### **Review Article**

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### Plant extracts as potential mosquito larvicides

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Mosquitoes act as a vector for most of the life threatening diseases like malaria, yellow fever, dengue fever, chikungunya ferver, filariasis, encephalitis, West Nile Virus infection, etc. Under the Integrated Mosquito Management (IMM), emphasis was given on the application of alternative strategies in mosquito control. The continuous application of synthetic insecticides causes development of resistance in vector species, biological magnification of toxic substances through the food chain and adverse effects on environmental quality and non target organisms including human health. Application of active toxic agents from plant extracts as an alternative mosquito control strategy was available from ancient times. These are nontoxic, easily available at affordable prices, biodegradable and show broad-spectrum target-specific activities against different species of vector mosquitoes. In this article, the current state of knowledge on phytochemical sources and mosquitocidal activity, their mechanism of action on target population, variation of their larvicidal activity according to mosquito species, instar specificity, polarity of solvents used during extraction, nature of active ingredient and promising advances made in biological control of mosquitoes by plant derived secondary metabolites have been reviewed.

 $\textbf{Key words} \text{ Insecticides - integrated mosquito management - larvicides - $LC_{50}$ - plant extracts}$ 

#### 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". 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. They act as a vector for most of the life threatening diseases like malaria, yellow fever, dengue fever, chikungunya ferver, filariasis, encephalitis, West Nile virus infection, *etc.*, in almost all tropical and subtropical countries and many other parts of the world.

To prevent proliferation of mosquito borne diseases and to improve quality of environment and public health, mosquito control is essential. The major tool in mosquito control operation is the application of synthetic insecticides such as organochlorine and organophosphate compounds. But this has not been very successful due to human, technical, operational, ecological, and economic factors. In recent years, use of many of the former synthetic insecticides in mosquito control programme has been limited. It is due to lack of novel insecticides, high cost of synthetic insecticides, concern for environmental sustainability, harmful effect on human health, and other non-target populations, their non biodegradable nature, higher rate of biological magnification through ecosystem, and

increasing insecticide resistance on a global scale<sup>2,3</sup>. Thus, the Environmental Protection Act in 1969 has framed a number of rules and regulations to check the application of chemical control agents in nature<sup>4</sup>. It has prompted researchers to look for alternative approaches ranging from provision of or promoting the adoption of effective and transparent mosquito management strategies that focus on public education, monitoring and surveillance, source reduction and environment friendly least-toxic larval control. These factors have resulted in an urge to look for environment friendly, cost-effective, biodegradable and target specific insecticides against mosquito species. Considering these, the application of eco-friedly alternatives such as biological control of vectors has become the central focus of the control programmme in lieu of the chemical insecticides.

One of the most effective alternative approaches under the biological control programme is to explore the floral biodiversity and enter the field of using safer insecticides of botanical origin as a simple and sustainable method of mosquito control. Further, unlike conventional insecticides which are based on a single active ingredient, plant derived insecticides comprise botanical blends of chemical compounds which act concertedly on both behavourial and physiological processes. Thus there is very little chance of pests developing resistance to such substances. Identifying bio-insecticides that are efficient, as well as being suitable and adaptive to ecological conditions, is imperative for continued effective vector control management. Botanicals have widespread insecticidal properties and will obviously work as a new weapon in the arsenal of synthetic insecticides and in future may act as suitable alternative product to fight against mosquito borne diseases.

Roark<sup>5</sup> described approximately 1,200 plant species having potential insecticidal value, while Sukumar *et al*<sup>6</sup> listed and discussed 344 plant species that only exhibited mosquitocidal activity. Shallan *et al* in 2005<sup>7</sup> reviewed the current state of knowledge on larvicidal plant species, extraction processes, growth and reproduction inhibiting phytochemicals, botanical ovicides, synergistic, additive and antagonistic joint action effects of mixtures, residual capacity, effects on non-target organisms, resistance and screening methodologies, and discussed some promising advances made in phytochemical research. Table I summarized the mosquitocidal activities of various herbal products from edible crops, ornamental plants,

trees, shrubs, herbs, grasses and marine plants according to the exaction procedure developed in eleven different solvent systems and the nature of mosquitocidal activities against different life stages of different vector species as a ready reference for further studies.

### **Phytochemicals**

Phytochemicals are botanicals which are naturally occurring insecticides obtained from floral resources. Applications of phytochemicals in mosquito control were in use since the 1920s8, but the discovery of synthetic insecticides such as DDT in 1939 side tracked the application of phytochemicals in mosquito control programme. After facing several problems due to injudicious and over application of synthetic insecticides in nature, re-focus on phytochemicals that are easily biodegradable and have no ill-effects on non-target organisms was appreciated. Since then, the search for new bioactive compounds from the plant kingdom and an effort to determine its structure and commercial production has been initiated. At present phytochemicals make upto 1 per cent of world's pesticide market<sup>9</sup>.

Botanicals are basically secondary metabolites that serve as a means of defence mechanism of the plants to withstand the continuous selection pressure from herbivore predators and other environmental factors. Several groups of phytochemicals such as alkaloids, steroids, terpenoids, essential oils and phenolics from different plants have been reported previously for their insecticidal activities7. Insecticidal effects of plant extracts vary not only according to plant species, mosquito species, geographical varities and parts used, but also due to extraction methodology adopted and the polarity of the solvents used during extraction. A wide selection of plants from herbs, shrubs and large trees was used for extraction of mosquito toxins. Phytochemicals were extracted either from the whole body of little herbs or from various parts like fruits, leaves, stems, barks, roots, etc., of larger plants or trees. In all cases where the most toxic substances were concentrated upon, found and extracted for mosquito control.

Plants produce numerous chemicals, many of which have medicinal and pesticidal properties. More than 2000 plant species have been known to produce chemical factors and metabolites of value in pest control programmes. Members of the plant families-Solanaceae, Asteraceae, Cladophoraceae, Labiatae, Miliaceae, Oocystaceae and Rutaceae have various

