



LARVICIDAL EFFECT OF SELECTED PLANT EXTRACTS ON *Aedes Aegypti* L. AND *Culex quinquefasciatus* SAY

Anju Viswan, K.¹, E. Pushapalatha¹

¹Biopesticides & Toxicology Laboratory, Department of Zoology, University of Calicut, Malappuram Dist, Kerala, 673635, India.

ABSTRACT

Usage of photochemical may serve as suitable alternative to synthetic insecticides, which causes various environmental consequences and resulted in development of resistance in insects. The larvicidal activity of leaf extracts of *Adhatoda vasica*, *Centella asiatica*, *Holarrhena antidysentrica*, *Hugonia mystax*, *Mentha spicata* on 3rd instar larvae of *Aedes aegypti* L. and *Culex quinquefasciatus* Say were determined in the present study. Methanol leaf extracts were used for the study and after 24 hours mortality were recorded and compared to find out the most active plant extracts. *M.spicata* leaf extract had shown more activity as compared with other plant extracts. The phytochemical screening was also done to find out the active compounds present in the methanolic extracts of *M.spicata*. The findings of the present study suggest that further purification and isolation of active principle could lead development of more potent and ecofriendly biocontrol agents against mosquitoes.

KEYWORDS: Larvicidal activity, *Aedes aegypti*, *Culex quinquefasciatus*, *Adhatoda vasica*, *Centella asiatica*, *Holarrhena antidysentrica*, *Hugonia mystax*, *Mentha spicata*.

INTRODUCTION:

Mosquitoes have a great influence on human well being than any other insects because of their involvement in transmitting dreadful diseases like malaria, filariasis, dengue, chikungunya, Japanese encephalitis, Zika virus, yellow fever etc., besides gorging blood from human beings. The most important thing to reduce the incidence of vector borne diseases is controlling the mosquito population. Vector control is facing a threat due to the emergence of resistance in vector mosquitoes to conventional synthetic insecticides, warranting either adopting counter measures or development of newer insecticides.

Plants acts as alternative agents for control of vectors because they are rich in bioactive chemicals, are active against specific target insects, and are biodegradable. Phytochemical insecticides considered to be more environmentally safer and biodegradable than synthetic insecticides. Numerous plants have shown insecticidal activities and in India nearly 2400 species of plants possess insecticidal properties (Bhaskaran & Narayanaswamy, 1995). The adulticides may only reduce adult population temporarily thus most of the mosquito control programmes target the breeding sites with larvicides (Knio, *et al.*, 2008).

The methanolic fraction of leaves of *Mentha piperita*, *Phyllanthus niruri*, *Leucas aspera* and *Vitex negundo* were against larvae of *Cx. quinquefasciatus* (Pandian *et al.*, 1994). The methanolic extracts of *Solanum surattense*, *Azadirachta indica* and *Hydrocotyle javanica* exhibited larvicidal activity against *Cx. quinquefasciatus* (Muthukrishnan *et al.*, 1997). Muthukrishnan and Pushpalatha (2001) evaluated the larvicidal activity of extracts from *Calophyllum inophyllum* (Clusiaceae), *Rhinacanthus nasutus* (Acanthaceae), *Solanum surattense* (Solanaceae) and *Samadera indica* (Simaroubaceae), *Myriophyllum spicatum* (Haloragaceae) against *Anopheles stephensi*. Jang *et al.*, 2002 evaluated the larvicidal activity of methanol extracts of *Cassia obtusifolia*, *Cassia tora* and *Viciatetra sperma* against early fourth stage larvae of *Ae. aegypti* and *Cx. pipiens*. Anju Viswan and Pushapalatha, 2019 studied on the larval and adulticidal activities of acetone and methanol leaf extracts of *Croton hirtus* L'Her. against *Cx. quinquefasciatus*.

