..	..	..
10	1.78	Butane, 2,2-dimethyl-	..	0.19	..	0.1
11	1.868	Pentane, 2-methyl-	..	32.97	0.69	34.91
12	1.901	Acetic acid	0.51	..	..	..
13	1.926	Pentane, 3-methyl-	..	32.13	0.71	34.99
14	1.992	n-Hexane	..	..	14.58	..
15	2.171	Cyclopentane, methyl-	…	17.09	0.8	15.52
16	2.349	Pentane, 3,3-dimethyl-	…	0.04	..	..
17	2.424	Cyclohexane	..	..	0.21	..
18	2.603	Cyclopentane, 1,3-dimethyl-	..	0.02	..	..
19	2.619	Hexane, 2,2-dimethyl-	0.29	..	..	..
20	3.631	Toluene	…	0.01	..	..
21	4.328	Glyceraldehyde	2.03	..	..	…
22	4.784	Ethylbenzene	..	..	..	0.23
23	4.881	o-Xylene	..	..	..	0.76
24	4.891	Benzaldehyde, 4-benzyloxy-3-methoxy-2-nitro-		0.01	..	..
25	5.047	Dihydroxyacetone	1.89	..	..	..
26	5.4	2-Cyclopenten-1-one, 2-hydroxy-	0.63	..	..	..
27	6.529	Benzene methanol, .alpha.-methyl-	..	..	..	0.11
28	6.567	1,3,5-Triazine-2,4,6-triamine	0.19	..	..	..
29	6.579	Acetophenone	..	..	..	0.2
30	6.607	Benzaldehyde, 3-methyl-	..	..	..	0.04
31	6.705	Ethanone, 1-(3-methylphenyl)-	..	..	..	0.03
32	6.761	Nonane, 3,7-dimethyl-	0.2	..	..	..
33	6.856	Maltol	0.65	..	..	..
34	7.006	3-Methylbenzyl alcohol	..	..	..	0.07
35	7.06	Benzene methanol, 2-methyl-	..	..	..	0.02
36	7.062	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl 2.08	4.45	..	..	..
37	7.396	5-Oxotetrahydrofuran-2-carboxylic acid	0.57	..	..	…
38	7.479	5-Hydroxymethylfurfural	1.13	..	..	..
39	7.561	1,2,3-Propanetriol, 1-acetate	1.69	..	..	..
40	7.697	2-Dodecenal, (E)-	..	..	..	0.04
41	7.869	4-Ethylcyclohexanol	..	..	..	0.03
42	7.99	2,4-Decadienal, (E,E)-	..	..	0.22	0.05
43	8.057	Butanedioic acid, 2-hydroxy-2-methyl-, (S)-	0.94	..	..	..
44	8.152	alpha.-Terpinyl acetate	..	..	..	0.03
45	8.207	1-Undecene, 9-methyl-	..	..	..	0.04
46	8.248	DL-Proline, 5-oxo-, methyl ester	0.58	..	..	..