Plant species	Family	Plant parts used	Target mosquito species	Lethal concentrations/ biological activity	References
Petroleum ether solve	nt extract				
Artemisia annua	Asteraceae	Leaf	Anopheles stephensi	$LC_{50}$ value was 16.85 ppm after 24 h and 11.45 ppm after 48 h of exposure	Sharma <i>et al</i> (2006) <sup>12</sup>
Acacia nilotica	Fabaceae	Leaf		$LC_{\rm 50}$ value was 55.72 ppm and $LC_{\rm 90}$ value was 194.58 ppm	& Daniel
Argemone mexicana	Papaveraceae	Leaf, seed		$LC_{50}$ value was 30.47 and 24.17; $LC_{90}$ values were 246.33 and 184.99 ppm for leaves and seeds respectively	$(2008)^{13}$
Jatropha curcas	Euphorbiaceae	Leaf		$LC_{50}$ value was 62.29 and $LC_{90}$ value was 454.18 ppm	
Withania somnifera	Solanaceae	Leaf		$LC_{50}$ value was 65.08 and $LC_{90}$ value was 266.39 ppm	
Citrullus colocynthis	Cucurbitaceae	Leaf		$LC_{50}$ values were 37.70 and $LC_{90}$ value was 52.62 ppm	
Aloe barbadensi	Liliaceae	Leaf		$LC_{50}$ values were 29.06 and 22.59 ppm for 24 and 48 h	Maurya <i>et al</i> (2007) <sup>14</sup>
Cannabis sativa	Moraceae	Leaf		$LC_{\rm 50}$ values were 376.58 and 1316.09 ppm for 24 and 48 h	
Eucalyptus globulus	Myrtaceae	Seed, leaf	Culex pipiens	Both the extracts at a dose of 1000 ppm caused 100 and 80% mortality to the tested larvae	Sheeren <i>et al</i> (2006) <sup>15</sup>
Solanum xanthocarpum	Solanaceae	Root	Cx. pipiens pallens	$LC_{50}$ and $LC_{90}$ values were 41.28 and 111.16 ppm after 24 h and 38.48 and 80.83 ppm after 48 h, respectively	Mohan et al (2006) <sup>16</sup>
Thymus capitatus	Lamiaceae	Leaf	Cx. pipiens	The volatile oil, Thymol, and the unsaponifiable portion proved high larvicidal potency (LC <sub>50</sub> value was 49.0 ppm)	Mansour <i>et a</i> (2000) <sup>17</sup>
Citrus aurantium <sup>17</sup>	Rutaceae	Fruit peel	Cx. quinquefasciatus	$LC_{90}$ values were 53.80 and 32.52 ppm after 24 and 48 h of treatment	Kassir (1989) <sup>18</sup>
Myrtus communis	Myrtaceae	Flower and Leaf	Cx. pipiens molestus	$LC_{50}$ value was 16 mg/ l. Thymol, carvacrol, (1 $R$ )-(+)pinene and (1 $S$ )-(-)pinene were the most effective toxic compounds with $LC_{50}$ values of 36-49 mg/l	
Origanum syriacum	Lamiaceae	Leaf		$LC_{50}$ value was 36 mg/ l at 24 h of exposure	
Mentha microcorphylla	Anacardiaceae	Leaf		$LC_{50}$ value was 39 mg /l at 24 h of exposure	
Pistacia lentiscus	Anacardiaceae	Leaf		$LC_{50}$ value was 70 mg /l at 24 h of exposure	
Lavandula stoechas	Lamiaceae	Leaf		LC <sub>50</sub> value was 89 mg/l at 24 h of exposure	
Jatropha curcas	Euphorbiaceae	Leaf	Cx. quinquefasciatus	$LC_{50}$ value was 11.34 and $LC_{90}$ value was 46.52 ppm	Rahuman <i>et</i> (2007) <sup>20</sup>
Pedilanthus tithymaloides				$LC_{50}$ value was 76.61 and $LC_{90}$ value was 307.07 ppm	
Phyllanthus amarus				$LC_{50}$ value was 113.40 and $LC_{90}$ value was 465.28 ppm	Contd