The purpose of the present investigation was to explore the larvicidal activity of methanol solvent extracts from leaves of five plant species, *Adhatoda vasica*, *Centella asiatica*, *Hugonia mystax*, *Justicia gendrussa*, *Mentha spicata* against the dengue vector *Aedes aegypti* and lymphatic filariasis vector *Culex quinquefasciatus*. The plants selected for the present study for screening their larvicidal activities against mosquito larvae have been reported for either insecticidal or medicinal properties. *A.vasica* had shown insecticidal activity against *Spodoptera littoralis* (Sadek, 2003). *A.vasica* also proved its medicinal property and in most of the ayurvedic treatments it has been using from the past several decades (Atul Kumar and Ashoke, 2014). *C.asiatica* leaves were evaluated for their larvicidal and adult emergence inhibition activities against *Cx. quinquefasciatus* and it was also known for its antibacterial activity.

(Rajkumar and Jabanesan, 2004). *H. antidysentrica* is known for its medicinal value and for its antimicrobial and antifungal activities (Raman, *et al.*, 2004). Govindarajan *et al.*, 2017, eco-friendly synthesised silver nanoparticles (AgNPs) with low-cost extract of *H. mystax* and proposed the *H. mystax*-mediated biosynthetic pathway as a future good sustainable route for nano mosquitoicide fabrication. Essential oil extracted from *M.spicata* is reported for their larvicidal activity against *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi* (Govindarajan *et al.*, 2012).

MATERIALS AND METHODS:

Collection and identification of Plants: The leaves of the selected plants were collected from in and around the Calicut University Campus and were identified from the Department of Botany, University of Calicut.

Plants used for the study: *Adhatoda vasica* (Acanthaceae), *Centella asiatica* (Apiaceae), *Holarrhena antidysentrica* (Apocyanaceae), *Hugonia mystax* (Linaceae), *Mentha spicata* (Lamiaceae) were the plants selected for the study.

Preparation of plant extracts: Fresh leaves of selected plants were thoroughly washed with water and shade dried at room temperature. The dried leaves were powdered mechanically, sifted through fine mesh sieve and extracted in the Soxhlet apparatus using analytical grade methanol (MeOH).

Test organisms: The 3rd instar larvae of *Ae.aegypti* and *Cx. quinquefasciatus* belongs to the family Culicidae and order Diptera used for the study were raised from laboratory culture maintained in standard cages at 27±2°C. Eggs laid by the females were collected and hatched larvae were fed with dog biscuit and yeast in the ratio 3:1.

Bioassay: The activity of methanol extracts of the selected plants against the larvae of *Ae.aegypti* and *Cx. quinquefasciatus* were done according to WHO protocol and % mortality were calculated using Abbott's formula and lethal concentration were calculated using the probit analysis developed by Finney, 1971.

Phytochemical analysis: Qualitative analysis was done to identify the presence of different secondary metabolites in the most active plant extract according to the methods proposed by Trease and Evans, 1989.

RESULTS AND DISCUSSION:

Data on 24hr LC₅₀ (ppm) and LC₉₀ (ppm) of methanol extracts of the selected plants on 3rd instar larvae of *Ae.aegypti* and *Cx. quinquefasciatus* is provided in table: 1. A comparison of 24hr LC₅₀ of the different plant extracts points out that *M. spicata* was more toxic than other plant extracts. The most active plant extract in the present study was *M.spicata* with LC₅₀ (ppm) 250.91 and 265.88 and least active plant extract was *A.vasica* with LC₅₀ (ppm) values 436.40 and 484.80 for *Ae. aegypti* and *Cx. quinquefasciatus* respectively.

Table 1: 24hr LC₅₀ (ppm) and LC₉₀ (ppm) of selected plant extracts treated against 3rd instar of *Ae.aegypti* and *Cx. quinquefasciatus*.

