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Chemical composition and larvicidal activities of the essential oil of *Zanthoxylum armatum* DC (Rutaceae) against three mosquito vectors

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Abstract

Background & objectives: In view of the recently increased interest in developing plant origin insecticides as an alternative to chemical insecticide, this study was undertaken to assess the larvicidal potential of the essential oil from the seeds of *Zanthoxylum armatum* DC [syn. *Z. alatum* Roxb] (Rutaceae) against three medically important species of mosquito vectors, *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus*.

Methods: Essential oil was hydro distilled in the laboratory from the seeds obtained from the market and the chemical constituents of the oil were determined using GC/GC-MS. Bioefficacy of the essential oil was evaluated under laboratory conditions using III instar mosquito larvae.

Results: Among the three mosquito species tested, *Cx. quinquefasciatus* was the most sensitive (LC_{50} = 49 ppm) followed by *Ae. aegypti* (LC_{50} = 54 ppm) and *An. stephensi* (LC_{50} = 58 ppm). GC-MS analysis of the oil revealed at least 28 compounds, consisting mainly of oxygenated monoterpenes (75%) and monoterpenes (22%). Linalool though constituted a major part (57%), failed to produce any appreciable mortality when tested alone.

Interpretation & conclusion: From the results it can be concluded that the larvae of the three mosquito species were susceptible to the essential oil composition. Such findings would be useful in promoting research aiming at the development of new agent for mosquito control based on bioactive chemical compounds from indigenous plant sources as an alternative to chemical larvicides.

Key words *Aedes aegypti* – *Anopheles stephensi* – *Culex quinquefasciatus* – larvicide – *Zanthoxylum armatum*

Introduction

Mosquitoes are the most important single group of insects well-known for their public health importance, since they act as vector for many tropical and subtropical diseases such as dengue fever, yellow fever, malaria, filariasis and encephalitis of different types including, Japanese encephalitis[1]. *Anopheles stephensi*, *Ae. aegypti and Cx. quinquefasciatus* (Diptera : Culicidae) are the major urban vectors of malaria, dengue and lymphatic filariasis, respectively. The approach to combat these diseases largely relied on interruption of the disease transmission cycle by either targeting the mosquito larvae through spraying of stagnant water breeding sites or by killing the adult mosquitoes using insecticides[2].

Larviciding is a successful way of reducing mosquito

densities in their breeding places before they emerge into adults. Larviciding largely depends on the use of synthetic chemical insecticides—organophosphates (*e.g.* temephos, fenthion), insect growth regulators (*e.g.* diflubenzuron, methoprene), etc. Although effective, their repeated use has disrupted natural biological control systems and sometimes resulting in the widespread development of resistance. These problems have warranted the need for developing alternative strategies using ecofriendly products. Plants offer an alternative source of insect-control agents because they contain a range of bioactive chemicals[3], many of which are selective and have little or no harmful effect on non-target organisms and the environment[3,4]. Much effort has, therefore, been focused on plant extracts or phytochemicals as potential sources of mosquito control agents or as lead compounds[4,5]. In this context, essential oils have received much attention as potentially useful bioactive compounds against insects[6] showing a broad spectrum of activity against insects, low mammalian toxicity and degrading rapidly in the environment. Studies of essential oils obtained from the plants, *Cymbopogan citratus*[5], *Tagetus minuta*[7], *Mentha piperita*[8], *Dalbergia sisoo*[9], *Lippia sidoides*[10], *Hyptis martiusii*[11] and many other plants[6,12–15]; have demonstrated promising larvicidal activities against mosquito vectors.

