GC-MS Analysis and Larvicidal Activity of *Sesame indicum* Oil Extract against Dengue Vector *Aedes aegypti* (L.) (Diptera: Culicidae)

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**Abstract:** The present study was carried out to investigate the larvicidal activity of *Sesame indicum* oil extract against the dengue vector *Aedes aegypti*. Sesame oil is recognized by a variety of regional names, including Kunjad and Til in Unani, Sesame and Gingelly in English, and Sneephyala and Tila in Ayurveda. Because of its medicinal and nutritional value, it is grown in tropical, subtropical, and southern temperate regions. For many years, Asian cultures have utilized sesame seed to boost health and battle illness. Because of its high content of mono and polyunsaturated fatty acids, vitamin E, phytosterols, fiber, and other nutraceutical components such as phenolic compounds, bioactive lignans, sesamin, episesamin, sesamol, and sesamolin, sesame is a unique nutritional source. The identification of the phytochemical compounds was confirmed based on the retention time, peak area, molecular formula, and molecular weight. Therefore this oil can be used as an effective controlling agent against *A. aegypti*

**Keywords:** GC-MS, Larvicidal activity, Seed oil extract, *A. aegypti*, *Sesame indicum*

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**Introduction**

Mosquitoes are carriers of diseases such as dengue fever, yellow fever, malaria, filariasis etc. They are responsible for high rate of mortality through transmission of diseases. Economic and social status of many countries is affected by mosquitoes and it is one of the important vectors causing illness to the human society. *Aedes aegypti* (L.), a vector of dengue and Chickungunya, is cosmopolitan in distribution, available in the tropical and subtropical zones. About two thirds of world’s populations are infested with dengue vectors, mainly *A. aegypti*. Dengue viruses, causative agents of dengue fever and more severe dengue hemorrhagic fever (DHF/Dengue Shock Syndrome) infect over 100 million people every year (Hahn *et al*., 2001). There is no vaccine for
mosquito borne diseases. Hence, vector control is the most commonly chosen solution available for reducing diseases (Hakim, 1996). The main approach for controlling mosquito borne diseases is prevention of disease transmission by preventing mosquitoes from biting humans. There are many methods used to control mosquitoes such as biocontrol, ovicidal, larvicidal and adulticidal.

Since mosquito breed in stagnant water, it is easy to control them in their natural habitat. Effective use of controlling agents lead to outbreak of mosquito species showing pesticide resistance. Even though chemical control programs have been carried on for long time, these mosquito vectors could not be completely eradicated because mosquitoes develop resistance against repeated use of synthetic products. Hence, researchers are trying to find out the biologically active natural chemical constituents which act as a larvicidal to reduce the risk of humans and harmful accumulated residues. This has necessitated the need for research and development of environmentally safe, bio-degradable and low cost indigenous method for vector control, which can be used with minimum care by individuals and communities in specific situation. During the recent past years, various studies have been made on natural plant products against mosquito vectors (Promsiri et al., 2006).

Sesame (Sesamum indicum L.) is an important seed oil crop (Khuong et al., 2023) in the genus Sesamum and family Pedaliaceae (Wei et al., 2022). It is mostly grown in Africa, with a little lesser number in India. Dehulling is required in places where sesame seeds are used by humans because the hulls contain considerable amounts of indigestible fibre and unpleasant oxalic acid, which gives sesame goods their black colour (Inyang and Nwadimkpa, 1992). Dehulling removes oxalates and is the first stage in making flour that is light in color, low in fiber, non-bitter, and rich in protein. Notably, dehulling increases both the nutritional content and the flavour of sesame products. (Inyang and Ekanem, 1996; Abou-Gharbia et al., 1997). The effectiveness of hull extract as a mosquitocide was investigated in this study.

**Materials and Methods**

**Collection of oil extract:**

The present study was carried out at Post Graduate and Research Department of Zoology, Raja Doraisingam Government Arts College, Sivagangai, Tamil Nadu, India from March, 2022 to July, 2022. The seed oil extract of S. indicum was obtained from the government recognized oil store, Madurai, Tamil Nadu, India. Bioactive substances of GC-MS analysis was done at Instrumentation Centre, ANJA College, Sivakasi, Viruthunagar, Tamil Nadu, India.