47	8.399	Hydrocoumarin	..	0.01	..	…
48	8.575	1,3-Propanediol, 2-(hydroxymethyl)-2-nitro-	..	..	2.79	..
49	8.63	Caryophyllene	..	..	..	0.04
50	8.656	1,3-Propanediol, 2-(hydroxymethyl)-2-nitro-	15.04	..	..	..
51	8.707	Coumarin	..	0.01	..	..
52	9.062	2-Butenedioic acid (Z)-, dibutyl ester	..	..	..	0.67
53	9.371	3-Deoxy-d-mannoic lactone	..	..	0.56	..
54	9.689	.alpha.-Methyl-l-sorboside	4.8	..	..	..
55	9.925	2-O-Methyl-d-xylose	4.51	..	..	..
56	10.248	4-O-Methylmannose	48.3	..	..	..
57	11.201	1-Oxacyclopentadecan-2-one, 15-ethenyl-15-methyl	..	0.01	..	..
58	11.232	2-(1,1-Dimethylethyl)-5-oxohexanal	..	0.01	..	..
59	11.714	Pentadecanoic acid, methyl ester	..	0.03	..	..
60	11.715	Hexadecanoic acid, methyl ester	0.57	..	4.8	0.17
61	11.897	n-Hexadecanoic acid	0.3	..	..	..
62	11.904	l-(+)-Ascorbic acid 2,6-dihexadecanoate	..	..	0.85	..
63	11.937	l-(+)-Ascorbic acid 2,6-dihexadecanoate	..	..	..	2.82
64	12.052	24-Noroleana-3,12-diene	..	0.17	..	..
65	12.309	Hexadecanoic acid, 15-methyl-, methyl ester	..	..	0.09	..
66	12.753	1,8,11-Heptadecatriene, (Z,Z)-	..	0.02	..	..
67	12.754	1,7-Hexadecadiene	0.21	..	..	..
68	12.757	Linoleic acid ethyl ester	..	..	6.55	..
69	12.757	9,12-Octadecadienoic acid (Z,Z)-, methyl ester				0.21
70	12.792	Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentylcycl	0.01	..	..	..
71	12.796	9-Octadecenoic acid, methyl ester, (E)-	..	..	14.82	0.41
72	12.868	Phytol	..	0.02	..	..
73	12.954	Butanoic acid, 2-methyl-	0.1	..	..	..
74	12.956	Methyl stearate	..	..	3.32	..
75	13.034	9,12-Octadecadienoic acid (Z,Z)-	..	2.97	..	..
76	13.042	cis-9-Hexadecenal	..	..	11.82	6.78
77	13.975	Benzyl .beta.-d-glucoside	0.8	..	..	..
78	14.178	Octanoic acid, 2-dimethylaminoethyl ester	..	..	0.37	..
79	15.971	6-Methyl-cyclodec-5-enol	..	..	..	0.02
80	15.987	Benzedrex	..	..	0.5	..
81	16.046	Fumaric acid, 2-dimethylaminoethyl nonyl ester	..	..	1.41	..
82	16.211	9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)-	0.22	21.21	0.03	..
83	16.295	Dimethyl-[2-(5-methylcyclohexa-1,4-dienyloxy)ethy	..	..	0.22	..
84	16.298	Dimethyl-[2-(5-methylcyclohexa-1,4-dienyloxy)ethy	..	0.03	..	..
85	16.36	Glycidyl oleate	..	0.02	0.21	..
86	16.422	Glycidyl oleate	..	0.09	..	..
87	16.522	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethy	..	..	3.42	0.12
88	16.701	Bis(2-ethylhexyl) phthalate	..	..	1.01	0.07
89	16.822	(R)-(-)-14-Methyl-8-hexadecyn-1-ol	..	..	..	0.02
90	17.304	9, 19-Cyclolanostan-3-ol, 24-methylene-, (3.beta.)-	..	..	..	0.06
91	17.749	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	..	..	21.21	1.46
92	17.766	Dotriacontane	1.11	3.11	..	..
93	17.768	Octacosane, 1-iodo-	0.43	..	..	..
94	17.895	Octadecanoic acid, 2,3-dihydroxypropyl ester	..	..	2.52	..
95	17.95	Tetracosanoic acid, methyl ester	..	..	0.46	..
96	18.395	Tetrapentacontane	..	0.17	0.43	..
97	18.877	(Z)-Decyl icos-9-enoate	..	2.81	..	..
98	19.166	.beta.-Amyrone	..	7.55	..	..
99	19.97	Behenic amide	..	..	0.31	..

GC-MS analysis and larvicidal bioassay using Eos of *Gliricidia sepium* and *Annona squamosa* were performed against second, third and fourth instar larvae of *Aedes aegypti.* Table: 1 gave information about nature and color of essential oils. The GC-MS studies of methanol and hexane essential oil of flower of *G. sepium* identified 32 and 26 components respectively whereas oils from seeds of *A. squamosa* in methanol and hexane solvent indicates the presence of 32 and 30 compounds respectively. Table 2 showed comparative analysis of EOs of *Gliricidia sepium* and *Annona squamosa* in different solvents. It was observed that few secondary metabolites were common to both plants extracts like 2-methyl pentane; 3-methyl pentane; methyl cyclopentane; Benzaldehyde; 2,4-decadienal; hexadecanoic acid; 9-octadecenoic acid; cis-9-hexadecenal.

During study, it was observed that EOs in methanol of both plants showed better results as compare to EOs in hexane solvent. More sensitivity to EOs was reported by second instar larval stages with more mortality as compared to later immature stages. Table: 3 and 4 showed larvicidal efficacy of essential oils from seeds of *Annona squamosa* and flowers of *Gliricidia sepium oil* extracted in methanol and hexane solvent respectively. The lowest LC_{50} values shown by second, third, fourth instar larvae in methanol extract of

Gliricidia sepium as 38.01ppm, 45.65 ppm, 60.25 ppm respectively whereas highest LC_{50} values were 77.62 ppm, 95.40 ppm, 128.82 ppm respectively in hexane Annona squamosa. The least LC_{90} dose for second, third, fourth in stars were found as 134.89 ppm, 346.73 ppm & 457.08 ppm respectively in hexane extract of Gliricidia sepium oil. Among the four EOs highest LC_{90} values shown by methanol extract of Annona squamosa. These LC_{90} values for second, third and fourth instar Aedes aegypti larvae were 281.83ppm, 436.515 ppm and 1023.29 ppm respectively. These larvicidal activities were contributed by various secondary metabolites present in Eos.