Zanthoxylum armatum DC [syn. *Z. alatum* Roxb] (Rutaceae) is extensively used in the Indian system of medicine, as carminative, stomachic and anthelmintic. The bark is pungent and stick from the plant is used in preventing toothache. The fruits and seeds are employed as an aromatic tonic in fever, dyspepsia, and expelling roundworms[16]. Mehta *et al*[17] has reported that the essential oil of fruits of *Z. armatum* exhibited good antibacterial, antifungal and anthelmintic activities. Kokate *et al*[18] reported that the petroleum extract of *Z. armatum*, showed significant insecticidal activity against *Culex* spp with LC_{50} value of 20.45 ppm. However, to the best of our knowledge, studies have not been conducted so far to evaluate quantitatively the activity of essential oil obtained from the seeds of this plant against the larvae of *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus*.

In the present paper we report the larvicidal activity of the essential oil extracted from the seeds of *Z. armatum* against three species of mosquito vectors, *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus*. The results of the present study would be useful in promoting research aiming at the development of new agent for mosquito control based on bioactive chemical compounds from indigenous plant source.

Material & Methods

Plant material and extraction of the essential oils: The dried seeds of *Z. armatum* were purchased from the local market in Delhi and identified at National Institute of Science Communication and Information Resource (CSIR), New Delhi, India. Seeds of the plant *Z. armatum* were subjected to hydro distillation for 6 h in a Clevenger-type apparatus. The essential oil obtained in batches was dried over anhydrous sodium sulfate and, after filtration, stored under refrigeration until tested and analysed.

Analysis of the essential oil: Gas chromatography (GC) analysis of the oil was performed on a Shimadzu GC 17A, using a fused silica capillary column (30 m × 0.25 mm i.d.), coated with 5% diphenyl dimethyl siloxane (DB-5), equipped with Flame Ionization Detector. Helium was used as carrier gas at a flow rate of 1.2 ml/min. Oven temperature was programmed from 60 to 200°C at 2°C/min and then held isothermal at 200°C for 20 min; injector temperature, 250°C; detector temperature, 250°C; 0.2 µl of sample injected in a split ration of 50%. Gas chromatography–Mass spectrometry (GC–MS) data were obtained on a Shimadzu QP–500, fitted with the same column and under similar temperature programme as mentioned above for GC analysis.

Identification of compounds: Compounds were iden-

tified by comparing the retention indices of peaks on DB-5 column with literature values[19,20], computer matching against the library spectra (NIST-1, NIST-2, Wiley and Adams Library). The Kokate's retention indices[19] were obtained from gas chromatograms by logarithmic interpolation between bracketing n-alkanes. The homologous series of n-alkanes (C8–C22; Poly Science Inc., Niles, USA) were used as standards.

Mosquito larvae: Larvae of the three mosquito species—*Ae. aegypti*, *Cx. quinquefasciatus* and *An. stephensi* were reared in the mosquito colony maintained at 26 ± 2ºC, 70 ± 10% RH and a photoperiod of 12:12, L:D at the National Institute of Malaria Research, Delhi.

Preliminary screening: Preliminary screening of essential oil or pure linalool (a major constituent in *Z. armatum* essential oil) was done at the two higher dosages (200 and 100 ppm) to check their larvicidal activity. To obtain test dosages of 200 and 100 ppm, individual essential oils or pure compound was dissolved in acetone @ 50 and 25 mg/ml respectively. In either of the case, 1 ml of the concentration was added in 249 ml of distilled water placed in beaker (500 ml) using a pipette with a disposable tips. Each sample was emulsified in distilled water with Triton X-100 added @ 1 ml/l.

Larval bioassis: Batches of 25 late III instar larvae of the desired species were gently placed in the beaker containing each test solution. With each experiment, a set of control sample containing only acetone/Triton X-100 solutions was run for comparison. For each concentration and control, three replicates were used and each test was repeated three times. Treated larvae were held for 24 h at the same conditions used for maintaining the mosquito colony in the laboratory. Mortality was recorded after 24 h of exposure, during which no food was given to the larvae. Larvae were considered dead if appendages did not move when probed with a needle in the siphon or cervical region. Larvae incapable of rising to the surface or not showing the characteristic diving reaction when water was disturbed, were considered moribund and added to the dead larvae for calculating percentage of mortality. Data were adjusted for control mortality using Abbott's formula[21], if mortality in the control sets exceeded 5%.