Plant species	Family	Plant parts used	Target mosquito species	Lethal concentrations/ biological activity	References
Argemone mexicana	Papaveraceae	Leaf	Cx. quinquefasciatus	Causes 100% mortality at 250 ppm of each extracts	Karmegan <i>et al</i> (1996) <sup>21</sup>
Jatropha curcus	Euphorbiaceae	Leaf			
Pergularia extensa	Asclepiadaceae	Leaf			
Withania somnifera	Solanaceae	Leaf			
Piper nigrum	Piperaceae	Seed	Cx. pipiens	LC <sub>50</sub> value was 2.6 mg/l	Shaalan <i>et al</i> (2005) <sup>7</sup>
Euphorbia hirta	Euphorbiaceae	Stem bark	Cx. quinquefasciatus	$LC_{50}$ value was 424.94 and $LC_{90}$ value was 1314.01 ppm	Rahuman <i>et</i> al (2007) <sup>22</sup>
E. tirucalli	Euphorbiaceae	Stem bark		$LC_{50}$ value was 5.52 and $LC_{90}$ value was 25.67 ppm	
Ocimum basilicum	Lamiaceae	Leaf	An. stephensi and Cx. quinquefasciatus	LC <sub>50</sub> value of 8.29 and 87.68 ppm respectively	Maurya <i>et al</i> (2009) <sup>23</sup>
Hexane solvent extract					
Momordica charantia	Cucurbitaceae	Fruit	An. stephensi	LC <sub>50</sub> value was 0.50 and LC <sub>90</sub> value was 1.54% concentration of the extract	Singh <i>et al</i> (2006) <sup>24</sup>
			Cx. quinquefasciatus	$LC_{50}$ value was 1.29 and $LC_{90}$ value was 4.11% concentration of the extract	
			Ae. aegypti	LC <sub>50</sub> value was 1.45 and LC <sub>90</sub> value was 4.46% concentration of the extract	
Kaempferia galanga	Zingiberaceae	Rhizome	Cx. quinquefasciatus	LC <sub>50</sub> value was 42.33 ppm	Choochote et al (1999) <sup>25</sup>
Khaya senegalensis	Meliaceae	Leaf	Cx. annulirostris	LC <sub>50</sub> value was 5.86 mg/l	Shaalan <i>et al</i> (2005) <sup>7</sup>
Daucus carota	Apiaceae	Leaves		LC <sub>50</sub> value was 77.19 mg/l	
Curcuma aromatica	Zingiberaceae	Rhizome	Ae. aegypti	LC <sub>50</sub> value was 36.30 ppm	Choochate et al (2005) <sup>26</sup>
Cybistax antisyphilitica	Bignoniaceae	Stem wood	Ae. aegypti	A natural quinone identified as 2-hydroxy-3-(3-methyl-2-butenyl)-1.4-naphthoquinone (lapachol) was quite potent with LC <sub>50</sub> value 26.3 μg/ml	Rodrigues et al (2005) <sup>27</sup>
Eucalyptus citriodora	Myrtaceae	Leaf	An. stephensi, Cx. quinquefasciatus, Ae. aegypti	The LC $_{50}$ values against IVth instar larvae of three species were 69.86, 81.12 & 91.76 ppm, respectively after 24 h and 26.7, 29.9 & 38.8 ppm, respectively after 72 h	Singh <i>et al</i> (2007) <sup>28</sup>
Solanum nigrum	Solanaceae	Dried fruit	An. Culicifacies, An. stephensi, Cx. quinquefasciatus, Ae. aegypti	The $LC_{50}$ values against IVth instar larvae of four species were 9.04, 6.25, 12.25 and 17.63 ppm respectively	Raghavendra et al (2009) <sup>29</sup>
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Plant species	Family	Plant parts used	Target mosquito species	Lethal concentrations/ biological activity	References
Acetone solvent extract	•				
Tridax procumbens	Compositae	Leaf	An. subpictus	LC <sub>50</sub> value of 39.98 mg/l	Kamaraj <i>et al</i> (2011) <sup>30</sup>
Ageratum conyzoides	Asteraceae	Leaf	Cx.	Potent larvicidal activity was noticed	Saxena et al
Cleome icosandra	Capparaceae	Leaf	quinquefasciatus		$(1992)^{31}$
Tridax procumbens	Compositae	Leaf			
Ageratina adenophora	Asteraceae	Twigs	Ae. aegypti and Cx. quinquefasciatus	At 24 h, $LC_{50}$ value of the extract was found to be 356.70 ppm for <i>Ae. aegypti</i> and 227.20 ppm for <i>Cx. quinquefasciatus</i>	Raj Mohan & Ramaswamy (2007) <sup>32</sup>
Feronia limonia	Rutaceae	Leaf	Cx. quinquefasciatus, An. stephensi, Ae. aegypti	LC <sub>50</sub> values of 129.24, 79.58 and 57.23 ppm for three mosquito species respectively	Rahuman <i>et a.</i> (2000) <sup>33</sup>
Millingtonia hortensis	Bignoniaceae	Leaf	An. stephensi, Ae. aegypti and Cx. quinquefasciatus	$LC_{50}$ values of 104.70, 138 and 83.18 ppm for $2^{nd}$ instar larvae of three species at 24 h of bioassay	Kaushik & Saini (2008) <sup>34</sup>
O. sanctum	labiate	Leaf	Ae. aegypti, Cx. quinquefasciatus	The LC <sub>50</sub> values of <i>O. sanctum</i> against the larvae of <i>Ae. aegypti</i> was 425.94, and against the larvae of <i>Cx. quinquefasciatus</i> was 592.60 ppm	Anees (2008) <sup>3</sup>
Carbon tetra chloride s	solvent extract				
Aloe barbadensis	Liliaceae	Leaf	An. stephensi	LC <sub>50</sub> values of 15.58 and 8.04 ppm after 24 and 48 h of exposure, respectively	Maurya <i>et al</i> (2007) <sup>14</sup>
S. xanthocarpum	Solanaceae	Root	Cx. pipiens pallens	$LC_{50}$ and $LC_{90}$ values were 64.99 and 252.43 ppm and 59.20 and 186.15 ppm after 24 and 48 h of exposure, respectively	Mohan <i>et al</i> (2006) <sup>16</sup>
E. globulus	Myrtaceae	Seed and leaf	Cx. pipiens	Both the extracts at a dose of 1000 ppm caused 100 and 80% mortality to the tested larvae	Sheeren (2006) <sup>15</sup>
Chloroform extract					
Plumbago zeylanica, P. dawei and P. stenophylla	Plumbaginaceae	Root	An. gambiae	LC <sub>50</sub> values were 4.1, 6.4 and 6.7 mg/ml respectively. LC <sub>90</sub> values were 10.6, 26.2 and 15.6 mg/ml, respectively	Maniafu <i>et al</i> (2009) <sup>36</sup>
Euphorbia tirucalli	Euphorbiaceae	Latex and stem bark	Cx. pipiens pallens	$LC_{50}value$ was 200.76 and $LC_{90}value$ was 343.515 mg/l	Yadav <i>et al</i> (2002) <sup>37</sup>
Nyctanthes arbortristis	Nyctantheceae	Flower	Cx. quinquefasciatus	$LC_{50}$ values were 25.67, 22.19; 38.60, 28.95; 53.14, 42. 14 and 72.60, 61.82 ppm and for the isolated compound NCS-2 were 73.31, 65.48; 83.02, 67.02; 97.26, 81.84 and 14.68, 99.02 ppm for $1^{st}$ , $2^{nd}$ , $3^{rd}$ and $4^{th}$ instar larvae, respectively at 24 and 48 h	Khatune <i>et al</i> (2001) <sup>38</sup>
				post-exposure	Contd

Plant species	Family	Plant parts used	Target mosquito species	Lethal concentrations/ biological activity	References
Citrus sinensis	Rutaceae	Fruit peel	An. subpictus	$LC_{50}$ value was 58.25 and $LC_{90}$ value was 298.31 ppm	Bagavan <i>et al</i> (2009) <sup>39</sup>
Aloe ngongensis	Asphodelaceae	Leaf	An. gambie	LC <sub>50</sub> value was 58.25 mg/ml	Matasyoh <i>et a</i> (2008) <sup>40</sup>
Millettia dura	Leguminosae	Seed	Ae. aegypti	Rotenoids, deguelin and tephrosin, isolated from the seeds of this plant showed potent activities, with LC $_{50}$ values of 1.6 and 1.4 $\mu g$ /ml at 24 h, respectively	Yenesew et al (2003) <sup>41</sup>
Cassia obtusifolia	Leguminosae	Seed	Ae. aegypti, Ae. togoi, and Cx. pipiens pallens	Showed a strong larvicidal activity of 100% mortality at 25 mg/l. The biologically active component was emodin. The $LC_{50}$ values of emodin were 1.4, 1.9, and 2.2 ppm respectively	
Methanol extract					
Atlantia monophylla	Rutaceae	Leaf	An. stephensi	$LC_{50}$ value of 0.05 mg/l. Insect growth regulating activity with $EI_{50}$ value 0.065 mg/l	Sivagnaname & Kalyanasundarar (2004) <sup>43</sup>
Dysoxylum malabaricum	Meliaceae	Leaf	An. stephensi	4% concentration of leaf extract killed more than 97% of first instars, 92% of fifth instars, 93% of pupae and 91% of adults	Senthil Nathar et al (2006) <sup>44</sup>
Melia azedarach	Meliaceae	Leaf and seeds	An. stephensi	The extract showed strong larvicidal activity	Senthil Nathar et al (2006) <sup>45</sup>
Moringa oleifera	Moringaceae	Bark	Cx. gelidus	$LC_{50}$ value was 38.47 $\mu g/ml$	Kamaraj & Rahuman (2010) <sup>46</sup>
Ocimum gratissimum	Lamiaceae	Leaf	Cx. gelidus	$LC_{50}$ value was 21.83 $\mu g/ml$	Kamaraj & Rahuman (2010) <sup>62</sup>
Solenostemma argel	Apocynaceae	Aerial parts	Cx. pipens	$LC_{50}$ values of 0.037, 0.031, 0.009 and 0.007 ppm and the $LC_{95}$ values were found as 0.394, 0.293, 0.065 and 0.030 ppm, after 1, 2, 4 and 7 days against the larva of $Cx$ . pipiens under laboratory conditions	Al-Doghairi et al (2004) <sup>47</sup>
S. xanthocarpum	Solanaceae	Root	Cx. pipiens pallens	$LC_{50}$ and $LC_{90}$ were 248.55 and 578.25 ppm and 215.52 and 562.72 ppm after 24 and 48 h of exposure, respectively	Mohan <i>et al</i> (2006) <sup>16</sup>
Chrysanthemum indicum	Asteraceae	Leaf	Cx. tritaeniorhynchus	$LC_{50}$ value was 42.29 mg/ml after 24 h	Kamaraj <i>et al</i> (2010) <sup>48</sup>
					Contd