Table 3: Larvicidal efficacy of essential oils from seeds of *Annona squamosa* extracted in methanol and hexane solvent.

Treatment	Solvent	Concentration	Corrected % Mortality	Probit	Regression Equation	LC 50	LC 90
L2	Methanol	50	65.53	5.41	y=3.126 x-0.26	47.86 ppm	120.22 ppm
		100	73.91	5.64			
		200	91.30	6.34			
		300	100.00	8.09			
	Hexane	50	39.44	4.72	y=2.24 x+0.77	77.62 ppm	281.83 ppm
		100	52.11	5.05			
		200	78.87	5.81			
		300	92.96	6.48			
L3	Methanol	50	46.67	4.8	y=1.56 x+2.12	69.18 ppm	457.08 ppm
		100	64.00	5.28			
		200	72.00	5.52			
		300	88.00	6.13			
	Hexane	50	33.81	4.59	y=1.94 x+1.17	95.49 ppm	436.515 ppm
		100	43.66	4.85			
		200	71.83	5.58			
		300	85.92	6.08			
L4	Methanol	50	45.33	4.77	y=0.99 x+3.13	75.85 ppm	1513.56 ppm
		100	60.00	5.18			
		200	66.67	5.36			
		300	74.67	5.58			
	Hexane	50	30.56	4.5	y=1.42x+2.01	128.82 ppm	1023.29 ppm
		100	38.89	4.72			
		200	62.5	5.33			
		300	70.33	5.55			

Table 4: Larvicidal efficacy of essential oils from flowers of *Gliricidia sepium* extracted in methanol and hexane solvent.

Treatment	Solvent	Concentration	Corrected % Mortality	Probit	Regression Equation	LC 50	LC 90
L2	Methanol	50	59.15	5.23	Y=1.48x+2.65	38.01 ppm	281.83 ppm
		100	73.24	5.61			
		200	77.47	5.74			
		300	94.37	6.55			
	Hexane	50	60.56	5.28	y=3.195x-0.53	56.23 ppm	134.89 ppm
		100	69.02	5.50			
		200	85.92	6.08			
		300	100.00	8.09			
L3	Methanol	50	52.12	5.05	y=0.77x+3.74	45.65 ppm	1949.84 ppm
		100	61.97	5.31			
		200	69.02	5.50			
		300	74.65	5.67			
	Hexane	50	53.52	5.10	y=1.53x+2.39	50.11 ppm	346.73 ppm
		100	61.97	5.31			
		200	80.28	5.88			
		300	90.14	6.28			
L4	Methanol	50	47.89	4.97	y=0.78x+3.61	60.25 ppm	2630.26 ppm
		100	54.93	5.13			
		200	64.79	5.39			
		300	71.83	5.58			
	Hexane	50	48.61	4.97	y=1.50x+2.28	64.56 ppm	457.08 ppm
		100	51.39	5.03			
		200	79.17	5.81			
		300	84.72	6.04			

4. Discussion

Heavy and rampant use of synthetic chemi-mosquitocides has ill effects on human health and also results in arising of issues related to environmental pollution. One of the best remedies to cope with such situation is to promote the use of bio-pesticides which will surely decrease overuse of stockpiled chemicals. From the literature, it is clear that scientists have made efforts in the light of bringing new eco-friendly

solutions using bio-pesticides to control mosquito larvae and thus adult population. The richness of botanical extracts can be traced back to last four decades for its use as ovicidal, larvicidal, and adult repellent.

