

Toxicological effects of *Sphaeranthus indicus* Linn. (Asteraceae) leaf essential oil against human disease vectors, *Culex quinquefasciatus* Say and *Aedes aegypti* Linn., and impacts on a beneficial mosquito predator

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Abstract Use of environmentally friendly, decomposable natural products for effective vector control has gained considerable momentum in modern society. In this study, essential oil of *Sphaeranthus indicus* (Si-EO) was extracted and further phytochemical screening revealed fourteen compounds with prominent peak area percentage of 24.9 and 22.54% in 3,5-di-tert-butyl-4-hydroxybenzaldehyde and benzene,2-(1,1-dimethylethyl)-1,4-dimethoxy, respectively. The Si-EO was further evaluated for their larvicidal response against *Culex quinquefasciatus* and *Aedes aegypti* at different dosages (62.5, 125, 250 and 500 ppm). The Si-EO displayed prominent larvicidal activity at higher concentration (500 ppm) against both species of mosquitoes. The LC₅₀ and LC₉₀ values of oils were observed at 130 and 350 ppm against *C. quinquefasciatus* larvae and at 140 and 350 ppm against *A. aegypti* larvae, respectively. Repellent bioassay established higher protection rate at 200 ppm up to 120 min against both the mosquitoes. However,

adulticidal response displayed higher mortality rate only at 700 and 800 ppm against *C. quinquefasciatus* and *A. aegypti*, respectively. Toxicological screening against mosquito predator *Toxorhynchites splendens* revealed that the Si-EO was harmless even at the concentration of 1500 ppm. Overall, these results suggest that the Si-EO plays a significant role as a new bio-rational product against ecological burden mosquito vectors which provides an eco-friendly alternative to synthetic pesticides.

Keywords Phytochemicals · Toxicity · Repellent · Adulticidal · Non-toxic · Eco-friendly

Introduction

Insect-transmitted illness plays a chief source of disease, and principally, mosquito-borne disease alone spreads over 2.5 billion population throughout the world (World Health Organization 2014). Mosquitoes play a significant role in transmitting several communicable diseases such as dengue, filariasis, malaria and yellow fever that are dangerous to humans and other organisms (Thanigaivel et al. 2012). *Aedes aegypti* Linn. (Diptera: Culicidae) is a most important vector in India and some of the West Asian countries which spreads deadly pathogens that cause diseases like dengue (Hemingway and Ranson 2000; Kalaivani et al. 2012; Thanigaivel et al. 2012; Benelli 2015a, b). *Culex quinquefasciatus* (Diptera: Culicidae) is a house-resting mosquito (i.e., endophilic mosquito that rests inside a human dwelling) principal in several tropical nations. Lymphatic filariasis is possibly the most rapidly spreading vector-borne disease of humans in the tropical region, infecting

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more than 146 million population due to transmission by the mosquito vector *C. quinquefasciatus* (World Health Organization 1992).

There is an urgent need for control of mosquito vectors to check transmission of pathogenic disease-causing agents, and this has been the principal topic of various novel researches over the past decades. The methods used in different management programmes usually depend on the different lifecycle phases (egg, larvae, pupae, or adult) that are most vulnerable in the habitat where control is needed (Gutierrez et al. 2014). Currently, several commercially formulated biological and chemical repellents are available in the market. The insects in general and mosquito species may have resistant individuals which can be selected by use of chemicals (World Health Organization 1992; Ranson et al. 2011). These issues have created a hunt for eco-friendly insecticides for the mosquito vectors (Murugan et al. 2007).

Nature provides several bioactive products against vector mosquitoes in the form of plant products, marine products, microbial products, and other biological derivatives. Use of natural products of plant origin is recommended because they are effective, biodegradable, environmentally friendly, inexpensive, readily available and popular and generally have a low mammalian toxicity (Senthil-Nathan et al. 2006a, b; Govindarajan 2011; Gahukar 2012; Senthil-Nathan 2013, 2015; Selin-Rani et al. 2016a, b). Plant-derived essential oils, after millions of years of plant evolution, have become a natural source of insecticides, pesticides, larvicides, fragrant odorants, and repellents (Oyedele et al. 2002; Rajkumar and Jebanesan 2010). Adverse effects of commercially available repellents can be reduced by incorporating components of plant-derived essential oils (EOs) into pest-management treatments (Senthil-Nathan 2007).

The herb *Sphaeranthus indicus* Linn. that belongs to the family Asteraceae has been used in certain regions in India for diverse systems of traditional medicines for human disease treatment and ailments of human beings such as asthma, bronchitis, rheumatism, and dermatitis and extensively studied for its various biological activities (Nemade et al. 2012). *S. indicus* plant is distributed all over the plains and wetlands in India and other sub-tropical nations (Gogate 2000). Several reports are available for isolation of medically important essential oil from aromatic herbs and spices by steam distillation for numerous studies (Kumar et al. 2014). Due to their potential biological activities, they were given keen interest by investigators worldwide (Mahajan et al. 2015). Mosquito larvicidal activity of various solvent extracts of *S. indicus* (Si-EO) has been reported (Kovendan et al. 2012); however, to our knowledge, there is lack of research on the larvicidal, repellent and adulticidal activities of Si-EO. Thus, the objectives of the present investigation comprise the following: (a) isolation and characterization of major chemical compounds from essential oils derived from fresh leaves of *S. indicus* (Si-EO); (b)

toxicological screening of Si-EO against two medically important mosquitoes *C. quinquefasciatus* and *A. aegypti*; and (c) evaluation of non-target impacts of Si-EO against the natural mosquito predator *Toxorhynchites splendens* (Wiedemann) compared with commercial pesticides. This may provide a novel and promising vector control agent that can be used in the development of cost-effective and practical means to protect humans from the environmental burden mosquitoes.

