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Full Length Research Paper

Biocidal potential of clove oils against *Aedes albopictus* – A comparative study

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The first phase of the study involved evaluation of leaf and bud oils of clove, *Syzygium aromaticum* for their biocidal (i.e. ovicidal and larvicidal) activity against *Aedes albopictus.* Eggs and fourth instar larvae were exposed to different concentrations of the oils to investigate their comparative efficacy against the target mosquito species. In the second phase of the study, biocidal activity of 'test formulation' derived from the leaf oil and commercial mosquitocide, Prallethrin were estimated by the same method to compare the efficacy of the former with popular mosquitocides. Mortality data were analyzed by a computerized Log-Probit analysis (StatsDirect). Ovicidal assay of the oil samples recorded EC_{50} values of 0.37 and 2.0 mg/ml respectively for leaf and bud oil. Larvicidal assay showed LC_{50} and LC_{95} values of leaf oil as 5.3 and 7.03 mg/ml respectively, while bud oil recorded LC_{50} and LC_{95} values of 17.84 and 23.99 mg/ml, respectively. The 'test formulation' recorded EC_{50} value of 1.63 mg/ml, LC_{50} of 3.67 mg/ml and LC_{95} of 13.49 mg/ml respectively, indicating levels comparable to that of prallethrin. Third phase of the study involved chemical characterization of the oil samples using GC-MS. Eugenol constituted the major component of both the oils. The study demonstrated the potential of clove oil as a biocide against *A. albopictus* and warrants further standardization of the 'test formulation'

Key words: *Aedes albopictus,* biocide, essential oil composition, eugenol, *Syzygium aromaticum,* test formulation.

INTRODUCTION

Mosquitoes represent one of the challenging groups of insects to mankind, owing to their established role as vectors in the transmission of wide range of human diseases such as malaria, lymphatic filariasis and viral diseases (Becker et al., 2003). *Aedes albopictus* (Skuse) also known as "The tiger mosquito" has become a major threat in many parts of the world due to its vector competency (Reiter et al., 2006).This species is an aggressive daytime biter, acting as a vector in transmission of arboviruses causing Dengue, Yellow Fever, several Encephalitis and Chikungunya (Becker et al., 2003; Zhang 1990; Savage et al., 1994; Cancrini et al., 1995; Mitchell et al., 1995; Gerhardt et al., 2001; Turell et al., 2001). Control of vector population attains strategic importance in public health, particularly in the case of communicable diseases against which effective vaccines have not been evolved. Incidentally, dengue and chikungunya belong to this category of diseases. Hence controlling the vectors concerned deserves special consideration for the prevention of these epidemics. Total dependence on synthetic pesticides for vector control is being reviewed due to the increasing tendency towards pesticide resistance by vectors (Turell, 1986) and environmental side effects. Insecticides of plant origin are gaining more popularity in this context, particularly due to their environmental feasibility. Of these, essential oils of various plants have been identified as potential sources of insecticides (Curtis et al., 1989; Sukumar et al., 1991; Tsao et al., 2002) which can be evolved into commercial formulations.

Syzygium aromaticum. (L.) Merr.and Perr., the clove is an evergreen tree, yielding cloves, clove oil and oleoresin as major commercial products. Major clove producing

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countries include Indonesia, Tanzania, Sri Lanka, Madagascar and to a limited extent India. Clove oil is found to have biological activities on wide range of organisms ranging from bacteria to human beings (Cai and Wu, 1996; Kim et al., 2003; Bhattacharjee, 2000; Betty et al., 2001; Han et al., 2004; Park and Shin, 2005; Yuwadee et al., 2005; Yoo et al., 2005; Prashar et al., 2006). Ovicidal and adulticidal effect of clove oils against *Pediculus capitis* has been previously reported (Yang et al., 2003). However, the potential of the clove essential oil as a biocidal agent against mosquitoes appears less explored. In consideration of the above facts, *S. aromaticum* has been chosen for the study involving comparative evaluation of the potential of leaf and bud oils against *A. albopictus* through bioassays for ovicidal and larvicidal activity.

MATERIALS AND METHODS

In the current study oils isolated from leaves and buds of *S. aromaticum* have been selected for investigation on their toxic effects on *Aedes albopictus,* targeting the eggs and the fourth instar larvae. The bioassay has initially been done with the oils and later with a formulation derived from the leaf oil.