**Gas chromatography mass spectroscopy:**

The GC-MS analysis of the sample was carried out on Agilent chromatography GC (Model 7820A series) fitted with detector VL-MSD (Model 5977E). The carrier gas Helium was flow at constant 2 ml/min; the GC oven temperature started at 100 ℃ for 1 min then increased at 10 ℃/min to 270 ℃ held for 30 min. 1 μl of the sample was automatically injected into the column (DB-5) with the injector temperature at 270 ℃. The injections were performed in split less mode. The compounds were identified based on the comparison of their retention indices (RI), retention time (RT), mass spectra of WILEY, NIST library data of the GC-MS system and literature data.

**Mosquito Rearing:**

The colony of A. aegypti mosquitoes was reared in the insectary at the Raja Doraisingam Govt. Arts College, Sivagangai using the standard procedures described by Manh et al. (2019, 2020). The insectary was kept at 27 ± 3 ℃, 70%–80% relative humidity with a photoperiod of 12 h light and 12 h dark. Larvae were placed in plastic trays and provided with cat food (Wiskcat), whereas adult mosquitoes were kept in breeding cages (30 cm ×
30 cm × 30 cm) and maintained on a 10% sucrose solution. The female mosquitoes were fed with blood of live mice for mosquito reproduction. These studies were conducted following the Guide for the Care and Use of Laboratory Animals of Raja Doraisingam Govt. Arts College, Sivagangai.

**Larvicidal activity:**

Standard WHO protocol with slight modifications was adopted for the study (WHO, 1996). The 250 ml of plastic cups contained 200 ml of water with different concentrations of extract (50, 100, 150, 200 and 250 ppm). Early fourth instar larvae were introduced to each concentration. Extract from the stock solution, the different concentrations of 50, 100, 150, 200 and 250 ppm were prepared. Early 20 fourth instar larvae were introduced in 250 ml plastic cups containing 200 ml of water with each concentration. A control was prepared by the addition of acetone to water. Mortality was recorded after 24 h. For each experiment, four replicates were maintained at a time. The observed percentage mortality was corrected by Abbott’s Formula (Abbott, 1925):

\[
\text{Per cent Mortality} = \frac{\% \text{ MT} - \% \text{ MC}}{100 - \% \text{ MC}} \times 100
\]

Where MC is mortality in control and MT is mortality in treated.

**Statistical analysis:**

Mortality was recorded after 24 h of exposure. Obtained values were subjected to logging probit regression analysis to obtain LC\(_{50}\) and LC\(_{90}\) values with a 95% confidence limit (Finney, 1971).

**Results**

The GC-MS characterization of seed oil extract of *S. indicum* was identified and presented in Table 1 and Figure 1. Totally, 12 major chemical compounds were identified, such as 3, 6-diamino-2-benzoyl-4-isopropylthiено [2,3-b] pyridine-5-carbonitrile (19.55 %); Benzamide, 3-bromo-N-(2,4-dimethoxyphenyl)- (3.72 %); dl-. Alpha.-Tocopherol (2.41%); 2-Ethylacridine, (1.94 %); 1H-Indole-2-carboxylic acid, 6-(4-ethoxyphenyl)-3-methyl-4-oxo-4, 5, 6, 7-tetrahydro-, isopropyl ester (10.42 %); Thieno[2, 3 -c] furan – 3 - carbonylitrile, 2- amino- 4, 6- dihydro- 4, 4, 6, 7-tetramethyl- (1.92%); 2,4-Cyclohexadien-1-one, 3, 5- bis (1, 1- dimethylthyl)- 4- hydroxy- (1.61%); Octasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl- (2.91 %); Disulfide, t-butyldipiperidino-(phenylimino)methyl)- (6.09 %); 3-Quinolinecarboxylic acid, 6,8-difluoro-4-hydroxy-, ethyl ester (3.44 %); 7-Diethylamino-3-[5-(4-fluorophenyl)-2-oxazolyl] coumarin (3.44 %); and Benzo (h) quinoline, 2,4-dimethyl- (6.35 %) were present in the seed oil extract of *S. indicum*.