Materials and methods

Plant harvesting and essential oil extraction

Leaves of *S. indicus* Linn. (Fig. 1a) were freshly collected from Virudhunagar district (11°00 and 12°00 N; 77°28 and 78°50 E) which is situated in the south-western part of Tamil Nadu, India. The collected plants were washed and air-dried between filter paper sheets. The leaves were cut into pieces, and steam distillation extraction was performed using a Clevenger apparatus, as described by Craveiro et al. (1976). The Si-EO was extracted and collected in sanitized glass vials. Anhydrous sodium sulphate was used to remove water traces, and the Si-EO samples were preserved at 4 °C for further experiments.

GC-MS analysis

The isolated Si-EO samples were dissolved in (1:1) ratio with ethyl alcohol. From this, 2 µl of crude and fractions were dissolved in HPLC-grade methanol and subjected to GC and MS JEOL GC mate equipped with secondary electron multiplier. Further, procedure of chemical characterization was carried out by our previous methodology (Thanigaivel et al. 2017). The molecular weight, molecular formula and structure of the compounds of test materials were ascertained by interpretation on mass spectrum of GC-MS using the database of the National Institute Standard and Technology (NIST).

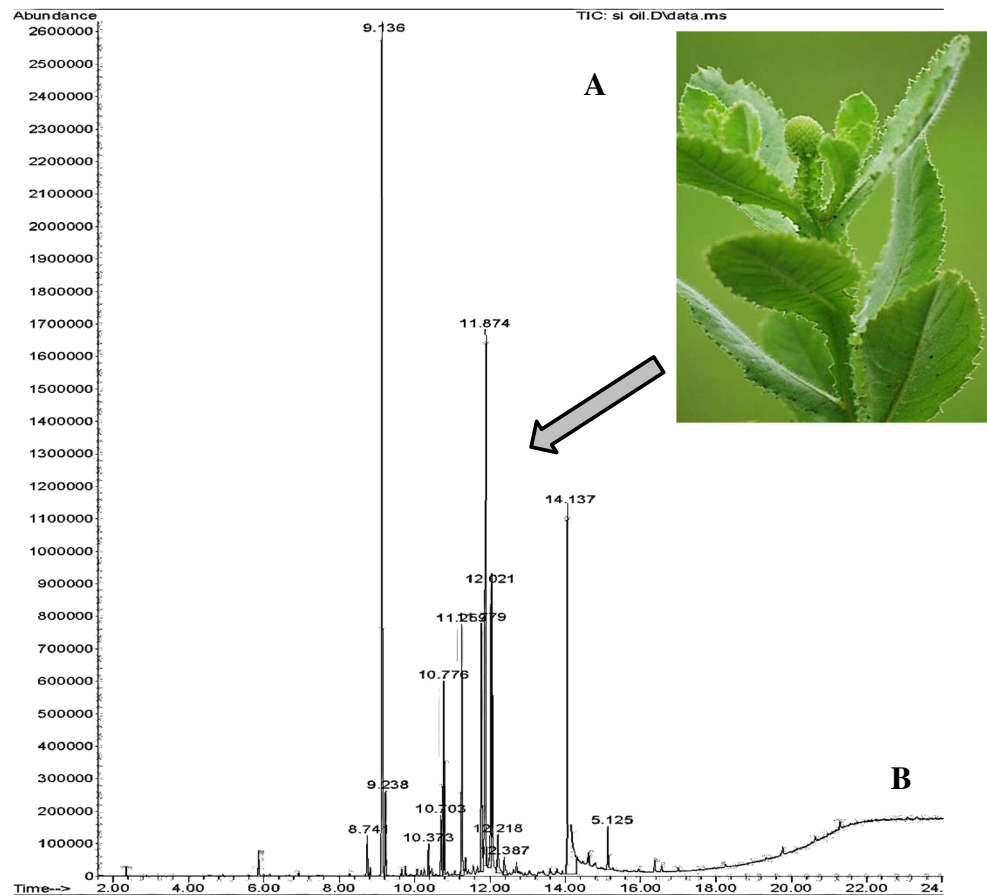
Mosquito rearing

The *A. aegypti* and *C. quinquefasciatus* culture has been maintained at the Biopesticides and Environmental Toxicology Laboratory (BET Lab) with our previous methodology (Kalaivani et al. 2012; Thanigaivel et al. 2012).

Larvicidal bioassay

Larvicidal activity was evaluated by following the methods of the World Health Organization (1981) with slight modifications (Thanigaivel et al. 2017). Twenty early fourth instars of *C. quinquefasciatus* and *A. aegypti* were introduced into a 250-ml glass beaker containing four different dilutions (62.5,

Fig. 1 External plant morphology of *Sphaeranthus indicus* (a). GC-MS chromatogram of Si-EO (b)



125, 250 and 500 ppm) of Si-EO. Control was maintained separately treated with acetone for EO. Throughout this experiment, no food was provided to the larvae and the rate of mortality was recorded after 24 h. The treatments were replicated five times, and each replicated set contained one control. The follow-up assays were carried out according to Finney's (1971) probit method. Percentage mortality (Eq. (1)) in the treatments was corrected when necessary for mortality in the controls using Abbott's (1925) equation (Eq. (2)).