Plant species	Family	Plant parts used	Target mosquito species	Lethal concentrations/ biological activity	References
Azadirachta indica	Meliaceae	Leaf	Cx. pipens	Showed an acute and chronic LC $_{50}$ and 95% CL at 824 and 265 ppm	El Hag <i>et al</i> (1999) <sup>65</sup>
Rhazya stricta	Apocynaceae	Leaf		Acute (2 d) and chronic (10 d) toxic effects, having an LC $_{50}$ and 95% CL at 251 and 140 ppm	
Momordica charantia	Cucurbitaceae	Leaf	Cx. quinquefasciatus	$LC_{50}$ value was 465.85; $LC_{90}$ value was 2421.46 ppm	Prabakar & Jebanesan (2004) <sup>49</sup>
Trichosanthes anguina				LC <sub>50</sub> value was 567.81; LC <sub>90</sub> value was 2915.48 ppm	
Luffa acutangula				$LC_{50}$ value was 839.81; $LC_{90}$ value was 3286.25 ppm	
Benincasa cerifera				$LC_{50}$ value was 1189.30; $LC_{90}$ value was 6528.5 ppm	
Citrullus vulgaris				$LC_{50}$ value was 1636.04; $LC_{90}$ value was 11473.92 ppm	
Vitex negundo	Verbenaceae	Leaf	Cx. quinquefasciatus	LC <sub>50</sub> value was 212.57 ppm	Krishnan <i>et al</i> (2007) <sup>50</sup>
V. trifolia				LC <sub>50</sub> value was 41.41 ppm	
V. peduncularis				LC <sub>50</sub> value was 76.28 ppm	
V. altissima				LC <sub>50</sub> value was 128.04 ppm	
Centella asiatica	Umbelliferae	Leaf	Cx. quinquefasciatus	$LC_{50}$ ranged between 6.84 ppm at 19°C and 1.12 ppm at 31°C. $LC_{90}$ varied from 9.12 to 3.63 ppm at the two temperatures, respectively	Rajkumar & Jebanesan (2005) <sup>51</sup>
Euphorbia tirucalli	Euphorbiaceae	Latex and stem bark	Cx. pipiens pallens	$LC_{50}$ value was 177.14; $LC_{90}$ value was 513.387 mg/l	Yadav <i>et al</i> (2002) <sup>37</sup>
Eucalyptus globulus	Myrtaceae	Seed and leaf	Cx. pipiens	At a dose of 1000 ppm caused 100% mortality of the tested larvae	Sheeren (2006) <sup>15</sup>
Atlantia monophylla	Rutaceae	Leaf	Cx. quinquefasciatus	Larvae were found susceptible with LC $_{50}$ value of 0.14 mg/l $$	Sivagnaname & Kalyanasundarar (2004) <sup>43</sup>
Pavonia zeylanica	Malvaceae	Leaf	Cx. quinquefasciatus	After 24 h of treatment the $LC_{50}$ values was 2214.7 ppm	Vahitha <i>et al</i> (2002) <sup>52</sup>
Acacia ferruginea	Leguminosae	Leaf	Cx. quinquefasciatus	After 24 h of treatment the $LC_{50}$ value was 5362.6 ppm	
Coccinia indica, Cucumus sativus, Momordica charantia	Cucurbitaceae	Leaf	Cx. quinquefasciatus and Ae. aegypti	$LC_{50}$ values of the respective plants were, 377.69, 623.80, 207.61 and 309.46, 492.73 and 199.14 ppm against the two vector species	Rahuman & Venkatesan (2008) <sup>22</sup>
Cassia tora	Caesulpinaceae	Seed	Ae. aegypti and Cx. pipiens	$LC_{50}$ value was 20mg/l for both the larval species	Jang et al (2002) <sup>73</sup>
			pallens		Contd

Plant species	Family	Plant parts used	Target mosquito species	Lethal concentrations/ biological activity	References
Atlantia monophylla	Rutaceae	Leaf	Ae. aegypti	Larval growth regulating activity of this extract was found to be pronounced with EI <sub>50</sub> value 0.002 mg/l	Sivagnaname & Kalyanasundaran (2004) <sup>43</sup>
Coccinia indica, Cucumis sativus, Momordica charantia	Cucurbitaceae	Leaf	Ae. albopictus	$LC_{50}$ value was 309.46, 492.73 and 199.14 ppm respectively	Rahuman & Venkatesan (2008) <sup>22</sup>
Aristolochia saccata	Aristolochiaceae	Root		$LC_{50}$ value was 14.52; $LC_{90}$ value was 42.68 ppm	Das <i>et al</i> (2007) <sup>54</sup>
Annona squamosa	Annonaceae	Leaf		$LC_{50}$ value was 20.26; $LC_{90}$ value was 86.59 ppm	
Gymnopetelum cochinchinensis	Cucurbitaceae	Fruit/ pericarp		$LC_{50}$ value was 50.67; $LC_{90}$ value was 155.12 ppm	
Caesalpinea sp.	Leguminosae	Bark		$LC_{50}$ value was 53.66; $LC_{90}$ value was 169.41 ppm	
Piper sp.	Piperaceae	Stem		$LC_{50}$ value was 144.22; $LC_{90}$ value was 357.32 ppm	
Chamaecyparis obtusa	Cupressaceae	Leaf	An. stephensi	The bioactive component in the leaf extract was characterized as beta-thujaplicin by spectroscopic analyses. The $LC_{50}$ value of beta-thujaplicin was 2.91 ppm	Jang et al (2005) <sup>55</sup>
Acalypha alnifolia	Euphorbiaceae	Leaf	An. stephensi, Ae. aegypti and Cx. quinquefasciatus	LC <sub>50</sub> values were 125.73, 127.98 and 128.55 ppm against 4 <sup>th</sup> instar larvae of three mosquito species at 24 h	Kovendan <i>et al</i> (2012) <sup>56</sup>
Chloroform: methanol	extract(1:1)				
Solanum villosum	Solanaceae	Leaf	An. subpictus	$LC_{50}$ values for all instars were between 24.20 and 33.73 ppm after 24 h and between 23.47 and 30.63 ppm after 48 h of exposure period	Chowdhury et al (2009) <sup>57</sup>
Cestrum diurnum	SolanaceaeLeaf		An. stephensi	The $LC_{50}$ value of the active ingredient was determined as 0.70, 0.89, 0.90 and 1.03mg/100mL, for 1st, 2nd, 3rd and 4th instar larva respectively in 24 h study period	Ghosh & Chandra (2006) <sup>58</sup>
			Cx. quinquefasciatus	$LC_{50}$ value of 0.29, 0.35, 0.57 and 0.65% for $1^{st},2^{nd},3^{rd}$ and $4^{th}$ instar larvae at 24 h	Ghosh <i>et al</i> (2008) <sup>59</sup>
Solanum villosum	Solanaceae	Berry	Ae. aegypti	$LC_{50}$ value of 5.97 ppm at 72 h of bioassay	Chowdhury et al (2008) <sup>60</sup>
Ethanol Extract					
Cassia obtusifolia	Leguminosae	Leaf	An. stephensi	$LC_{50}$ and $LC_{90}$ values were 52.2 and 108.7 mg/ l	Rajkumar & Jebanesan (2009) <sup>61</sup>
Azadirachta indica	Meliaceae	Leaf	Cx. fatigans	In comparison with malathion (LC <sub>50</sub> value was 0.45 ppm) the LC <sub>50</sub> value of neem fraction (NLX) was found to be higher to the third instar larvae at 390 ppm	Azmi <i>et al</i> (1998) <sup>62</sup>
				and mind install fail vac at 570 ppm	Contd