Dose-response bioassay: Test materials were subjected to dose response bioassay to determine lethal concentration at which larvae showed 50% (LC_{50}) and 95% (LC_{95}) mortality level. Test materials were prepared in acetone in the range of concentration, 50, 37.5, 25, 12.5, 6.25, and 2.5 mg/ml either separately or by serial dilution from the solution of higher concentration. One ml of each concentration was added in 249 ml of distilled water placed in the beaker (500 ml) to obtain test dosage of 200, 150, 100, 50, 25 and 10 ppm, respectively. Each sample was emulsified in distilled water with Triton X-100 added @ 1 ml/l. With each experiment, a set of control sample containing only acetone/Triton X-100 solutions was run for comparison. Each sample was tested three times in three replicates. Temephos, chemical larvicide used commonly for controlling mosquito larvae, was tested at a range of concentration (0.005–0.1 ppm) as positive control.

Data analysis: LC_{50} and LC_{95} values (concentration that caused 50 and 95% larval mortality, respectively) were determined by log-probit regression using SPSS 10.0 for Windows/Microsoft Excel programme.

Results

Hydro distillation of the seeds yielded 1.3 ± 0.2% w/w. GC-MS revealed at least 28 components which could be identified representing 97.6% of the oil (Table 1). The essential oil consisted mainly of oxygenated monoterpenes (75%) and monoterpenes (22%). Among the oxygenated monoterpenes, linalool (57%) was the major component followed by E-carveol

Table 1. Percentage composition of essential oil from the seeds of *Z. armatum*

Compounds	Percentage	RRI Cal	RRI Lit
α-thujone	0.1	927	931
α-pinene	0.1	930	939
Sabinene	0.1	972	976
β pinene	0.1	975	980
myrcene	1.3	987	991
o-cymene	0.2	1012	1022
Limonene	19.8	1020	1031
1,8- cineole	t	1024	1033
α-terpinene	0.4	1045	1062
Z-sabinene hydrate	t	1053	1068
Z- linalool oxide	0.8	1061	1072
E-linalool oxide	1.0	1078	1088
Linalool	57.0	1101	1098
Z-pinene hydrate	0.5	1114	1121
Terpin-4-ol	2.3	1166	1177
α–terpineol	1.1	1180	1189
E-carveol	2.6	1208	1217
Nerol	0.3	1219	1228
Cuminaldehyde	0.3	1226	1239
Carvone	0.4	1232	1242
Piperitone	0.3	1243	1252
Geraniol	0.4	1250	1255
Phellandral	1.3	1280	–
E-methyl cinnamate	5.7	1375	1379
E-nerolidol	0.6	1509	1534
Methyl palmitate	t	1900	1927
Palmitic acid	0.9	2021	–
Oleic acid	t	–	–
Total	97.6		

Compounds are listed in the order of elution on DB-5 column; RRI Cal: Relative retention indices, calculated; RRI Lit: Relative retention indices, reported in literature; and t : Traces.

(2.6%), Terpin-4-ol (2.3%), Phellandral (1.3%), α-terpineol (1.1%) and E-linalool oxide (1%). Other oxygenated monoterpenes were represented in the range 0.1–0.8%. Among the monoterpenes, Limonene (19.8%) was the major component followed by myrcene (1.3%) and other monoterpenes were present in the range 0.1–0.4%. The oil is also characterised by the presence of an aromatic ester, E-methyl cinnamate, in appreciable quantity (5.7%) and sesquiterpene (E-caryophyllene) and oxygenated sesquiterpene (E-nerolidol) accounting ~ 0.6%.