Percentage of mortality

$$= \frac{\text{Number of dead larvae}}{\text{Number of larvae introduced}} \times 100 \quad (1)$$

Correct percentage of mortality

$$= \left(1 - \frac{n \text{ in } T \text{ after treatment}}{n \text{ in } C \text{ after treatment}} \right) \times 100 \quad (2)$$

where T is the test treatment and C is the control treatment in the treated group.

Repellent assay

The Si-EO were tested for its repellent activity against *C. quinquefasciatus* and *A. aegypti* mosquitoes at

different concentrations of 75, 100, 150 and 200 ppm carried out using method cited in the World Health Organization (2009). One hundred gravid female mosquitoes (mated, 5–7 days post emergence) starved for 24 h without a blood meal but previously fed on 10% sucrose solution were kept in net cages (45 cm × 35 cm × 45 cm). An area of 3 × 10 cm on each forearm of three human volunteers was marked out with a permanent marker and the remaining area was covered with a paper sleeve with a hole corresponding to the marked area. Further, on the day of the assay, the volunteers had no contact with lotions and perfumed soaps. As a blank control, ethanol was placed on one forearm of the same volunteer with the same process as the test repellents, whereas the other forearm was untreated. *A. aegypti* was tested from 06.00 h to 14.00 h. Whereas, *C. quinquefasciatus* was treated from 18.00 h to 02.00 h. The control and treated arms were introduced simultaneously into the mosquito cage, and by gently tapping the sides of the experimental cages, the mosquitoes were activated. Each test concentration was repeated three times. The volunteers conducted their test of each concentration by inserting the treated and control arms into the cages at the same time for one full minute every 5 min. The mosquitoes that land on the hand were recorded and then shaken off before they

Table 1 Chemical composition of Si-EO

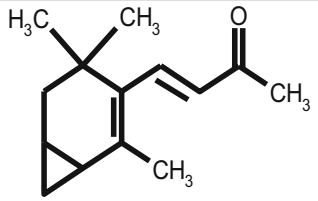
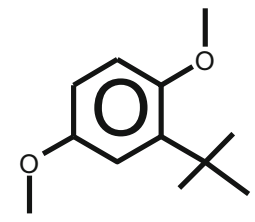
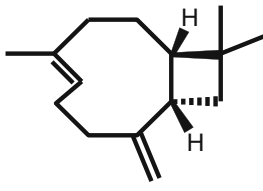
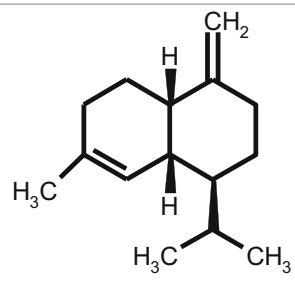
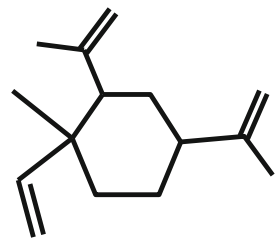
Peak no.	RT time (min)	Peak area	Peak area (%)	Compound name	Quality	Structure
1	8.741	186451	1.07	4-(2',4',4'-Trimethyl-bicyclo[4.1.0]hept-2'-en-3'-yl)-3-buten-2-one	93	
2	9.136	393764	22.54	Benzene, 2-(1,1-dimethylethyl)-1,4-dimethoxy	90	
3	9.238	398175	2.28	Caryophyllene	99	
4	10.373	139727	0.80	.γ.-Muuroleone	96	
5	10.703	274667	1.57	Cyclohexane, 1-methyl-2,4-bis(1-methylethenyl)-, (1.alpha.,2.beta.,4.beta.)-	52	

Table 1 (continued)

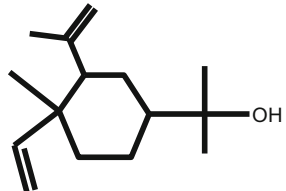
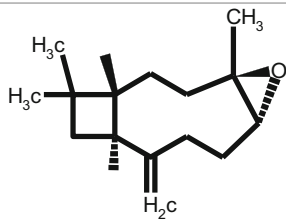
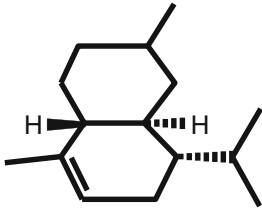
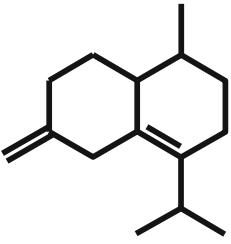
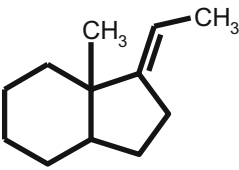
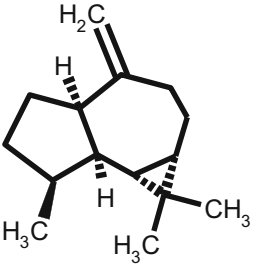
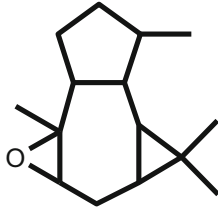
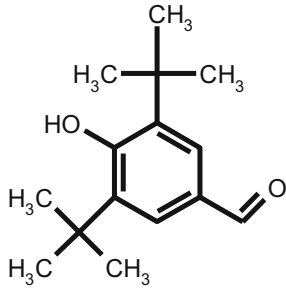
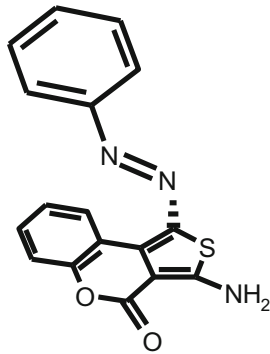
6	10.77 6	851121	4.87	Cyclohexanemethanol, 4-ethenyl-.alpha.,.alpha.,4-trimethyl-3-(1-methylethenyl)-, [1R-(1.alpha.,3.alpha.,4.be	87	
7	11.25 9	116183 4	6.65	Caryophyllene oxide	92	
8	11.77 9	137442 1	7.87	Naphthalene, 1,2,4a,5,8,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1.alpha.,4a.beta.,8a.alpha.)-(./-)-	96	
9	11.87 4	260215 2	14.8 9	Bicyclo[4.4.0]dec-1-ene, 2-isopropyl-5-methyl-9-methylene-	94	
10	12.02 1	149317 9	8.55	1H-Indene, 1-ethylideneoctahydro-7a-methyl-, cis-	93	
11	12.21 8	309780	1.77	Alloaromadendrene	89	