Plant species	Family	Plant parts used	Target mosquito species	Lethal concentrations/ biological activity	References
Piper retrofractum	Piperaceae	Unripe and ripe fruit	Cx. quinquefasciatus	The ripe fruit extract (002/3) was somewhat less active than ripe fruit extract (001/4) with lesser larvicidal activity	Chansang <i>et a</i> (2005) <sup>63</sup>
Citrus reticulata	Rutaceae	Seed	Cx. quinquefasciatus and Ae. aegypti	LC <sub>50</sub> value against <i>Ae. aegypti</i> and <i>Cx. quinquefasciatus</i> larvae was 2,267.71, and 2,639.27 ppm respectively	Sumroiphon et al (2006) <sup>64</sup>
Azadirechta indica	Meliaceae	Leaf	Ae. aegypti	LC <sub>50</sub> value is 8.32 mg/ml	Mgbemena (2010) <sup>65</sup>
Azadirechta indica, Ocimum gratissimium and Citrus citratus	Meliaceae, Lamiaceae and Rutaceae respectively	Leaf	Ae. aegypti	A. indica showing the greatest toxicity having $LC_{50}$ at $8.32$ mg/ml, while on the other hand O. gratissimum and C. citratus had $LC_{50}$ 19.50mg/ml and 34.67mg/ml respectively	Mgbemena (2010) <sup>65</sup>
Apium graveolens	Umbelliferae	Seed	Ae. aegypti	$LD_{50}$ and $LD_{95}$ values of 81.0 and 176.8 mg/l, respectively for fourth instar larvae	Choochate et al (2004) <sup>66</sup>
Rhizophora mucronata	Rhizophoraceae	Bark, pith, stem wood	Ae. aegypti	LC <sub>50</sub> values of 157.4, 168.3 and 1003.4 ppm for bark, pith and stem wood at 48 h respectively	Kabaru & Gichia (2001) <sup>67</sup>
Piper longum	Piperaceae	Fruit exocarp	Ae. aegypti	LC <sub>50</sub> value of 2.23 ppm	Chaithong et al (2006) <sup>68</sup>
P. ribesoides	Piperaceae	Fruit exocarp	Ae. aegypti	LC <sub>50</sub> value of 8.13 ppm	
P. sarmentosum	Piperaceae	Fruit exocarp	Ae. aegypti	LC <sub>50</sub> value of 4.06 ppm	
Annona crassiflora	Annonaceae	Root wood	Ae. aegypti	LC <sub>50</sub> value was 0.71; LC <sub>90</sub> value was 5.12 µg/ml	Omena <i>et al</i> (2007) <sup>69</sup>
		Root bark		$LC_{50}$ value was 8.94; $LC_{90}$ value was 39.00 $\mu g/ml$	
		Stem		LC <sub>50</sub> value was 16.1; LC <sub>90</sub> value was 54.8 μg/ml	
A. glabra	Annonaceae	Seed		$LC_{50}$ value was 0.06; $LC_{90}$ value was 2.75 $\mu g/ml$	
A. muricata	Annonaceae	Root		$LC_{50}$ value was 42.3; $LC_{90}$ value was 200 $\mu g/ml$	
A. squamosa	Annonaceae	Root		$LC_{50}$ value was 31.9; $LC_{90}$ value was 66.2 $\mu g/ml$	
		Leaf		$LC_{50}$ value was 169; $LC_{90}$ value was 748 $\mu g/ml$	
Denis sp.	Leguminoseae	Root		$LC_{50}$ value was 8.54; $LC_{90}$ value was 15.2 $\mu g/ml$	
Erythrina mulungu	Leguminoseae	Stem bark		$LC_{50}$ value was 67.9; $LC_{90}$ value was 15.2 $\mu g/ml$	
Pterodon polygalaeflorus	Leguminoseae	Seed		$LC_{50}$ value was 35.7; $LC_{90}$ value was 63.6 $\mu g/ml$	
Tagetes minuta	Asteraceae	Aerial parts	Ae. fluviatilis	$LC_{90}$ of 1.5 mg/l and $LC_{50}$ of 1.0 mg/l.	Macedo <i>et al</i> (1997) <sup>70</sup>
					Contd