Our aim is to find bio-pesticides which itself yields less toxic by-products during degradation or products which can biodegrade easily. All these issues had triggered the minds of scientists to search and develop more eco-friendly mosquito control strategies. Narumon Komalmisra screened and evaluated the larvicidal activity of methanolic and petroleum ether extracts from *Rhinacanthus nasutus*, *Derris elliptica* against late third and early fourth instar larvae of *Aedes aegypti* and found to be highly effective with $LC_{50}$and LC_{90} values of 8.1&18.46 ppm and 3.93 & 18.51 ppm for *R. nasutus* whereas *D. elliptica* showed values of 11.17 &27.74 mg/L and 13.17 and 32.22 mg/L for PE and methanol extract respectively [25]. The investigation of mosquito larvicidal efficacy of *A. calamus* against III and IV instar larvae of *Aedes aegypti* showed LC_{50} and LC_{90} values 57.32 &120.13 ppm for petro-ether extract while 64.22 ppm in ethyl acetate extract [24]. Ephantus *et al.* [6] examined ovicidal and larvicidal effects of garlic and asafetida EOs and found more toxicity of garlic extract than asofoetida. The LC_{50} values for *Cx. restuans and Cx. pipiens* larvae were 2.7& 7.5 ppm for garlic essential oil and 10.1&13.5 ppm for asafoetida essential oil. The co-toxicity coefficient values for *Cx. pipiens and Cx. restuans* were 80.35 & 46.9 5 respectively suggesting that both oils interacted in antagonistic manner. Sarita Kumar *et al.* [21] (2017) investigated impact of *Ocimum basilicum* leaf extract against fourth instar larvae of *Aedes aegypti* (LC_{50}= 141.95ppm and LC_{90} =445.95 ppm) Comparable to this, in present study, *Gliricidia sepium* oil in methanol showed LC_{50} <50ppm of 38.01ppm and 45.65ppm for second and third instar larvae of *Aedes aegypti* respectively. Cheng *et al.* [3] suggested that if LC_{50} <50 ppm then biolarvicide possess excellent toxicity. The fourth instar larvae had minimum LC_{50} value of 60.25ppm in *Gliricidia sepium* oil in methanol. The *Gliricidia sepium* oil in methanol, hexane and *Annona squamosa* methanol oil also shown LC_{50} <100ppm against second, third, fourth instar larvae.

The findings of present study can also be comparable to other mosquito species. Kaliyamoorthy K.(2012) [35] investigated that the leaf extract of *Gliricidia sepium* possessed remarkable larvicidal, ovicidal, pupicidal activity against malarial vector *Anopheles stephensi* with LC_{50} values of 144.25ppm and 121.79ppm against third instar larvae of *Aedes aegypti* in ethyl acetate and ethanol oil respectively. Kamaraj *et al.* (2011) [29] reported LC_{50} values of 93.80 ppm in methanol oil of *Annona squamosa* leaf extract against Anopheles subpictus which are higher than evaluated in our present study. Magadula JJ revealed that the leaf extracts of *A. squamosa and A. senegalensis* possess cytotoxic and larvicidal activities [31]. Their potential application in managing mosquito larvae would therefore proved to be a promising undertaking. The more sensitivity of EOs to early instar in present study could correlates the similar results given by Kaushik R& Saini P (2008) [32]. The bioactivity of plant based insecticides against mosquito larvae varies significantly according to solvent used in extraction and the mosquito species tested (Shaalan *et al*., 2005) [33]. Bioactive toxic secondary metabolites including fatty acids linoleic acid [29, 34], hexadecanoic acid; 9-octadecenoic acid [28]; hydrocarbons (Siddique *et al*., 2004) [27] 2-methyl pentane; 3-methyl pentane; methyl cyclopentane; aldehydes Benzaldehyde; 2,4-decadienal; cis-9-hexadecenal; coumarin [36, 37]; were present in *G. sepium and A. squamosa* oils.

The activity in *Gliricidia sepium* was found to be highly effective in methanol than any other essential oils under investigation. Plants are known for their insecticidal property and are popular as phytopesticides [38]. India, a mega-diversity country, plants can be used as phyto-pesticide to control mosquito species.

5. Conclusion

The larvicidal efficacy may be contributed by major constituents present in EOs. The results of present study prove to be useful in the direction of developing eco-friendly, cheap and effective mosquito bio-control strategies using aromatic plant extract.

6. Conflict of Interest: The Author do not have any conflict of interest.

7. Acknowledgement

The authors express their sincere gratitude to the Principal J.D. Kabra for his support during study period. First author is grateful to MIT-CARS, Aurangabad for providing the facility of GCMS in their institute and also to Dr. Arvind Dhabe, Professor and Head, Department of Botany, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad for identification of study plants.

