



Received on 25 January 2020; received in revised form, 29 June 2020; accepted, 23 August 2020; published 01 October 2020

## CHEMICAL COMPOSITION AND PUPICIDAL ACTIVITY OF ESSENTIAL OIL FROM *MENTHA SPICATA* AGAINST *AEDES AEGYPTIL*. (DIPTERA: CULICIDAE)

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### Keywords:

Essential oil, *Mentha spicata*, Phytochemicals, Pupicidal activity, *Aedes aegypti*, Vector control

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**ABSTRACT:** Mosquito-borne diseases are serious health problems in India, and mosquito vector control is a fundamental approach to control various diseases. Essential oils represent eco-friendly alternatives to costly synthetic pesticides for mosquito control. The essential oil of *Mentha spicata* in India was used for its activity against *Ae. aegypti*. The essential oil showed pupicidal activity against this mosquito. The results indicated that essential oil from *M. spicata* induced 29, 49.5, 60.8, 67.76, and 81.36% mortality at 100, 200, 300, 400 and 500 ppm essential oil concentration, respectively. The essential oil was rich for various phytochemicals, including estragole, thymol, (-) - 8 - p - Menthen - 2 - yl, acetate, trans, and 3, 5-Heptadienal, 2-ethylidene-6-methyl-. This essential oil may serve as a low-cost vector control agent for mosquito-borne diseases.

**INTRODUCTION:** Mosquitoes involved in the transmission of various human diseases such as filariasis, yellow fever, dengue fever, and malaria, which mainly consider them among the serious health problems across the world<sup>1</sup>. *Anopheles* species are considered as vectors of various human diseases such as certain arboviruses and filariasis<sup>2</sup>. *Anopheles stephensi*, an oriental malaria vector, is distributed in the Indo-Persian area from India, Pakistan, and Iran, to countries around the Persian Gulf<sup>3</sup>.

Although there are various methods for control of *Anopheles* mosquitoes, however resistance and the environmental effect are an essential concern. Synthetic pyrethroids, which are mainly considered as highly effective insecticides against *Anopheles* are generally expensive and beyond the financial resources of various developing countries. Botanical insecticides can apply as an alternative to costly synthetic chemical formulations.

Most botanical insecticides are breakdown easily and rapidly acting on the organisms. The extract of essential oil of some plants and the whole leaf has been studied against various disease-causing mosquito vectors<sup>4-6</sup>. Plant secondary metabolites have activity against predator insects, and microorganisms are natural candidates for the novel discovery of products of combat *A. aegypti*. Many studies focused on various natural products to

<p><b>QUICK RESPONSE CODE</b></p> 	<p><b>DOI:</b> 10.13040/IJPSR.0975-8232.11(10).5158-62</p> <hr/> <p>This article can be accessed online on <a href="http://www.ijpsr.com">www.ijpsr.com</a></p> <hr/> <p>DOI link: <a href="http://dx.doi.org/10.13040/IJPSR.0975-8232.11(10).5158-62">http://dx.doi.org/10.13040/IJPSR.0975-8232.11(10).5158-62</a></p>
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control *Aedes* mosquitoes as larvicides and insecticides<sup>7, 8</sup>. The repellent activity of essential oil from and clove<sup>9</sup> and orange peel<sup>10</sup> was reported. The essential oil from *Cymbopogon citrates*<sup>11</sup>, and *Lippia sidoides*<sup>12</sup> showed auspicious larvicidal activity. In this study, the essential oil of *Mentha spicata* was used to test its activity against the pupae of *Ae. aegypti*.

## MATERIALS AND METHOD:

**Collection and Rearing of Selected Mosquito:** In the present study mosquito stock culture was established as per the method prescribed by Al – Mashhadani *et al.*<sup>13</sup> Freshly moulted *Aedes aegypti* pupae were selected from the stock culture to analyze pupicidal activity. *Ae. aegypti* colony was maintained at the laboratory for analysis.