Plant species	Family	Plant parts used	Target mosquito species	Lethal concentrations/ biological activity	References
Eclipta paniculata	Asteraceae	Aerial parts	Ae. fluviatilis	$LC_{90}$ of 17.2 mg/l $$ and $LC_{50}$ of 3.3 mg/l $$	
Benzene extract					
Citrullus vulgari	s Cucurbitaceae	Leaf	Ae. stephensi	100% mortality was exerted at 250 ppm and the corresponding LC <sub>50</sub> value was 18.56 ppm	Mullai <i>et al</i> (2008) <sup>71</sup>
Acalypha indica	Euphorbiaceae	Leaf	An. stephensi	LC $_{50}$ value was 19.25 ppm at 24 h	Govindarajan et al (2008) <sup>72</sup>
C. vulgaris	Cucurbitaceae	Leaf	Ae. aegypti	LC <sub>50</sub> value was 42.76 ppm	Mullai <i>et al</i> (2008) <sup>73</sup>
Ethyl acetate ext	ract				
Dysoxylum malabaricum	Meliaceae	Leaf	An. stephensi	Fourth instars were more susceptible to the extract when compared with pupae and adults	Senthil Nathan et al (2008) <sup>74</sup>
D. beddomei					
Aloe turkanensis	Asphodelaceae	Leaf	An. gambiae	100% mortality was achieved at a concentration of 0.2 mg/ml and it had a $LC_{50}$ value of 0.11mg/ml	Matasyoh <i>et a</i> (2008) <sup>40</sup>
Solanum nigrum	Solanaceae	Leaf	Cx. quinquefasciatus	$LC_{50}$ value was 17.04 ppm against $4^{th}$ instar larvae after 24 h $$	Rawani <i>et al</i> (2010) <sup>75</sup>
Ocimum gratissi	mum Lamiaceae	Leaf	Cx. gelidus and Cx. quinquefasciatus	$LC_{50}$ values were 39.31 and 66.28 $\mu g/ml$ against $4^{th}$ instar larvae after 24 h	Kamaraj & Rahuman (2010) <sup>48</sup>
Annona squamos	sa Annonaceae	Bark	Cx. quinquefasciatus and An. stephensi	LC <sub>50</sub> values of 28.18 and 43.07 ppm against <i>An. stephensi</i> and <i>Cx. quinquefasciatus</i> respectively	Kamaraj <i>et al</i> (2010) <sup>48</sup>
O. sanctum	Labiates	Leaf	Ae. aegypti, Cx. quinquefasciatus	The LC <sub>50</sub> values of <i>O. sanctum</i> against the larvae of <i>Ae. aegypti</i> was 425.94, and against the larvae of <i>Cx. quinquefasciatus</i> was 592.60 ppm	Anees (2008) <sup>3</sup>
Aqueous extract					
Carica papaya	Caricaceae	Seed	Cx. quinquefasciatus	$LC_{50}$ value of 0.15, 0.11. 0.07 and 0.20 % against 1 <sup>st</sup> , 2 <sup>nd</sup> , 3 <sup>rd</sup> and 4 <sup>th</sup> instar larvae	Rawani <i>et al</i> (2009) <sup>76</sup>
Murraya paniculata	Rutaceae	Fruit		$LC_{50}$ value of 0.05, 0.06, 0.08 and 0.31% against $1^{st}$ , $2^{nd}$ , $3^{rd}$ and $4^{th}$ instar larvae	
Cleistanthus collinus	Euphorbiaceae	Leaf		$LC_{50}$ value of 0.21, 0.27, 0.29 and 0.40 % against $1^{st},2^{nd},3^{rd}$ and $4^{th}$ instar larvae	
			An. gambiae	$LC_{50}value$ was $409.77$ and $LC_{90}value$ was $831.08\;ppm$	
Hemidesmus indicus	Asclepiadaceae	Root	Cx. quinquefasciatus	80% mortality was observed in 5% concentration after 1 day of exposure	Khanna & Kannabiran
Gymnema sylvestre	Asclepiadaceae	Leaf	Cx. quinquefasciatus	6.6% mortality was observed in 5% concentration after 1 day of exposure	$(2007)^{77}$
Eclipta prostrata	Asteraceae	Leaf, root	Cx. quinquefasciatus	78.3% mortality was observed in 5% concentration after 1 day of exposure	Contd

Plant species	Family	Plant parts used	Target mosquito species	Lethal concentrations/ biological activity	References
Artimisia cina	Compositeae	Leaf	Cx. pipens	The EC <sub>50</sub> for the mosquito at 24 h after treating with extract was 4.0 g/l	Aly & Bardan (1986) <sup>78</sup>
Cleome droserifolia	Capparidaceae	Leaf	Cx. pipens	The EC $_{50}$ for the mosquito at 24 h after treating with extract was 4.7 g/l	
Piper retrofractum	Piperaceae	Un ripe and ripe fruit	Cx. quinquefasciatus and Ae. aegypti	LC <sub>50</sub> value of 135 against <i>Cx.</i> quinquefasciatus and 79 ppm against <i>Ae.</i> aegypti	Chansang <i>et al</i> (2005) <sup>63</sup>
Solanum villosum	Solanaceae	Leaf	An. stephensi, Cx. quinquefasciatus and Ae. aegypti	The protein compound responsible for mosquitocidal property was isolated with a $LC_{50}$ value of 644.75, 645.75 and 747.22 ppm	Chowdhury <i>et al</i> (2008) <sup>79</sup>
Solanum nigrum	Solanaceae	Dried fruit		The LC <sub>50</sub> of <i>An. culicifacies</i> species A was the lowest while that of <i>Ae. aegypti</i> was highest in the order, <i>An. culicifacies</i> species A (208.5 ppm) > <i>An. stephensi</i> (242.5 ppm) > <i>An. culicifacies</i> species C (251.7 ppm) > <i>Cx. quinquefasciatus</i> (337.2 ppm) > <i>Ae. aegypti</i> (359 ppm)	Raghavendra et al (2009) <sup>29</sup>
Steam distillatio	n				
Paullinia clavigera	Sapindaceae	Leaf	An. benarrochi	LC <sub>50</sub> (24 h) value was 0.81; LC <sub>50</sub> (12 h) value was 1.19%	Iannacone & Pérez (2004) <sup>80</sup>
Tradescintia zebrina	Commelinaceae		An. benarrochi	$LC_{50}(24 \text{ h})$ value was 0.81; $LC_{50}(12 \text{ h})$ value was 7.83%	

types of larval, adulticidal or repellent activities against different species of mosquitoes<sup>7</sup>.

# Application of phytochemicals as mosquito larvicide: An essential component of IMM

Human beings have used plant parts, products and secondary metabolites of plant origin in pest control since early historical times. Vector control has been practiced since the early 20th century. During the pre-DDT era, reduction of vector mosquitoes mainly depended on environmental management of breeding habitats, *i.e.*, source reduction. During that period, some botanical insecticides used in different countries were Chrysanthemum, Pyrethrum, Derris, Quassia, Nicotine, Hellebore, Anabasine, Azadirachtin, d-limonene camphor, Turpentine, *etc*<sup>7</sup>.

From the early 1950s, DDT and other synthetic organochloride and organophosphate insecticides were extensively used to interrupt transmission of vector borne diseases by reducing densities, human-vector

contact and, in particular, the longevity of vector mosquitoes. In the mid-1970s, the resurgence of vector borne diseases, along with development of insecticide resistance in vector population, poor human acceptance of indoor house spraying and environmental concerns against the use of insecticides led to a rethinking in vector control strategies<sup>10</sup>. As a result, emphasis was given on the application of alternative methods in mosquito control as part of the Integrated Mosquito Management (IMM)<sup>11</sup>. Integrated Mosquito Management (IMM) is a decision-making process for the management of mosquito populations, involving a combination of methods and strategies for long-term maintenance of low levels of vectors. The purpose of IMM is to protect public health from diseases transmitted by mosquitoes, maintain healthy environment through proper use and disposal of pesticides and improve the overall quality of life through practical and effective pest control strategies. The main approaches of IMM include: (i) Source reduction and habitat management

by proper sanitation, water management in temporary and permanent water bodies, and channel irrigation. Vegetation management is also necessary to eliminate protection and food for mosquito larvae; (ii) Larviciding by application of dipteran specific bacteria, insect growth regulators, surface films and oils, expanded polystyrene beads, phytochemicals, organophosphates and organochlorides, (iii) Adulticiding by application of synthetic pyrethroids, organophosphates and synthetic or plant derived repellents, insecticide impregnated bed nets, genetic manipulations of vector species, etc., (iv) Use of mosquito density assessment in adult and larval condition and disease surveillance; and (v) Application of biological control methods by using entomophagous bacteria, fungi, microsporidians, predators and parasites.