8. References

1. Shinde L, Goyal M, Bayas R, Patil S. Review on Potential Eco-friendly Biolarvicides against Dengue Vector: *Aedes aegypti* (Linn) (Diptera: Culicidae) International Journal of Life Sciences Research 2018;6(3):61-80. Available at: www.researchpublish.com
2. Intirach J, Junkun A, Tueton B, Choochote W, Chaithong U, Jitpakdi A *et al.* Chemical constituents and combined Larvicidal effects of selected essential oils against Anopeles cracens (Diptera: Culicidae) Hindawi Pub. House, Psyche, 2012, 11.
3. Cheng SS, Chang HT, Chang ST, Tsai KH, Chen WJ. Bioactivity of selected plant essential oils against the yellow fever mosquito Aedes aegypti larvae. Biores Technol. 2003; 89:99-102.
4. Pavela R. Essential oils for the development of eco-friendly mosquito larvicides: A review. Industrial Crops and Products. 2015; 76:174-187.
5. Mital PK. Biolrvicides in vector control: Challenges and Prospects. J Vec. borne diseases. 2003; 1(2):20-32.
6. Ephantus J. Muturi JL, Ramirez, Zilkowski B, Lina B, Weiler F *et al*. Ovicidal and Larvicidal Effects of Garlic and Asafoetida Essential Oils Against West Nile Virus Vectors Journal of Insect Science. 2018; 18(2);43:1-6.
7. Mahendran S, Pravin Y, Saranya M, Sivakumar T, Mohanraj RS, Dhanakkodi B. Ovipositional deterrence, Ovicidal and developmental effects of *Pithecellobium dulce* (Roxb.) Benth (Fabaceae) Aqueous leaf extract against Dengue vector mosquito *Aede*s *aegypti* (Diptera; Culicidae) Int. J Current. Res. Biosci. Plant Boil. 2015; 2(5):79-91.
8. Iwu MW, Dunkan AR, Okunjio CO. New antimicrobials of plant origin. Alexandria VA: ASHS press, 1999, 457-462.