The identification of the phytochemical compounds was confirmed based on the retention time, peak area, molecular formula and molecular weight.

Larvicidal activity of *S. indicum* against fourth instar larvae of *A. aegypti* was estimated. The larval mortality of the fourth instar larvae of *A. aegypti* was found to be increased with increasing concentrations of essential oil extract of *S. indicum* LC\(_{50}\) and LC\(_{90}\) values were 144.7 ppm (91.5 – 138.7), and 272.3 ppm (210.0 – 445.3), respectively (Table 2).

**Discussion**

Pesticides must be safe for the environment and do not damage non-target organisms (Kabarui and Kichiya, 2001). A key component of vector-borne disease prevention is the use of larvicides to reduce mosquito larvae. A practical and desirable alternative for community-level mosquito control is the use of larval-repellent plants. Common poisons for humans and mosquito larvae are phytochemicals produced by plants. Some chemokines or growth inhibitors can also act as attractants or repellents.

There are many reports of the insecticidal or repellent properties of plant extracts. However, some plant therapies have shown real benefits in the management of mosquitoes (Sun *et al.*, 2006). Essential oils from natural plants are one of the most important alternatives since they are easy to procure and low cost compared to synthetic...
Table 1: GC-MS analysis of essential oil of *S. indicum*

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Retention Time</th>
<th>Area</th>
<th>Compounds</th>
<th>Formula</th>
<th>Molecular weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12.914</td>
<td>19.55</td>
<td>3,6-diamino-2-benzoyl-4-isopropylthieno[2,3-b]pyridine-5-carbonitrile</td>
<td>C_{18}H_{16}N_{4}O_{5}S</td>
<td>336.04</td>
</tr>
<tr>
<td>2</td>
<td>13.686</td>
<td>3.72</td>
<td>Benzamide, 3-bromo-N-(2,4-dimethoxyphenyl)-</td>
<td>C_{15}H_{14}BrNO</td>
<td>336.18</td>
</tr>
<tr>
<td>3</td>
<td>16.144</td>
<td>2.41</td>
<td>dl-Alpha-Tocopherol</td>
<td>C_{29}H_{50}O_{2}</td>
<td>430.07</td>
</tr>
<tr>
<td>4</td>
<td>16.442</td>
<td>1.94</td>
<td>2-Ethylacridine</td>
<td>C_{15}H_{13}N</td>
<td>207.27</td>
</tr>
<tr>
<td>5</td>
<td>17.229</td>
<td>10.42</td>
<td>1H-Indole-2-carboxylic acid, 6-(4-ethoxyphenyl)-3-methyl-4-oxo-4,5,6,7-tetrahydro-, isopropyl ester</td>
<td>C_{26}H_{20}NO_{4}</td>
<td>355.4</td>
</tr>
<tr>
<td>6</td>
<td>17.433</td>
<td>1.92</td>
<td>Thieno[2,3-c]furan-3-carbonitrile, 2-amino-4,6-dihydro-4,6,6-tetramethyl-</td>
<td>C_{11}H_{14}N_{2}S</td>
<td>222.31</td>
</tr>
<tr>
<td>7</td>
<td>17.575</td>
<td>1.61</td>
<td>2,4-Cyclohexadien-1-one, 3,5-bis(1,1-dimethyllethyl)-4-hydroxy-</td>
<td>C_{14}H_{22}O_{2}</td>
<td>222.33</td>
</tr>
<tr>
<td>8</td>
<td>17.896</td>
<td>2.91</td>
<td>Octasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-</td>
<td>C_{26}H_{48}O_{7}Si_{8}</td>
<td>577.2</td>
</tr>
<tr>
<td>9</td>
<td>18.152</td>
<td>6.09</td>
<td>Disulfide, t-butyl-[piperidino-(phenylimino)methyl]-</td>
<td>C_{16}H_{21}N_{2}S_{2}</td>
<td>308.5</td>
</tr>
<tr>
<td>10</td>
<td>18.970</td>
<td>3.44</td>
<td>3-Quinolinecarboxylic acid, 6,8-difluoro-4-hydroxy-, ethyl ester</td>
<td>C_{12}H_{16}F_{2}NO_{3}</td>
<td>253.2</td>
</tr>
<tr>
<td>11</td>
<td>20.449</td>
<td>6.35</td>
<td>7-Diethylamino-3-[5-(4-fluorophenyl)-2-oxazolyl] coumarin</td>
<td>C_{15}H_{19}F_{2}N_{3}O</td>
<td>378.4</td>
</tr>
<tr>
<td>12</td>
<td>20.567</td>
<td>5.69</td>
<td>Benzo [h] quinoline, 2,4-dimethyl-</td>
<td>C_{15}H_{14}N</td>
<td>207.27</td>
</tr>
</tbody>
</table>