Of the above avenues of IMM, larviciding approach is the more proactive, proenvironment, target specific and safer approach than controlling adult mosquitoes. Application of larvicide from botanical origin was extensively studied as an essential part of IMM, and various mosquito control agents such as ocimenone, rotenone, capllin, quassin, thymol, eugenol, neolignans, arborine and goniothalamin were developed<sup>7</sup>.

### Variation of larvicidal potentiality according to mosquito species, plant parts and polarity of solvents used

The efficacy of phytochemicals against mosquito larvae can vary significantly depending on plant species, plant parts used, age of plant parts (young, mature or senescent), solvent used during extraction as well as upon the available vector species. Sukumar et al6 have described the existence of variations in the level of effectiveness of phytochemical compounds on target mosquito species vis-à-vis plant parts from which these were extracted, responses in species and their developmental stages against the specified extract, solvent of extraction, geographical origin of the plant, photosensitivity of some of the compounds in the extract, effect on growth and reproduction. Changes in the larvicidal efficacy of the plant extracts occurred due to geographical origin of the plant (in Citrus sp<sup>18,39,64,65</sup>, Jatropha sp<sup>13,20,21</sup>, Ocimum sanctum<sup>22,35,65,82</sup>, Momordica charantia<sup>22,24,49</sup>, Piper sp<sup>54,63,89,95</sup> and Azadirechta indica<sup>65</sup>); response in the different mosquito species (in Curcuma domestica<sup>26</sup>, Withania somnifera<sup>13</sup>, Jatropha curcas<sup>13,20</sup>, Piper retrofractum<sup>63</sup>, Cestrum diurnum<sup>58</sup>, Citrullus vulgaris<sup>50,71</sup>, and Tridax procumbens<sup>30,31</sup>); due to variation in the species of plant examined (in Euphorbia sp<sup>22,28,37,51</sup>, Phyllanthus

sp<sup>20</sup>, Curcuma sp<sup>36</sup>, Solanum sp<sup>16,29,57,60,75,79,96</sup>, Ocimum  ${\rm sp}^{23,35,65,82}$ , Eucalyptus  ${\rm sp}^{22,28,37,51}$ , Plumbago  ${\rm sp}^{20}$ , *Vitex* sp<sup>50,93</sup>, *Piper* sp<sup>54,63,89,95</sup>, *Annona* sp<sup>48,54,69</sup>, and Cleome sp<sup>31,78</sup>) and between plant parts used to study the larvicidal efficacy (in Euphorbia tirucalli<sup>28,51</sup>, Solanum xanthocarpum<sup>16</sup>, Azadirechta indica<sup>65</sup>, Solanum villosum<sup>57,60,79,96</sup>, Annona squamosa<sup>48,54,69</sup>, Withania somnifera<sup>13</sup>, Melia azedarach<sup>45</sup>, Moringa oleifera<sup>46</sup> and Ocimum sanctum<sup>35,82</sup>). However, the principal objective of the present documentation is to report the changes in larvicidal potentiality of the plant extracts due to change of the particular solvent used during extraction. Variation of the larvicidal potential of the same plant changed with the solvents used as evidenced in case of Solanum xanthocarpum<sup>16</sup>. Euphorbia tirucalli<sup>28,51</sup>, Momordica charantia<sup>22,24,49</sup>. Eucalyptus globules<sup>14,15,28,83</sup>, Citrullus colocynthis<sup>13</sup>, Azadirechta indica<sup>65</sup>, Annona squamosa<sup>48,54,69</sup> and Solanum nigrum<sup>29,75</sup>

It has been shown that the extraction of active biochemical from plants depends upon the polarity of the solvents used. Polar solvent will extract polar molecules and non-polar solvents extract non-polar molecules. This was achieved by using mainly eleven solvent systems ranging from hexane/ petroleum ether, the most non polar (polarity index of 0.1 that mainly extracts essential oil) to that of water, the most polar (polarity index of 10.2) that extracts biochemical with higher molecular weights such as proteins, glycans, etc. Chloroform or ethyl acetate are moderately polar (polarity index of 4.1) that mainly extracts steroids, alkaloids, etc. It has been found that in most of the studies solvent with minimum polarity have been used such as hexane or petroleum ether or that with maximum polarity such as aqueous/ steam distillation. However, those biochemical that were extracted using moderately polar solvents were also seen to give good results as reported by a few bioassay. Thus, different solvent types can significantly affect the potency of extracted plant compounds and there is difference in the chemo-profile of the plant species. In Table I, the lowest LC<sub>50</sub> value was reported in Solenostemma argel against Cx. pipiens<sup>47</sup>. Several other plants such as Nyctanthes arbotristis<sup>38</sup>, Atlantia monophylla<sup>57</sup>, Centella asiatica<sup>40</sup>, Cryptotaenia paniculata<sup>76</sup> were also reported with promising LC<sub>50</sub> values. These extracts may be fractioned in order to locate the particular bioactive toxic agent responsible for larval toxicity. Table I also reported that most of the studies were carried out on Culex mosquitoes and Aedes was the least frequently chosen mosquitoes for all the experiments. In several studies, instead of a particular