**Essential Oil:** In this study, the essential oil was extracted from *M. spicata* and stored in an amber colour bottle at room temperature for its pupicidal activity against the freshly moulted pupae of *Ae. aegypti*.

**Pupicidal Activity of Essential Oil from *M. spicata*:** The pupicidal activity of the *M. spicata* essential oil was assessed by using the standard method as prescribed by WHO<sup>14</sup>. The concentrations of 100, 200, 300, 400 & 500 ppm were prepared and tested against the pupae of *Ae. aegypti*. DMSO in water was treated as control. The pupae of these mosquito species (n=30) were introduced in 250-ml containers, and the required amount of plant oils were added individually for each experiment. The pupae mortality was recorded after 24 h treatment. For each experiment, five replications were maintained, and the percentages of mortality were calculated by the prescribed formula<sup>15</sup>.

$$\text{Mortality (\%)} = \% A - \% B / 100 - \% B \times 100$$

Where, % A = % pupal mortality in treatment and % B = % pupal mortality in control. The LC<sub>50</sub> and LC<sub>90</sub> values were calculated using probit analysis (SPSS version 20, 2016).

**GC-MS Analysis of Essential oil from *M. spicata*:** The GC–MS analysis of the oil was performed on Perkin Elmer Clarus 500 gas chromatography, coupled to VG Analytical 70-250S mass spectrometer.

The GC was equipped with a fused capillary column Elite-5 (Column length 30 mm; Column id: 250 µm; Crossband 5% Phenyl 95% dimethylpolysiloxane). Helium was used as carrier gas at a flow rate of 1 mL/min. The oven program started with an initial temperature of 160 °C held for 2 min, and then the oven temperature was heated at 10 °C/min to 200 °C and finally held isothermally for 20 min; an electron ionization system for GC-MS detection with ionization energy of 70 eV was used. A scan rate of 0.6 s (cycle time: 0.2 s) was applied, covering a mass range from 40 to 450 amu. The identification of the compounds was made based on the comparison of retention indices and mass spectra of most of the compounds with data generated under identical experimental conditions by applying a two-dimensional search algorithm, considering the retention index, as well as mass spectral similarity or with those of authentic compounds available in NIST 2005 libraries. Moreover, special software, namely Turbo Mass software (Ver 5.2.0) was used for the processing and interpretation of mass

## RESULTS AND DISCUSSION:

**Pupicidal Activity:** Pupicidal activity of *M. spicata* essential oils was tested against the pupae of *Ae. aegypti*, *An. stephensi*. Uniformed size, hale, and healthy pupae were subjected to different concentrations *viz.*, 100, 200, 300, 400 and 500 ppm concentrations. The results indicated that essential oil from *M. spicata* induced 29, 49.5, 60.8, 67.76, and 81.36% mortality at 100, 200, 300, 400, and 500 ppm essential oil concentration **Table 1**. Larvicidal, pupicidal, and repellent activities of *Gaultheria* oil against the filarial vector, *C. quinquefasciatus* were investigated by Aruna *et al.*<sup>16</sup>

Bezerra-Silva *et al.*,<sup>17</sup> determined the evaluation of the activity of essential oils of three cultivars of *Etilingera elatior* from an ornamental flower against AEA electrophysiology, molecular dynamics and behavioral assays. Ephantus *et al.*<sup>18</sup> examined the chemical composition of garlic and asafoetida essential oils and their individual and combined toxicity against larvae of *C. pipiens* and *C. restuans*, West Nile virus vectors. Andrade-Ochoa *et al.*<sup>19</sup> tested the larvicidal activity of essential oils, and their major components are tested against *C. quinquefasciatus*.