Fig. 1: GC-MS analysis of seed oil extract of *S. indicum.*
Table 2: Larvicidal activity of S. indicum essential oil against A. aegypti larvae after 24 h exposure

<table>
<thead>
<tr>
<th>Concentration (ppm)</th>
<th>% Mortality</th>
<th>LC₅₀</th>
<th>LCL-UCL</th>
<th>LC₉₀</th>
<th>LCL-UCL</th>
<th>χ²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.0</td>
<td>144.7</td>
<td>91.5-138.7</td>
<td>272.3</td>
<td>210.0-445.3</td>
<td>6.24</td>
</tr>
<tr>
<td>50</td>
<td>19.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>33.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>150</td>
<td>51.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>80.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>250</td>
<td>99.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

LC₅₀= Lethal Concentration brings out 50% mortality and LC₉₀= Lethal Concentration brings out 90% mortality. LCL= Lower Confidence Limit, UCL= Upper Confidence Limit, χ²=Chi-square

chemical products. Moreover, they do not leave residues in the environment because they are extracted from renewable sources and decompose quickly (Hazarika et al., 2018; Al-Rashidi et al., 2022).

Furthermore, our GC-MS analysis of the polyunsaturated fatty acid in sesame oil showed the presence of 3, 6-diamino-2-benzoyl-4-isopropylthieno [2,3-b] pyridine-5-carbonitrile in highest amount of percentage 19.55 %. High monounsaturated fatty acids diets exhibited various benefits on cardiovascular disease risk factors beyond those associated with plasma lipids and lipoproteins (Gillman et al., 1997). A diet rich in monounsaturated fat has beneficial effects on blood pressure and glucose metabolism (Rasmussen et al., 1993)

In this study S. indicum oil recorded LC₅₀ and LC₉₀ values of 144.7 and 272.3 ppm against the larvae of A. aegypti, respectively. A similar observation was reported by Manimaran et al. (2012). However, our results for LC₉₀ were more effective compared to those reported by Manimaran et al. (2012). LC₅₀ of lavender essential oil has been recorded as 140 ppm against Cx. pipiens larvae and a concentration of 800 ppm caused larval mortality of 100% (El-Akhal et al., 2021). Tennyson et al. (2013) observed that LC₅₀ was eight times higher (138.36 ppm) for A. aegypti than that presented herein. According to Warikoo et al. (2012), the larvicidal bioassay on A. aegypti larvae yielded LC₅₀ values of 446.84 ppm and LC₉₀ values of 370.96 ppm. The hexane leaf extract of Citrus sinensis was demonstrated to be a highly efficient irritant to the dengue vector in addition to its mild larvicidal activities.

Conclusion

In this study, we have evaluated environmentally acceptable compounds for vector mosquito control. S. indicum essential oil can provide safer alternatives to today's poisonous, fatal man-made toxins. More research on the effect of active principles of medicinal herbs on larvae is needed to understand the mechanism of action of active principles against mosquito larvae. The findings suggest that affordable and readily available medicinal herbs might be used to control mosquitoes as part of an integrated vector management strategy. Therefore this oil can be used as an effective controlling agent against A. aegypti.

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References