Sl. No:	Plant	Mosquito larvae	LC ₅₀ (ppm)	LFL-UFL	LC ₉₀ (ppm)	LFL-UFL
1	<i>Adhatoda vasica</i>	<i>Ae.aegypti</i>	436.40	361.20-503.40	656.50	566.50-942.50
		<i>Cx. quinquefasciatus</i>	484.80	408.24- 565.44	708.88	685.50-730.60

Sl. No:	Plant	Mosquito larvae	LC ₅₀ (ppm)	LFL-UFL	LC ₉₀ (ppm)	LFL-UFL
2	<i>Centella asiatica</i>	<i>Ae. aegypti</i>	295.10	225.30-372.80	488.90	401.80-718.70
		<i>Cx. quinquefasciatus</i>	320.20	288.50-380.80	510.40	480.86-656.50
3	<i>Holarrhena antidysentrica</i>	<i>Ae. aegypti</i>	389.90	286.70- 460.80	605.10	524.40-785.40
		<i>Cx. quinquefasciatus</i>	434.80	389.60- 469.90	552.40	509.10-652.50
4	<i>Hugonia mystax</i>	<i>Ae. aegypti</i>	431.20	365.80- 508.10	625.60	538.70-845.50
		<i>Cx. quinquefasciatus</i>	482.50	416.80-554.20	658.44	578.70-765.51
5	<i>Mentha spicata</i>	<i>Ae. aegypti</i>	250.91	231.72-350.94	455.49	344.27-467.16
		<i>Cx. quinquefasciatus</i>	265.88	225.30-361.20	462.40	401.86-562.50

Phytochemical screening results of the methanol extract of *Mentha spicata*, the most active plant extracts of the present study was given in Table 2. The results indicated the presence of tannin, steroids, flavonoids, terpenoids, phenols and cardiac glycosides while saponins and anthraquinones were not determined in the plant extract.

Table 2: Phytochemical composition of *M. spicata*

Phytochemical Components	Inference
Tannins	+
Terpenoids	+
Flavonoids	+
Cardiac glycosides	+
Phenols	+
Steroids	+
Saponins	-
Anthraquinones	-

+ = presence, - = absence

Ansari & Razdan, 1995 reported that *Mentha* plant spp. oil have shown larvicidal effect on *Culex*, *Aedes* and *Anopheles* mosquitoes. Phytochemical and antimicrobial activities of the leaf oil extract of *M. spicata* and its efficacy in repelling mosquito was reported by Modupe *et al.*, 2017.

The detrimental effects on mosquitoes and their larvae by the plant extracts was mainly due to the phytochemicals present in it (Samidurai *et al.*, 2009). The Phytochemicals present in the plants were grouped into two categories, primary metabolites such as Amino acids, chlorophyll etc. and secondary metabolites which are Alkaloids, flavonoids, Tannins, Saponins etc. (Dharmesh & Natvarlal, 2010). Phytochemicals present in the plant extracts have been found to have insecticidal, antimicrobial, anticonspicative, antispasmodic and antioxidant activities (Edeogo *et al.*, 2005 and Tamilselvan *et al.*, 2015).

The findings of the present study, reveals that *M. spicata* is significantly efficacious as larvicides. Larval mortality increased significantly as the concentration of *M. spicata* extract and exposure period increased. The activity of methanol extracts of *M. spicata* leaves were more on *Ae. aegypti* than with *Cx. quinquefasciatus*. *Ae. aegypti* used to breed in fresh water habitat and that may be the reason for all the plant extracts used for the study shown more the activity on *Ae. aegypti* than *Cx. quinquefasciatus*, the polluted water breeding mosquito.

CONCLUSION:

The present study reveals that there is a scope to use *M. spicata* to control the mosquito larvae of *Ae. aegypti* and *Cx. quinquefasciatus*. It was not having any adverse effect on other insects. Further studies like partial purification, isolation of active compounds are going in our laboratory. So finding out the most active compound from the plant may serve as an alternative to synthetic pesticides

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