**TABLE 1: PUPICIDAL ACTIVITY OF *MENTHA SPICATA* AGAINST THE FRESHLY MOULTED PUPAE OF *AEDES AEGYPTI***

Concentrations (ppm)	Number of Pupae Subjected to the Experiment (n)	Mortality**	
		Pupal Mortality	% Pupal Mortality
	30		
100	30	8.70±1.21 <sup>b</sup>	29.00
200	30	14.85±1.82 <sup>c</sup>	49.50
300	30	18.24±1.61 <sup>d</sup>	60.80
400	30	20.33±1.47 <sup>e</sup>	67.76
500	30	24.41±1.53 <sup>f</sup>	81.36
Control	30	1.32±0.22 <sup>a</sup>	4.40

The value represents the mean ±S.D. of five replications.\* Number of pupae subjected to the experiment. \*\*Mortality of the pupae observed after 7 days of the exposure period (WHO, 2005 and Abbott, 1925). Values in the column with a different superscript alphabet are significantly different at p<0.05 (Tukey's Test).

**TABLE 2: CHEMICAL COMPOSITION OF *M. SPICATA* ESSENTIAL OIL**

Peak	Compound name	Compound Formula	Retention Time	% Peak Area
1	Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)-	C <sub>10</sub> H <sub>16</sub>	5.98	1.55
2	Camphene	C <sub>10</sub> H <sub>16</sub>	6.39	0.66
3	Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, (1S)-	C <sub>10</sub> H <sub>16</sub>	7.22	0.41
4	α-Pinene	C <sub>10</sub> H <sub>16</sub>	7.67	21.31
5	(+)-4-Carene	C <sub>10</sub> H <sub>16</sub>	8.59	0.79
6	Benzene, 1-methyl-2-(1-methylethyl)-	C <sub>10</sub> H <sub>14</sub>	9.36	10.59
7	Cyclohexene, 1-methyl-4-(1-methylethylidene)-	C <sub>10</sub> H <sub>16</sub>	10.53	6.81
8	Bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methylethyl)-, (1à,2à,5à)-	C <sub>10</sub> H <sub>18</sub> O	10.83	0.07
9	5,7-Octadien-2-ol, 2,6-dimethyl-	C <sub>10</sub> H <sub>18</sub> O	11.34	0.15
10	1,6-Octadien-3-ol, 3,7-dimethyl-	C <sub>10</sub> H <sub>18</sub> O	12.41	4.93
11	Camphor	C <sub>10</sub> H <sub>16</sub> O	13.77	0.15
12	Cyclopentane, 1-methyl-2-acetyl-3-(1-methylethenyl)-	C <sub>11</sub> H <sub>18</sub> O	14.37	0.01
13	Borneol	C <sub>10</sub> H <sub>18</sub> O	15.04	0.14
14	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-	C <sub>10</sub> H <sub>18</sub> O	15.35	0.11
15	3-Cyclohexene-1-methanol, à,à4-trimethyl-	C <sub>10</sub> H <sub>18</sub> O	16.11	0.05
16	Benzene, 1-methoxy-4-methyl-2-(1-methylethyl)-	C <sub>11</sub> H <sub>16</sub> O	18.01	0.21
17	Phenol, 5-methyl-2-(1-methylethyl)-, acetate	C <sub>12</sub> H <sub>16</sub> O <sub>2</sub>	20.92	0.01
18	Benzenemethanol, 4-(1-methylethyl)-	C <sub>10</sub> H <sub>14</sub> O	23.96	6.26
19	2,6-Octadien-1-ol, 3,7-dimethyl-, acetate, (E)-	C <sub>12</sub> H <sub>20</sub> O <sub>2</sub>	24.89	0.09
20	Caryophyllene	C <sub>15</sub> H <sub>24</sub>	25.85	43.00
21	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-, (1à,4aà,8aà)-	C <sub>15</sub> H <sub>24</sub>	28.14	0.04
22	Cyclohexene, 1-methyl-4-(5-methyl-1-methylene-4-hexenyl)-, (S)-	C <sub>15</sub> H <sub>24</sub>	29.30	0.29
23	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-, (1à,4aà,8aà)-	C <sub>15</sub> H <sub>24</sub>	29.90	0.05
24	Caryophyllene oxide	C <sub>15</sub> H <sub>24</sub> O	32.44	1.44
25	.tau.-Cadinol	C <sub>15</sub> H <sub>26</sub> O	34.84	0.05
26	1R,3Z,9S-2,6,10,10-Tetramethylbicyclo[7.2.0]undeca-2,6-diene	C <sub>15</sub> H <sub>24</sub>	36.13	0.06
27	Phenol, 2,3,5-trimethyl-	C <sub>9</sub> H <sub>12</sub> O	43.49	0.04
28	Naphthalene, 1,2,3,4,4a,5,6,7-octahydro-4a-methyl-	C <sub>11</sub> H <sub>18</sub>	44.42	0.21
29	2,6,11,15-Tetramethyl-hexadeca-2,6,8,10,14-pentaene	C <sub>20</sub> H <sub>32</sub>	45.10	0.08
30	(E,E,E)-3,7,11,15-Tetramethylhexadeca-1,3,6,10,14-pentaene	C <sub>20</sub> H <sub>32</sub>	46.21	0.07
31	Benzene, 2-methoxy-1,3,5-trimethyl-	C <sub>10</sub> H <sub>14</sub> O	47.39	0.02
32	7,11-Epoxy-megastigma-5(6)-en-9-one	C <sub>13</sub> H <sub>20</sub> O <sub>2</sub>	47.74	0.14
33	2,5,5,8a-Tetramethyl-1,2,3,5,6,7,8,8a-octahydronaphthalen-1-ol	C <sub>14</sub> H <sub>24</sub> O	48.64	0.07
34	Ethanone, 1-(6,6-dimethylbicyclo[3.1.0]hex-2-en-2-yl)-	C <sub>10</sub> H <sub>14</sub> O	49.70	0.06
35	Phenanthrene, 1,2,3,4,4a,9,10,10a-octahydro-6-methoxy-1,1,4a-trimethyl-7-(1-methylethyl)-, (4aS-trans)-	C <sub>21</sub> H <sub>32</sub> O	51.14	0.08
Total percentage of chemical compositions				100.0000