Table 1 (continued)

12	12.38 7	136992	0.78	Isoaromadendrene epoxide	72	
13	14.13 7	435757 0	24.9 4	3,5-Di-tert-butyl-4- hydroxybenzaldehyde	58	
14	15.12 5	246577	1.41	4H-Thieno[3,4- c]benzopyran-4-one, 3- amino-	53	

imbibe any blood. The repellency was calculated by the following Eq. (3):

$$\text{Repellency (\%)} = [(Ta - Tb) / Ta] \times 100 \quad (3)$$

where *Ta* is the number of mosquitoes in the control group and *Tb* is the number of mosquitoes in the treated group.

Adulticidal activity

Sugar-fed adult female mosquitoes (4 to 7 days old) were used for adult toxicity studies. The mosquitoes were treated with impregnated filter paper (140 × 120 mm) diluted with different concentrations of Si-EO (400, 500, 600, 700 and 800 ppm). A blank paper consisting of only ethanol was used as control. The papers were left to dry at room temperature to evaporate off the ethanol overnight. Impregnated papers were prepared fresh prior to testing. The bioassay was conducted in an experimental kit consisting of two cylindrical plastic tubes both measuring

120 × 45 mm following with our previous procedure (Thanigaivel et al. 2017). One tube served to expose the mosquitoes to the seed extracts, and another tube was used to hold the mosquitoes before and after the exposure periods. The impregnated papers were rolled and placed in the exposure tube. Each tube was closed at one end with a 15-mesh size wire screen. Sucrose-fed and blood-starved mosquitoes (25) were released into the tube, and the mortality effects of the extracts were observed every 10 min for 3-h exposure.

Effects on non-target organisms

The toxic effect of Si-EO against beneficial aquatic predator was performed with adapted methodology of Sivagnaname and Kalyanasundaram (2004) with slight modifications (Thanigaivel et al. 2017). *T. splendens* were collected in the field at the same aquatic habitat of *A. aegypti* and maintained separately in containers containing water (45 cm in diameter

and 15 cm in depth) maintained with 26 ± 2 °C and 85% RH (relative humidity). The non-target *T. splendens* were tested against the EO in comparison with the commercially used pesticides Temephos (Sigma-Aldrich, PESTANAL®, analytical standard). The different concentrations of Si-EO (900, 1000 and 1500 ppm) and Temephos (0.2, 0.4 and 0.5 ppm) were tested for its non-target effect. For each tested concentration, ten replicates were performed along with the five replicates of the untreated controls. Mortality rate was recorded after 24 h.

Data analysis

Mortality data from the treatments were exposed to variance analysis (ANOVA of arcsine-, logarithmic- and square root-transformed percentages), and mean of five replicates was expressed in the data. Tukey's multiple-range test was used to determine the significant differences between the different treatments (significance at $P < 0.05$) using Minitab®17. Further, MicroCal software (SigmaPlot 12) was used to determine the enzyme activity. The lethal concentrations required to kill 50% (LC_{50}) of the larvae in 24 h were calculated by probit analysis with a dependability interval of 95% using the Minitab®17 programme.

Results

The yield percentage of the Si-EO samples was 1.5 ml/kg of leaves. The GC-MS chromatogram of the Si-EO was displayed in Fig. 1b. The essential oil volatile compounds, expressed as percentage from the total area, were shown in Table 1. It can be seen that fourteen volatile compounds up to 99.1% total area were recorded. The peak area was prominent for 3,5-di-tert-butyl-4-hydroxybenzaldehyde and benzene,2-(1,1-dimethylethyl)-1,4-dimethoxy with 24.94 and 22.54%, respectively.