Rearing of mosquitoes

An uninfected, wild strain of *A. albopictus* was reared in the laboratory by standard rearing procedures. About 150 larvae were reared in plastic trays until the adults emerged. Adults were maintained in a cage (60 × 30 × 30 cm) at 27 ± 2˚C and 75 – 85% RH. The females were fed with blood every alternate day whereas the males were fed with 10% glucose solution soaked on cotton pad, which were hung in the middle of the cage. A beaker with strips of moistened filter paper was kept for oviposition. The eggs laid on paper strips were dehydrated at room temperature for 15 days. The dehydrated eggs were put into plastic tray containing tap water to hatch and yeast pellets served as food for the emerging larvae.

Essential oils and stocks

The leaf and bud oils of clove were purchased from a commercial supplier 'The Plant lipid Ltd.', Colenchery, Kerala, India. The samples were stored in brown bottles at room temperature. The stock solution (10 mg/ml) of oils and test formulation was always prepared fresh. Measured volume of the stock was added to 100 ml of tap water to get oil concentrations ranging from 1 – 50 mg/ml.

Formulation of clove leaf oil (test formulation)

The leaf oil which showed a promising result in the initial laboratory trial was formulated into water soluble emulsified gel. This formulation contained a stabilizer, gelling agent, Na_2SO_4 and emulsifier, referred as "Test formulation" hereafter, was tested for its Ovicidal and Larvicidal potential under laboratory conditions after two weeks of preparation. The formulation was stored in a cool and dark place till use.

Ovicidal and larvicidal activity of essential oils

The bioassay method by Prajapati et al. (2005) was followed with some modifications for evaluating the ovicidal effect of essential oils. Twenty five dehydrated eggs of *A. albopictus* were exposed to five concentrations of clove leaf and bud oils in vials containing 5.0 ml of test solutions of respective concentrations and allowed to hatch. Trials on each concentration were carried out in triplicate. Tap water with ethanol served as control. Data were analyzed statistically for effective concentration (EC_{50}) inhibiting egg viability, using the Log – Probit Analysis Software, StatsDirect.

The bioassay specified by the WHO (2005) was followed with certain modifications for assaying the larvicidal activity of the oil samples. The fourth instar larvae were used in the bioassay. They were transferred by using a camel hair brush into the test solution. Each test solution comprised thirty larvae in 100 ml of tap water with appropriate amount of the stock solutions to get concentrations ranging from 1 - 50 mg/ml. The experiment was conducted nine times. The concentrations giving the best results were chosen for further trials. Mortality was observed after 24 h interval and recorded. Control larvae were kept in 100 ml tap water with ethanol (5 ml). Lethal concentration (LC_{50} and LC_{95}) was determined by using Log – Probit Analysis Software, StatsDirect.

Ovicidal and larvicidal activity of prallethrin and test formulation

In the second phase of the study, an attempt was made to compare the 'test formulation' with that of a commercial mosquitocide, Prallethrin (liquid formulation). Ovicidal and Larvicidal activity of the above samples were estimated by the same method illustrated for the oils.

Chemical analysis of oils

Chemical composition of clove leaf and bud oils was analysed by GC-MS using Perkin Elmer Clarus Gold 500 apparatus equipped with a capillary column (SGE's BPX-5) of 30 m length and 0.25 mm ID and 0.25 mm film thickness. Oven temperature was programmed at 40 to 270ºC. Helium was used as carrier gas at a flow rate of 1 ml/min. Compounds were identified by GC retention time and mass spectrum using NIST library as reference.

Statistical analysis of data

Concentration – mortality lines and Linear Regression equation were evolved by a computerized Log – Probit analysis (StatsDirect). The 95% confidence intervals (CI) at the lethal concentration (LC) and effective concentration (EC) of 50% and 95% were used to measure differences between the oils.