**GC-MS Analysis:** *M. spicata* essential oil revealed the presence of 28 different compounds. These include, 1) Estragole; 2) Thymol 3) (-)-8-p-Men-

then -2-yl, acetate, trans; 4) 3,5-Heptadienal, 2-ethylidene-6-methyl- ; 5) 2-Cyclohexen-1-ol, 2-methyl-5-(1-methylethenyl)-, acetate, (1R-cis)- ; 6)

4 - Acetyl - 1 - methylcyclohexene; 7)  $\alpha$ -Bourbonene; 8) Caryophyllene; 9)  $\gamma$ -Muurolene; 10) Humulene; 11)  $\beta$ -copaene; 12) Naphthalene, 1, 2, 3, 5, 6, 8a - hexahydro - 4, 7 dimethyl - 1 - (1-methylethyl)-, (1S-cis)-; 13)  $\gamma$ -Elemene; 14) 1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, (E)-; 15) Caryophyllene oxide; 16) Ledol; 17) Aromadendrene oxide-(2) 18) Tetracyclo [6.3.2.0 (2,5). 0(1,8)] tridecan-9-ol, 4,4-dimethyl-; 19) 2-Naphthalenemethanol, decahydro- $\alpha,\alpha$ ,4-trimethyl-8-methylene-, [2R - (2 $\alpha$ , 4 $\alpha$ , 8 $\beta$ )]-; 20) Tetradecanoic acid; 21) Kaur-16-ene; 22)  $\alpha$ -inene; 23) Androstan-17-one, 3-ethyl-3-hydroxy-, (5 $\alpha$ )-; 24) Octadecanoic acid; 25) Cyclohexanone, 5-ethenyl-5 - methyl - 4 - (1methylethenyl) - 2 - (1-methylethylidene)-, cis- ; 26) 3,7,7-Trimethyl - 1-penta-1, 3-dienyl-2oxabicyclo [3.2.0] hept-3-ene; 27) and 2, 6, 11, 15 - Tetramethyl - hexadeca-