The Si-EO showed prominent larval mortality with 96.80 and 96.31% at the highest concentration (500 ppm) against *C. quinquefasciatus* and *A. aegypti*, respectively. Dose-dependent mortality was observed in both the vectors (Fig. 2). The prominent mortality at 500 ppm concentration ($F_{3,16} = 80.87$, $P \leq 0.0001$) was different significantly from the 250 ($F_{3,16} = 80.87$, $P \leq 0.035$), 125 ($F_{3,16} = 80.87$, $P \leq 0.0001$), and 62.5 ppm treatments ($F_{3,16} = 80.87$, $P \leq 0.0001$) and control ($F_{3,16} = 80.87$, $P \leq 0.0001$) with 82.00, 43.40, 21.00 and 5.00% mortality rate, respectively, against *C. quinquefasciatus*. Correspondingly, the mortality rate for the fourth instar larvae of *A. aegypti* at 500 ppm ($F_{3,16} = 68.19$, $P \leq 0.0001$) was significantly different from the 250 ($F_{3,16} = 68.19$, $P \leq 0.0049$), 125 ($F_{3,16} = 68.19$, $P \leq 0.0001$), and 62.5 ppm treatments ($F_{3,16} = 68.19$, $P \leq 0.0001$) and control ($F_{3,16} = 68.19$, $P \leq 0.0001$) with 80.42, 46.73, 25.31 and 5.32% mortality rate, respectively. The lethal concentrations (LC_{50} value) were recorded at 130 and 140 ppm against fourth instar larvae of

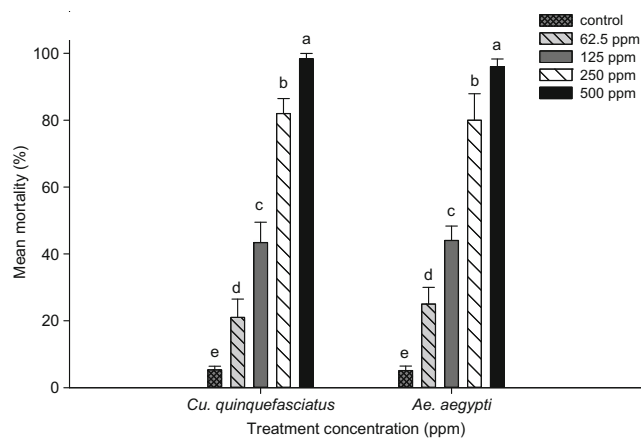


Fig. 2 Percentage mortality of fourth instar larvae of *C. quinquefasciatus* and *A. aegypti* treated with Si-EO. Means (\pm (SE) standard error) followed by the same letters above bars indicate no significant difference ($P \leq 0.05$) in a Tukey's test

C. quinquefasciatus (Fig. 3a) and *A. aegypti* (Fig. 3b), respectively.

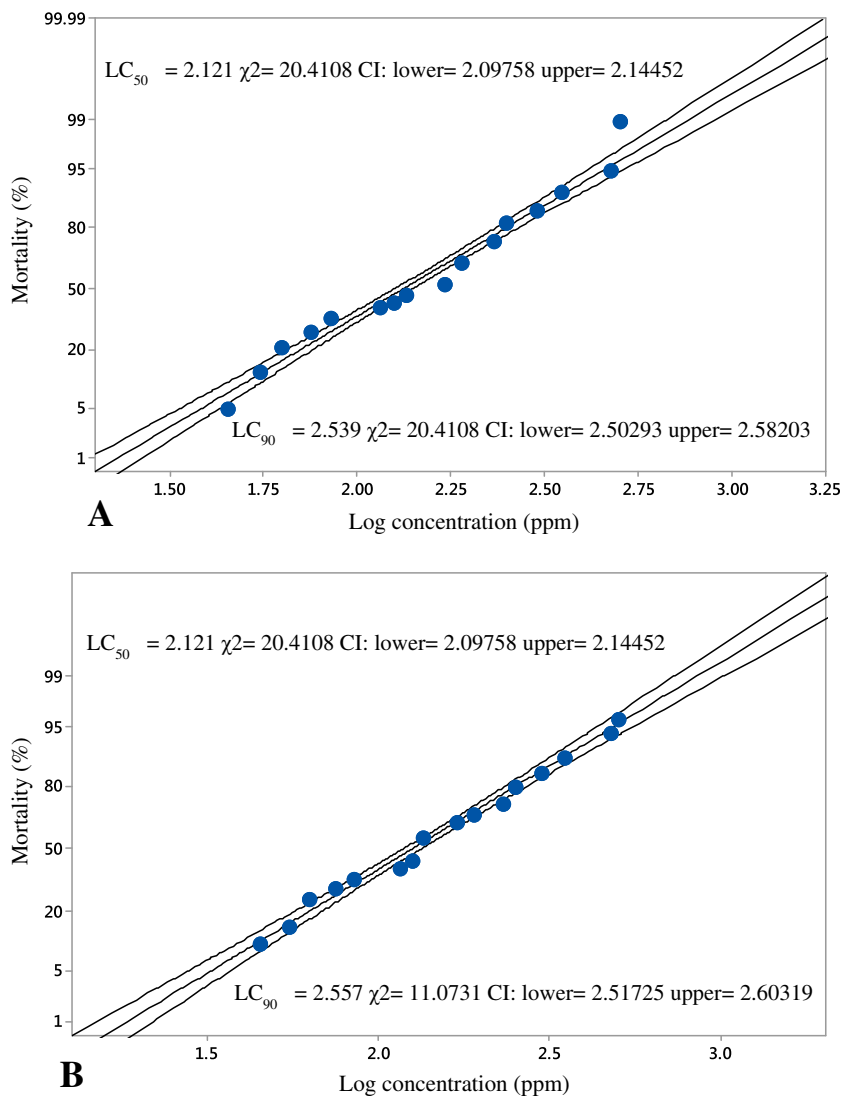
The Si-EO gave a significant protection time against *C. quinquefasciatus* and *A. aegypti* at different dosages (Fig. 4a). Among the different concentrations tested, the prominent repellent activity was observed at 200 ppm with protection up to 210 min for both the mosquitoes. The higher concentration of 200 ppm ($F_{4,20} = 14.11$, $P < 0.004$) displayed significant difference in the repellent activity to 150 ($F_{4,20} = 17.23$, $P < 0.001$), 100 ($F_{4,20} = 21.31$, $P < 0.002$) and 75 ppm ($F_{4,20} = 14.11$, $P < 0.004$) against *C. quinquefasciatus*. Likewise, repellent activity of 200 ppm ($F_{4,20} = 15.55$, $P < 0.004$) was found to be statistically different against other treatment concentrations of Si-EO against *A. aegypti* (Fig. 4b). Our results indicate that protection time was dose-dependent in both the vectors. The major phytoconstituents of the Si-EO showed complete protection against both the vectors. The Si-EO displayed 100% adulticidal mortality rate at 700 and 800 ppm against *C. quinquefasciatus* (Fig. 5a) and *A. aegypti* (Fig. 5b), respectively, within 30 min. However, other treatments did not produce $>90\%$ rates of mortality even at 90 min.