RESULTS

During the first part of the study, eggs and fourth instar larvae of *A. albopictus* were subjected for concentration-dependent toxicity bioassay of the two oils. Results of the ovicidal assay are illustrated in Table 1. From the data it is evident that the hatchability of the eggs is affected by the presence of the oils. The EC_{50} values for the leaf and bud oils were found to be 0.37 and 2 mg/ml, respectively. Treated eggs failed to hatch even at 48 h of exposure, whereas all eggs in the control and the untreated vials hatched in 24 h. Results of the acute toxicity test of the leaf and bud oils against larvae are presented in Table 2. The LC_{50} and LC_{95} values of leaf oil were found to be 5.3

Table 1. Ovicidal activity of clove oil against *A. albopictus.*

Plant parts	EC_{50} (mg/ml)	Regression equation	95% CL (mg/ml)		Chi^2 test
			LCL	UCL	
Leaf	0.37	Y=0.065+15.818X	0.07	0.76	52.07*
Bud	2.00	Y=0.095+8.362X	0.98	3.45	23.39*

*Significant at P< 0.05 level.
EC, Effective concentration; CL, Confidence limit; LCL, Lower confidence limit; UCL, Upper confidence limit; Sample size n, 25

Table 2 . Larvicidal activity of clove oil against *A. albopictus.*

Plant parts	LC_{50} (LC_{95}) (mg/ml)	Regression equation	95% CL (mg/ml)		Chi^2 test
			LCL	UCL	
Leaf	5.30 (7.03)	Y=0.233+3.915X	5.05 (6.55)	5.56 (7.89)	52.07*
Bud	17.84 (23.99)	Y=0.052+8.829X	16.62 (22.07)	18.96 (35.35)	2.69

*Significant at P< 0.05 level.
EC, Effective concentration; CL, Confidence limit; LCL, Lower confidence limit; UCL, Upper confidence limit; Sample size n, 30.

Table 3. Ovicidal and Larvicidal activity of Parallethrin and Test formulation against *A. albopictus.*

Formulation	EC_{50}/LC_{50} (LC_{95}) (mg/ml)	Regression equation	95% CL (mg/ml)		Chi^2 test
			LCL	UCL	
Prallethrin (O)	0.02	Y=14.665+11.995X	0.01	0.02	1.81
Test formulation (O)	1.63	Y=0.114+8.365X	0.41	5.77	20.67*
Prallethrin (L)	0.03 (0.12)	Y=6.505+16.297X	0.03(0.09)	0.04(0.21)	4.97
Test formulation (L)	3.67 (13.49)	Y=0.241+0.743X	1.96(4.02)	6.77(59.54)	8.64*

*Significant at P< 0.05 level.
CL, Confidence limit; (O), Ovicidal activity; (L), Larvicidal activity; EC, Effective concentration; LCL, Lower confidence limit; UCL, Upper confidence limit; Sample size n, 25.

and 7.03 mg/ml, respectively. Bud oil was proved to be less toxic than leaf oil with LC_{50} and LC_{95} values of 17.84 and 23.99 mg/ml, respectively. Symptoms of toxicity on larvae were manifested by a coiling movement of the individuals along with tremor and convulsion, as an instant reaction to exposure to the concentration of LC_{95} of each oil. Twenty minutes after exposure to this concentration, all the larvae were found killed and sank to the bottom of the beaker. From the results of toxicity assays, it is evident that the leaf oil is more potent than the bud oil with reference to the biocidal parameters studied, namely ovicidal and larvicidal effects.

In the second part of the study eggs and fourth instar larvae were subjected to toxicity bioassay of commercial samples of synthetic mosquitocide Prallethrin and 'Test formulation' derived from the leaf oil (Table 3). From the results it is evident that the 'test formulation' from *S. aromaticum* leaf is effective as an ovicide (EC_{50} = 1.63 mg/ml) and larvicide (LC_{50} = 3.67 mg/ml, LC_{95} = 13.49 mg/ml) at levels comparable to that of Prallethrin, indicating the biocidal potential of the former against the target mosquito species.

Results of the chemical elucidation of clove leaf and bud oils of *S. aromaticum* have been illustrated in Table 4. Leaf oil has yielded a total of 20 compounds, eugenol representing the major component accounting for 62.85% and Caryophyllene constituted the second largest component accounting for 14.12%. All the remaining compounds contributed for less than 6%. Bud oil recorded a total of 15 compounds with eugenol forming the major component accounting for 40.64% and Palatinol A representing the second largest component forming 38.24% of the oil content. The later was however not found in the leaf oil. Caryophyllene constituted the third abundant compound in bud oil with a concentration of 10.4%.