2,6,8,10,14- pentaene; 28) Squalene **Fig. 1, Table 2.** Our present findings are in attribute to the earlier findings of several authors. Aurelie *et al.*<sup>20</sup> evaluated the Chemical composition and biocide properties of *Clausena anisata* essential oil against developmental stages of the malaria vector *Anopheles coluzzii*. Andrade-Ochoa *et al.*<sup>19</sup> tested the larvicidal activity of *Cinnamomum verum*, *Citrus aurantifolia*, *Cuminum cyminum*, *Syzygium aromaticum*, *Laurus nobilis*, *Lippia berlandieri*, *Pimpinella anisum* and their major components are tested against larvae and pupae of *CLQ*. Cotchakaew and Soonwera<sup>21</sup> studied the efficacies of essential oils from Liliaceae and Zingiberaceae plants as oviposition deterrent, ovicidal and adulticidal agents against females *Ae. albopictus* and *An. Minimus*.

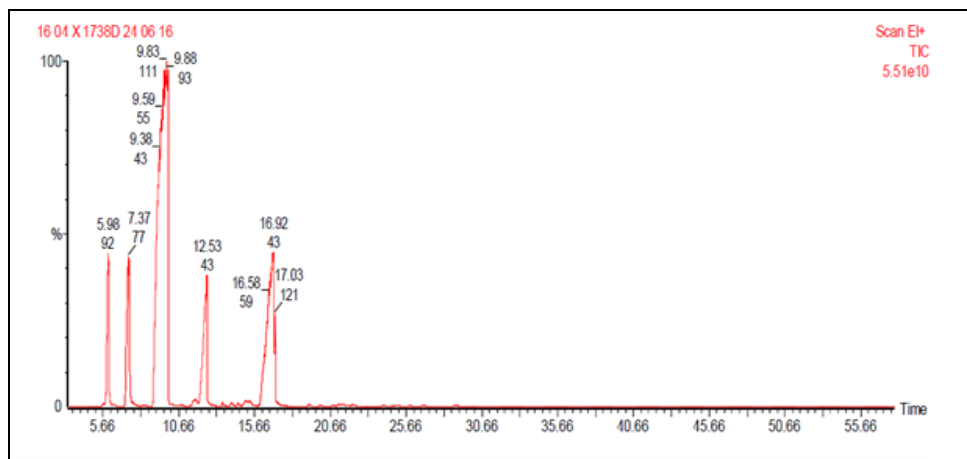


FIG. 1: GC-MS CHROMATOGRAM OF *MENTHA SPICATA* ESSENTIAL OIL

**CONCLUSION:** *Mentha spicata* is one of the medicinal plants that grow in India. Mosquito pupicidal screening of this species indicates good pupicidal activity, which can be significantly attributed to their major bioactive components. In light of these present findings, we report the importance of analyzing the composition of phytochemicals and various biological properties of essential oils. The present finding would help to develop suitable pupicidal agents to control *Ae. Aegypti* population.

**ACKNOWLEDGEMENT:** Authors are very much grateful to UGC, New Delhi for the financial assistance, and also thankful to the Principal and Head, Department of Zoology, Government Arts College for Men (Autonomous), Nandanam, Chennai - 35 for providing the laboratory facilities.

**CONFLICTS OF INTEREST:** None of the conflicts of interest.

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**How to cite this article:**

Paulraj S, Selvamohan T and Kumaraswamy K: Chemical composition and pupicidal activity of essential oil from *Mentha spicata* against *Aedes aegypti*. (Diptera: culicidae). *Int J Pharm Sci & Res* 2020; 11(10): 5158-62. doi: 10.13040/IJPSR.0975-8232.11(10).5158-62.

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