The non-target effects of the Si-EO on non-target *T. splendens* were low even at concentrations of 1500 ppm with mortality rate of 15.4% ($F_{3,16} = 23.37$, $P \leq 0.001$). However, Temephos at 0.5 ppm showed 97.34% mortality rate against *T. splendens* (Fig. 6). Besides, the development and behaviour of the mosquito predator were altered significantly after treatment with Temephos.

Discussion

Plant products have been used worldwide as vector control agents due to the diversity of biologically active compounds

Fig. 3 Median lethal concentrations (LC₅₀ and LC₉₀) of Si-EO against *C. quinquefasciatus* (a) and *A. aegypti* (b) fourth instars by using probit analysis



and reduced human toxicity compared to many synthetics (Murugan et al. 2007; Senthil-Nathan et al. 2007; Lija-Escaline et al. 2015). Screening of plants which were locally available for its mosquitocidal properties would generate eco-friendly and cost-effective means and stimulate natural efforts to improve public health against the disease-spreading vectors (Senthil-Nathan et al. 2008a, b; Benelli and Mehlhorn 2016).

Large number of plant-derived essential oils (EOs) have been reported as promising larvicidal agents against diverse mosquito vectors (Kamsuk et al. 2006; Senthil-Nathan 2007; Kalaivani et al. 2012; Benelli 2015a, b). Besides, phytochemicals derived from the plant source also displayed significant toxic responses against different mosquito vectors (Nemade et al. 2012; Pradeepa et al. 2014, 2015, 2016; Edwin et al. 2016; Govindarajan and Benelli 2016). In support to the above statement, phytochemicals derived from the Si-EO displayed promising mosquitocidal response against both the vectors. Chemical screening revealed that the peak area was prominent for 3,5-di-tert-butyl-4-hydroxybenzaldehyde and

benzene,2-(1,1-dimethylethyl)-1,4-dimethoxy. Kaila et al. (2007) evaluated that 3,5-di-tert-butyl-4-hydroxybenzaldehyde showed an anti-inflammatory action and could exert actions of multiple benefits in atherosclerosis. Similarly, bicyclo[4.4.0]dec-1-ene (8.2%) compound is listed as having an antimicrobial activity (Naidoo et al. 2014). Further, previous research also proved caryophyllene as potent anti-inflammatory (Fernandes et al. 2007; Chavan et al. 2010), anti-cancer (Legault and Pichette 2007) and anti-fungal agent (Yang et al. 2000).

Controlling of vectors at the larval stage is more preferable compared to other life stages which reduces pesticide usage (Tennyson et al. 2012). The hexane fractions of *S. indicus* showed 100% mortality against three mosquito vectors at 10 mg/l (Vidhya and Mathew 2014). Similar to the above findings, 500 ppm concentration of Si-EO delivers approximately 97 and 95% larval mortality against *C. quinquefasciatus* and *A. aegypti* mosquitoes, respectively. It is generally accepted that botanical extracts with