The oil demonstrated promising activities against the larvae of all the tested mosquito species. The regression parameters of probit analysis and LC_{50}, LC_{95} for mortality of the larvae are presented in Table 2. The results showed that among all the three species tested, *Cx. quinquefasciatus* was the most sensitive with LC_{50} and LC_{95} values, that were 49 and 146 ppm, respectively followed by *Ae. aegypti* and *An. stephensi* with LC_{50} values in the range of 54–58 ppm. Linalool alone did not show promising activity in the dose response bioassay against any of the test larvae (mortality >50% was observed only at the highest test dose). Temephos (used as positive control) caused 100% mortality against all the larvae at very low test dose (≥0.625 mg/l).

Discussion

In the present study the chemical composition of the oil is comparable to that of the previous reports[22] with some variation in the constituents. The observed chemical variations in the composition of the essential oil obtained from the same species are not uncommon. This could be due to different chemotypes for the same species[23] or may result from environmental, developmental or other differences[24].

Table 2. Regression parameters of probit analysis for mortality of the larvae of three mosquito species to the essential oil of *Z. armatum*

Mosquito species	LC_{50} (95%, FL)	LC_{95} (95%, FL)	Slope ± SE
Cx. quinquefasciatus	49 (40–59)	146 (112–216)	3.46 ± 0.42
Ae. aegypti	54 (43–65)	171 (130–259)	3.27 ± 0.42
An. stephensi	58 (47–70)	183 (140–278)	3.28 ± 0.42

The study has shown larvicidal potential of essential oils of *Z. armatum* against three mosquito species with varied activities. The larval sensitivity towards the oil was found in the same order (*Cx. quinquefasciatus* >*Ae. aegypti* >*An. stephensi*) as for some other essential oils tested against these mosquito species[8,9]. However, some studies showed that sensitivity of *Culex* spp was lesser towards many essential oils[25,26]. The variations in the toxicity of essential oil against different mosquito species are not uncommon[5], due to qualitative and quantitative variations of constituents like monoterpenes in the essential oil composition. Larvicidal activity of commercially available pure linalool, a major constituent (57%) in the *Z. armatum* essential oil, was also studied to compare its activity with that of the *Z. armatum* oil. Surprisingly this compound when tested alone failed to produce promising activity against any of the mosquito larvae (mortality >50% was observed only at the higher test dosages). Available reports on mosquitocidal activities indicate that linalool, although demonstrated mosquito repellent activity[15,27], its activity against mosquito larvae was reported at comparatively much higher dose when tested individually[14,28]. Chantraine *et al*[29], studied the toxic effect of linalool against *Ae. aegypti* larvae and found that linalool could not cause larval mortality at the test doses ranging between 10 and 100 ppm. In the present study, GC-MS data (Table 1) reveals that besides linalool there are many other oxygenated monoterpenes and related compounds present in the oil. Thus the activity of the oil against the mosquito larvae (LC_{50} = 49 to 58) may be attributed to the additive or synergistic or blend effect of many/some of the constituents. Such an effect has been previously observed with some essential oils where the activity was due to the combination of the major constituents, none of which was found to exhibit significant activity, individually[30,31]. Overall, the bioactivity of the *Z. armatum* oil was comparable with many essential oils reported recently as mosquito larvicide[6,13–15].

Recently, promising larvicidal activities of many essential oils and their compositions[5, 6, 8,11,13–15,28,32] against mosquito vectors have reemphasised the need to explore the possibility of using essential oil-based products as supplementary and complimentary measures for mosquito borne diseases.

The findings of the present studies, therefore, suggest the use of the whole essential oil from the seeds of *Z. armatum* as a local resource in controlling mosquito larvae. The source constraint may not allow their practical utility in larger breeding habitat; however, the plant source may be utilised by local people for controlling mosquito larvae in small breeding places like water coolers, tree holes, abandoned wells, drums and containers in and around the rural/suburban dwellings. Such practice would not only reduce the chemical burden on the environment but also promote sustainable utilisation of locally available bioresource by rural communities. Further studies may be directed towards enhancing the efficacy of such oils with the use of potentiating/synergistic agents, developing suitable formulations and their bioefficacy evaluation in real field conditions.

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