9. Chengyao M, Yayun Chen, Chen J, Xiang Li, Chen Y. A Review on *Annona squamosa* L. Phytochemicals and Biological Activities. The American Journal of Chinese Medicine. 2017; 45(5):1-32.
10. James R, Malcolm K, Dariel B, Joanna V. Using Soxhlet Ethanol Extraction to Produce and Test Plant Material (Essential Oils) for Their Antimicrobial Properties Journal of Microbiology and Biology Education. 2014, 45-46.
11. Abbott WS. A method of computing the effectiveness of insecticides. J Ecol Entomol. 1925; 18:265-267.
12. Finney DJ. Probit Analysis Cambridge University Press, London, (London), 1971, 68-78.
13. Tyagi V, Patel R, Hazarika H, Dey P, Goswami D, Chattopadhyay P. Chemical composition and bioefficacy for larvicidal and pupicidal activity of essential oils against two mosquito species. International Journal of Mosquito Research. 2017; 4(4):112-118.
14. Deepa M, Palanisamy K, Krishnappa K, Elumalai K. Mosquitocidal activity of Polygala arvensis Wild against Aedes aegypti (Linn.), Anopheles stephensi (Liston.) and Culex quinquefasciatus (Say.) (Diptera: Culicidae). International Journal of Mosquito Research. 2014; 1(4):30-34.
15. Mulla M, Activity ST. Biological effects of neem products against arthropods of medical and veterinary importance. J Am Mosq Control Assoc. 1999; 15:133-52.
16. Fillinger U, Lindsay SW. Larval source management for malaria control in Africa: myths and reality. Malar J. 2011; 10:353.
17. Rana MR, Amin NI, Naser A, Islam N. Screening of Calophyllum inophylum L. leaf extracts for cytotoxic, larvicidal, insect repellent and antimicrobial activities Journal of Pharmacognosy and Phytochemistry. 2017; 6(3):612-616.
18. Khatiwora E, Adsul VB, Pawar P, Joseph M, Deshpande NR, Kashalkars RV. Larvicidal activity of Ipomoea carnea stems extracts and its active ingredient dibutyl phthalate against Aedes aegypti and Culex quinquifasciatus Scholars Research Library Der Pharma Chemical. 2014; 6(1):155-161. (http://derpharmachemica.com/archive.html)
19. Rathy MC, Sajith U, Harilal CC. Plant diversity for mosquito control: A preliminary study International Journal of Mosquito Research. 2015; 2(1):29-33.
20. Bushra S, Tariq M. Use of essential oils for the management of different species of dengue mosquitoes. International Journal of Advances in Biology (IJAB). 2014; 1(1):29-41.
21. Kumar S, Warikoo R, Mishra M, Samal R, Shrankhla, Panmei K *et al*. Impact of Ocimum basilicum leaf essential oil on the survival and behavior of an Indian strain of Dengue vector, *Aedes aegypti* (L.) Vector Biol. J. 2017; 2:2.
22. Mgbemena IC. Comparative evaluation of larvicidal potentials of three plant extracts on Aedes aegypti. Journal of American Science. 2010; 6:435-440.
23. Araujo AFD, Ribeiro-Paes JT, de Deus SC, de Holanda C, de Souza RN, Alves PB *et al.* 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. 2016; 111(7):443-449.
24. Imam H, Zarnigar, Sofi G. Mosquito Larvicidal efficacy of Acorus calamus extracts against Aedes aegypti larvae. Asian Pac J Trop Dis. 2014; 4(1):S181-S185.
25. Komalamisra N, Trongtokit Y, Rongsriyam Y, Apiwathnasorn C. Screening for Larvicidal Activity in some Thai Plants against four Mosquito Vector Species. Southeast Asian J. Trop Med. Public Health. 2005; 36(6):1412-1422.
26. Theodore GA, Thomas MC, Shepard JJ. Identification guide to the mosquitoes of Connecticut, Bulletin, 2005, 966.
27. Siddiqui BS, Rasheed M, Ilyas F, Gulzar T, Tariq RM, Naqvi SNH. Analysis of Insecticidal Azadirachta indica, Z Naturforsch C, 2004; 59:104-112.
28. Kaushik R, Saini P. Screening of some semi-arid region plants for larvicidal activity against Aedes aegypti mosquitoes. J Vector Borne Dis. 2009; 46:244-246.
29. Vijaya Kumar S, Panagal Mani, John Bastin TMM, Ravikumar G. Mosquito Larvicidal, Oviposition deterrent and Repellent properties of Acalypha indica L extracts against Aedes aegypti, Anopheles stephensi, and Culex quinquefasciatus Int J Med Biosci. 2012; 1(3):33-41.
30. Yajima A, Saitou F, Sekimoto M, Maetoko S, Nukada T, Yabuta G. Synthesis of cordiaquinone J and K via B-alkyl Suzuki-Miyaura coupling as a key step and determination of the absolute configuration of natural products. Tetrahedron 2005; 61:9164-9172.
31. Joseph JM, Ester I, Joseph NO. Mosquito larvicidal and cytotoxic activities of 3 Annona species and isolation of active principles. Journal of Medicinal Plants Research. 2009; 3(9):674-680.
32. Kaushik R, Saini P. Larvicidal activity of leaf extract of Millingtonia hortensis (Family:Bignoniaceae) against Anopheles stephensi, Culex quinquefasciatus and Aedes aegypti. J Vector Borne Dis. 2008; 45:66-69.
33. Shaalan E, Canyon D, Faried MW, Abdel WH, Mansour A. A review of botanical phytochemicals with mosquitocidal potential. Environ Int. 2005; 31:1149-1166.
34. Ramsewak RS, Nair MG, Murugesan S, Mattson WJ, Zasada J. Insecticidal Fatty Acids and Triglycerides from Dirca palustris. J Agric. Food Chem. 2001; 49:5852-5856.
35. Kaliyamoorthy K, Dhanasekaran S, Elumalai K. Larvicidal, ovicidal, and pupicidal activities of Gliricidia sepium (Jacq.) (Leguminosae) against the malarial vector, Anopheles stephensi Liston (Culicidae: Diptera). Asian Pacific Journal of Tropical Medicine. 2012, 598-604.
36. Molykutty MK, Arif MM, Jirovetz L, Shafi PM. Essential oil composition of Gliricidia sepium (Leguminosae) leaves and flowers. Ind. J Chem. 2007; 46(8):1359-1360.
37. Simone MS, Franco DM, Artur SJ. Antibacterial activity of coumarins. Z. Naturforsch. 2005; 60C:693-700.
38. Gbolade AA, Oyedele AO, Sosan MB, Adewayin FB, Soyela OL. Mosquito repellant activities of essential oils from two Nigerian Ocimum species. J Trop. Med. Plants. 2000; 1:146-148.
39. World Health Organization. Guidelines for laboratory and field testing of mosquito larvicides. Geneva: World Health Organization. [Online] Available from: http://apps.who.int/iris/bitstream/10665/69101/1/WHO_CDS_WHOPES_GCDPP_2005.13.pdf, 2005-2015