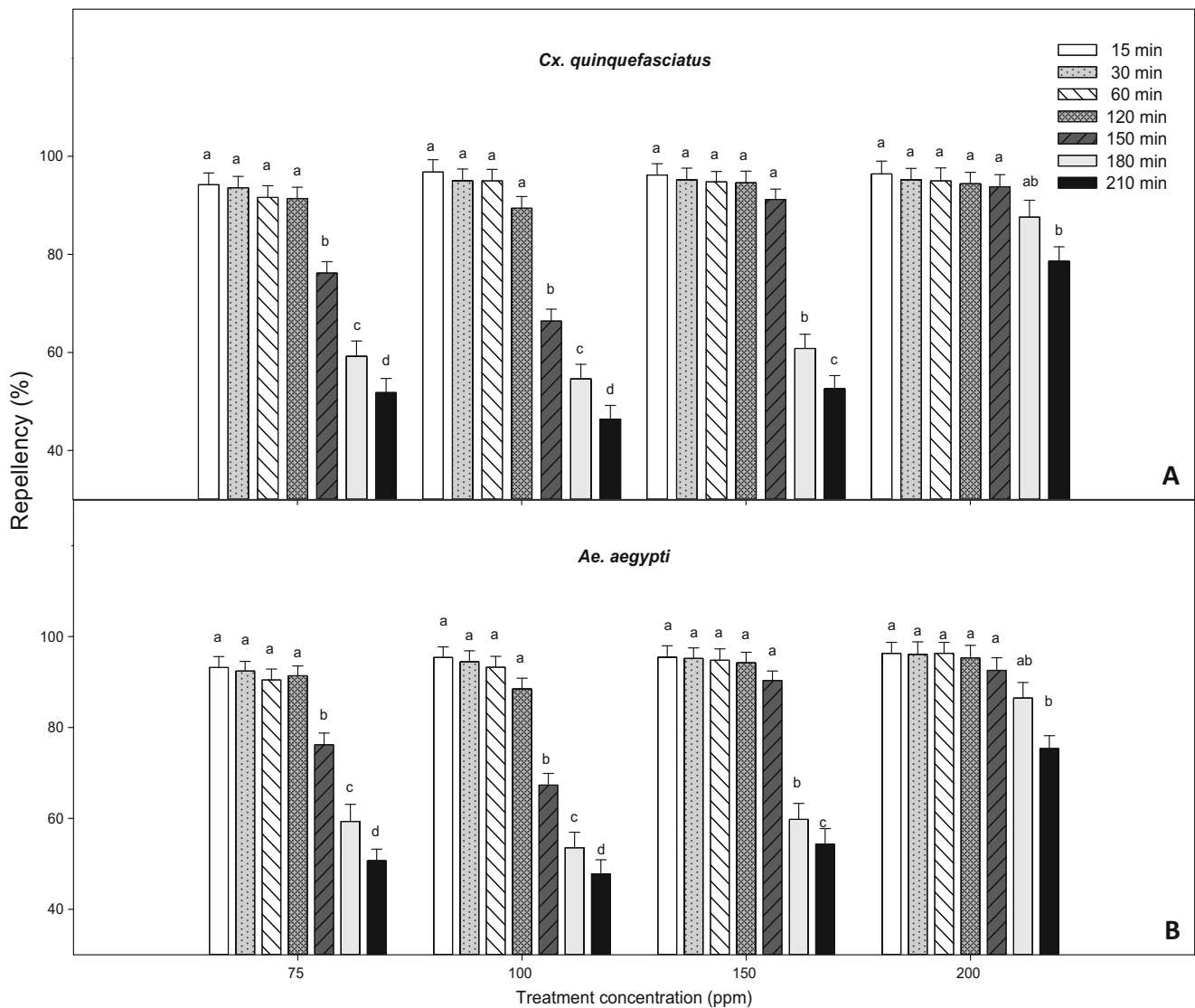


Fig. 4 Repellency of Si-EO against *C. quinquefasciatus* (a) and *A. aegypti* (b) where vertical bars that indicate standard error (\pm SE) followed by the same letters above bars indicate no significant difference ($P \leq 0.05$) in a Tukey's test

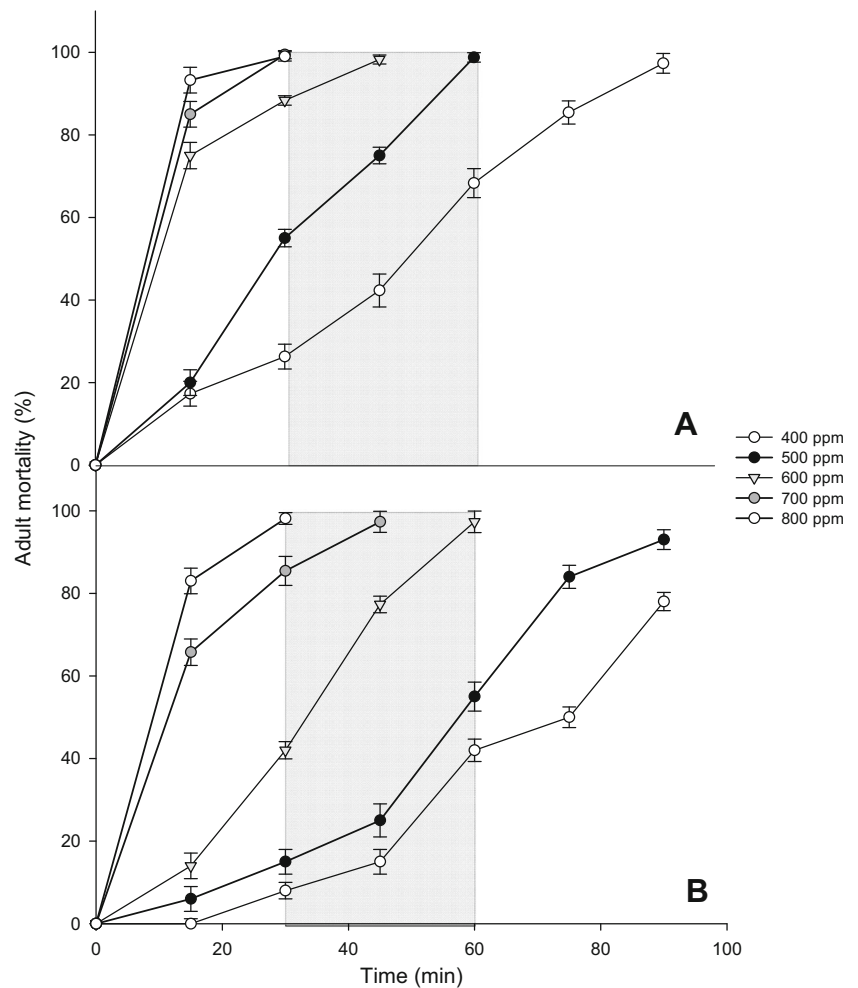
LC₉₀ > 10 ppm are not effective for management of field populations (Pavela 2015). Consequently, it would be beneficial to identify and increase the fractions of compounds with the greatest effectiveness in future Si-EO studies. It should also be noted that the dosage needed to attain larval mortality depends on several factors, including larval stage, suitable temperature, and capability of the phytochemicals to enter inside the cuticle, as well as the mode and site of action (Rattan 2010; Pavela 2015).