Active ingredient	Mosquito	Plants	LC/LD values	References
Octacosane	Cx. quinquefasciatus	Moschosma polystachyum	LC <sub>50</sub> value of 7.2±1.7 mg/l	Rajkumar & Jebanesan (2004) <sup>81</sup>
( <i>E</i> )-6-hydroxy-4,6- dimethyl-3- heptene-2-one	Ae. aegyptii	Ocimum sacnctum	$LD_{100}$ value of 6.25 $\mu\text{g/ml}$	Kelm & Nair (1998) <sup>82</sup>
α-terpinene	Ae. aegypti	Eucalyptus camaldulensis	$LC_{50}$ value of 14.7 $\mu$ g/mL	Jantan et al (2005)83
Geranial	Ae. aegypti	Magnolia salicifolia	LD <sub>100</sub> value of 100 ppm	Kelm et al (1997) <sup>84</sup>
Germacrene D	An. gambiae, Cx. quinquefasciatus and Ae. aegypti	Chloroxylon swietenia	$LD_{50}$ values of 1.8, 2.1 and $2.8{\times}10^{\text{-}3}$	Kiran and Devi (2007) <sup>85</sup>
Hugorosenone	An. gambiae	Hugonia castaneifolia	$LC_{50}$ values of 0.3028 mg/ml	Baraza <i>et al</i> (2008) <sup>86</sup>
Azadirachtin	An. stephensi	Azadirachta indica	EC <sub>50</sub> values of 0.014, 0.021, 0.028 and 0.034 ppm against first, second, third and fourth instar larvae respectively	Senthil Nathan <i>et al</i> (2005) <sup>87</sup>
Dioncophylline-A	An. stephensi	Triphyophyllum peltatum	$LD_{50}$ values of 0.5, 1.0 and 2.0 mg/L concentrations at 3.33, 2.66 and 1.92 h	François <i>et al</i> (1996) <sup>88</sup>
N-methyl-6β-(decal', 3',5'-trienyl)-3-β-methoxy- 2-β-methylpiperidine	Ae. aegypti	Microcos paniculata	LC <sub>50</sub> value of 2.1 ppm	Bandara <i>et al</i> (2000) <sup>89</sup>
Stemocurtisine, stemocurtisinol and oxyprotostemonine	An. minimus	Stemona curtisii	LC <sub>50</sub> values of 18, 39 and 4 ppm, respectively	Mungkornasawakul <i>et a</i> (2009) <sup>90</sup>
Plumbagin	An. gambiae	Plumbago zeylanica	LC <sub>50</sub> value of 1.9 μg/ml	Maniafu et al (2009) <sup>36</sup>
Pachyrrhizine	An. gambiae	Neorautanenia mitis	LC <sub>50</sub> value 0.007 mg/ml	Joseph et al (2004)91
Marmesin	An. gambiae	Aegle marmelos	$LC_{50}$ and $LC_{90}$ values of 0.082 and 0.152 mg/l	Joseph et al (2004) <sup>91</sup>
Neoduline, 4-methoxyneoduline, and nepseudin	An. gambiae	Neorautanenia mitis	$LD_{50}$ values 0.005, 0.011 and 0.003 mg/ml	Breytenbach & Rall (1980) <sup>92</sup>
Methyl-p-hydroxybenzoate	Cx. quinqaefasciatus and Ae. aegypti	Vitex trifolia	$LC_{50}$ values of 5.77 and 4.74 ppm, respectively	Kannathasan <i>et al</i> (2011) <sup>93</sup>
β-sitosterol	Ae. aegypti, An. stephensi and Cx. quinquefasciatus	Abutilon indicum	LC <sub>50</sub> value of 11.49, 3.58 and 26.67 ppm, respectively	Rahuman et al (2008)94
Pipernonaline	Ae. aegypti and Cx. pipiens	Piper longum	LC <sub>50</sub> values of 0.25 and 0.21 mg/l, respectively	Lee (2000) <sup>95</sup>

solvent, combination of solvents or serial extraction by different solvents according to their polarity has also been tried and good larvicidal potentiality found as a result<sup>96</sup>.

# Nature of active ingredients responsible for larval toxicity

The plant world comprises a rich untapped pool of phytochemicals that may be widely used in place of synthetic insecticides in mosquito control programme. Kishore *et al*<sup>97</sup> reviewed the efficacy of phytochemicals against mosquito larvae according to their chemical nature and described the mosquito larvicidal potentiality of several plant derived secondary materials, such as, alkanes, alkenes, alkynes and simple aromatics, lactones, essential oils and fatty acids, terpenes, alkaloids, steroids, isoflavonoids, pterocarpans and lignans. They also documented the isolation of several bioactive toxic principles from various plants and reported their toxicity against different mosquito species (Table II).

# Mode of action of phytochemicals in target insect body

Generally the active toxic ingredients of plant extracts are secondary metabolites that are evolved to protect them from herbivores. The insects feed on these secondary metabolites potentially encountering toxic substances with relatively non-specific effects on a wide range of molecular targets. These targets range from proteins (enzymes, receptors, signaling molecules, ion-channels and structural proteins), nucleic acids, biomembranes, and other cellular components<sup>98</sup>. This in turn, affects insect physiology in many different ways and at various receptor sites, the principal of which is abnormality in the nervous system (such as, in neurotransmitter synthesis, storage, release, binding, and re-uptake, receptor activation and function, enzymes involved in signal transduction pathway)<sup>98</sup>. Rattan<sup>98</sup> reviewed the mechanism of action of plant secondary metabolites on insect body and documented several physiological disruptions, such as inhibition of acetylecholinestrase (by essential oils). GABA-gated chloride channel (by thymol), sodium and potassium ion exchange disruption (by pyrethrin) and inhibition of cellular respiration (by rotenone). Such disruption also includes the blockage of calcium channels (by ryanodine), of nerve cell membrane action (by sabadilla), of octopamine receptors (thymol), hormonal balance disruption, mitotic poisioning (by azadirachtin), disruption of the molecular events of morphogenesis and alteration in the behaviour and memory of cholinergic system (by essential oil), *etc*. Of these, the most important activity is the inhibition of acetylcholinerase activity (AChE) as it is a key enzyme responsible for terminating the nerve impulse transmission through synaptic pathway; AChE has been observed to be organophosphorus and carbamate resistant, and it is well-known that the alteration in AChE is one of the main resistance mechanisms in insect pests<sup>99</sup>.

# Scope for future research: isolation of toxic larvicidal active ingredients

Several studies have documented the efficacy of plant extracts as the reservoier pool of bioactive toxic agents against mosquito larvae. But only a few have been commercially produced and extensively used in vector control programmes. The main reasons behind the failure in laboratory to land movements of bioactive toxic phytochemicals are poor characterization and inefficiency in determining the structure of active toxic ingredients responsible for larvicidal activity. For the production of a green biopesticide, the following steps can be recommended during any research design with phytochemicals: (i) Screening of floral biodiversity in search of crude plant extracts having mosquito larvicidal potentiality; (ii) Preparation of plant solvent extracts starting from non-polar to polar chemicals and determination of the most effective solvent extract; (iii) Evaporation of the liquid solvent to obtain solid residue and determination of the lethal concentration (LC<sub>50</sub>/LC<sub>100</sub> values); (iv) Phytochemical analysis of the solid residue and application of column chromatography and thin layer chromatography to purify and isolate toxic phytochemical with larvicidal potentiality; (v) Determination of the structure of active principle by infra red (IR) spectroscopic, nuclear magnetic resonance (NMR) and gas chromatography and mass spectroscopy (GCMS) analysis; (vi) Study of the effect of active ingredient on non target organisms; and (vii) Field evaluation of the active principle before its recommendation in vector control programme and commercial production.

#### Conclusion

Today, environmental safety is considered to be of paramount importance. An insecticide does not need to cause high mortality on target organisms in order to be acceptable but should be eco-friedly in nature. Phytochemicals may serve as these are relatively safe, inexpensive and readily available

in many parts of the world. Several plants are used in traditional medicines for the mosquito larvicidal activities in many parts of the world. According to Bowers et al<sup>100</sup>, the screening of locally available medicinal plants for mosquito control would generate local employment, reduce dependence on expensive and imported products, and stimulate local efforts to enhance the public health system. The ethno-pharmacological approaches used in the search of new bioactive toxins from plants appear to be predictive compared to the random screening approach. The recently developed new isolation techniques and chemical characterization through different types of spectroscopy and chromatography together with new pharmacological testing have led to an interest in plants as the source of new larvicidal compounds. Synergestic approaches such as application of mosquito predators with botanical blends and microbial pesticides will provide a better effect in reducing the vector population and the magnitude of epidemiology.

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