DISCUSSION

Potential of plant essential oils as source insecticides has been worked out and reported with reference to various pests (Curtis et al., 1989; Sukumar et al., 1991; Tsao et

Table 4. Composition of leaf and bud oils of *S. aromaticum.*

S/N	CAS. No	Compounds present	% in Leaf oil	% in Bud oil
1	3856-25-5	Copaene	0.108	0.3
2	97-53-0	Eugenol	62.849	40.64
3	87-44-5	β-Caryophyllene	14.196	10.4
4	93-16-3	Isohomogenol	0.078	nil
5	5951-61-1	Cadinene	0.085	nil
6	97-54-1	Isoeugenol	0.398	nil
7	22567-17-5	δ-Gurjunene	0.151	nil
8	473-13-2	α-Selinene	0.271	nil
9	483-77-2	Calamenene	0.195	0.092
10	16728-99-7	Linolenin	0.067	0.05
11	1139-30-6	Caryophyllene oxide	5.266	0.993
12	84-66-2	Neatine	1.157	nil
13	2778-68-9	Carane	0.597	nil
14	88-84-6	Guaiene	nil	0.06
15	6785-38-2	α-Muurolene	nil	0.05
16	19784-98-6	propenyl guaiacol	nil	0.105
17	5986-49-2	Palustrol	nil	0.102
18	84-69-5	Palatinol A	nil	38.24
19	639-15-6	Acovenosigenin	nil	0.145
20	32509-55-0	Decyl stearate	nil	0.02
21	6753-98-6	α-Humulene	2.952	1.904
22	2306-78-7	Nerolidol	0.087	nil
23	21284-22-0	Cubenol	0.374	nil
24	19888-34-7	Pinane	0.541	nil
25	109119-91-7	Aromadendrene	0.274	nil
26	502-61-4	α-Farnesene	0.27	0.211
27	51371-47-2	Globulol	0.241	nil
Total %			90.16%	93.31%

al., 2002). During recent times reports on their efficacy against mites has also been confirmed from different parts of the world (Kim et al., 2003; Steven and Brentwood, 2004; Srivastava et al., 2005; Bhat and Kempraj, 2008). Therefore it may be feasible to view these oils as possible source for broad spectrum pesticides against arthropods. While discussing the toxic effect of clove oils on *P. capitis,* Yang et al. (2003) have indicated the safety of these oils on non target organisms including man. Therefore, formulations derived from the oil would be more feasible from environmental perspective than synthetic mosquitocides. This advantage of the 'test formulation' evolved during the current study would outweigh its comparatively lower efficacy with reference to Prallethrin. Chemical elucidation of clove oils during the current study has indicated eugenol and β-carryophyllene as the dominant constituents of both the oils. This result is in consensus with earlier reports on chemical composition of these oils from different parts of the world (Zhu et al., 1995; Raina et al., 2001; Ghorab and Massry, 2003; Park and Shin, 2005; Leopold et al., 2006). Mosquitocidal efficacy of eugenol and β-carryophyllene analyzed in comparison with bark and leaf oils of *Cinnamomum zeylanicum* against adult mosquitoes of *Anopheles tessellates*, *Culex quinquefasciatus* and *Aedes aegypti* has been reported from Sri Lanka (Samarasekera and Kalhari, 2005). In the light of this finding, the biocidal activities, i. e. ovicidal and larvicidal effects of clove oils against *A. albopictus* observed during the current investigation can be attributed to eugenol and β- carryophyllene contained in these oils. Furthermore the higher level of biocidal activity observed with reference to clove leaf oil must be due to the higher concentration of the above two compounds in the leaf oil when compared to that of the bud. The current study has ascertained the potential of clove oil as a source of mosquitocidal product. However, more trials on 'test formulation' prepared using eugenol/ β- carryophyllene are warranted for further standardization/optimization of the current technology for practical utility.

Conclusion

The present study has confirmed the biocidal potential of

S. aromaticum leaf oil against *A. albopictus*. Environmental feasibility and plentiful availability of the oil at reasonable market price provides an extra edge for promotion of the test formulation into a commercial product. However, further research work for optimization of the formulation for field condition and large scale application is required to upgrade the product for commercial exploitation.

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