Generally, EOs possess strong repellent properties against the mosquito vectors (Sritabutra and Soonwera 2013). However, it is important to consider that EOs used in repellent activity are not constantly harmless comparable to commercial repellents (Nerio et al. 2010; Champakaew et al. 2015). In contrast, our results did not reveal any harmful effects to the treated volunteers in the complete study period. The presence of diverse phytoconstituents increases the repellence activity

of EO against diverse mosquito vectors (Trongtokit et al. 2005). Repellent activity of several EOs was linked with the presence of sesquiterpenes and monoterpenoids (Jaenson et al. 2006). Our results clearly illustrate that major compounds present in the Si-EO influenced the repellence of both the mosquitoes strongly up to 210 min. Similar to our results, Choochote et al. (2007) reported that EO derived from *Zanthoxylum piperitum* (L.) DC showed maximum protection period up to 1 h against *A. aegypti*. Likewise, Govindarajan et al. (2016a) proved *Zingiber nimmonii* (J. Graham) Dalzell EO showed protection up to 150 min against different mosquito vectors.

The adult mosquitoes of both the species were found to be susceptible against Si-EO treatment. The adulticidal activity of the Si-EO was more prominent at 700 and 800 ppm concentrations against both the species. Similar to our results, EO derived from *Lantana camara* L. established adulticidal

Fig. 5 Adulticidal activity of Si-EO against *C. quinquefasciatus* (a) and *A. aegypti* (b) where vertical bars indicate standard error (\pm SE)



response at 0.06 mg/cm² against *A. aegypti* (Dua et al. 2010). However, Si-EO requires higher concentrations (800 ppm) to produce adulticidal actions on mosquitoes than that required for larval mortality (500 ppm). Ajaegbu et al. (2016) stated that most of the botanical EOs proved as a good larvicidal agent but failed to display their adulticidal potential against

the mosquito vectors. However, in our results, Si-EO displayed decent larvicidal and adulticidal responses against both the vectors.

EO and their compounds were found to be toxic against non-targets only at higher concentrations or exposure for a longer time (Pavela 2015; Seo et al. 2012). However, our results did not exhibit significant mortality against *T. splendens* even at 1500 ppm concentration. This might be due to the fact that *Toxorhynchites* species are generally larger in size than our target mosquitoes (Benelli et al. 2016). Similarly, *Pinus kesiya* EO was safer towards *Anisops bouvieri*, *Diplonychus indicus* and *Gambusia affinis* aquatic organisms with LC₅₀ readings ranging from 4135 to 8390 mg/ml (Govindarajan et al. 2016b). Synthetic pesticides exhibit direct harmful effects towards beneficial insects and display toxic effects on their development and behaviour (Vasanth-Srinivasan et al. 2016, 2017; Ponsankar et al. 2016a, b). Correspondingly, Temephos produce severe toxic response against *T. splendens* at 0.5 ppm. Therefore, the current results suggest that the Si-EO can be considered completely safe against the mosquito predator.

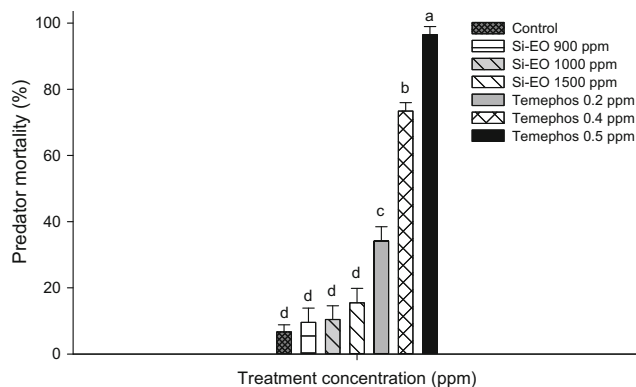


Fig. 6 Effects of Si-EO and Temephos against non-target organism *T. splendens*. Means (\pm SE) followed by the same letters above bars indicate no significant difference ($P \leq 0.05$) by using probit analysis

Conclusion

The application of botanical pesticide against mosquito vectors will not only diminish our reliance on chemical pesticides but also encourage eco-friendly and sustainable means of vector control. In conclusion, the EO extracted from the bio-rational plant *S. indicus* was analysed by GC-MS and evaluated for its larvicidal, repellent and adulticidal activities against *C. quinquefasciatus* and *A. aegypti*. Both mosquito larvae are susceptible to Si-EO at the higher dosage of treatments. Further, the repellent and adulticidal actions were promising. Moreover, the toxicological screening against mosquito predator also confirms that the Si-EO can be considered completely safer on the non-target insects. Overall, the present investigation encourages other scientific circles to move forward in the EO research efforts against medically challenging mosquito vectors.

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Compliance with ethical standards All applicable international and national guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

Conflict of interest The authors declare that they have no conflict of interest.

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