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Synergistic action of essential oil of *Ageratum conyzoides*, *Cymbopogon citratus*, *Eucalyptus globulus*, and synthetic insecticides against the mosquito vector, *Aedes albopictus* Skuse (Diptera: Culicidae)

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Abstract

Background *Aedes albopictus* is an epidemiologically important vector for the transmission of viral pathogens of yellow fever, dengue fever, chikungunya, zika, and filariasis. Plant-based products can provide environmentally safe mosquito control agents. Therefore, the efficacy of essential oils (EOs) extracted from *Eucalyptus globulus*, *Ageratum conyzoides*, and *Cymbopogon citratus* was determined against the larvae of *A. albopictus*.

Results The highest larvicidal action was illustrated by the EO of *E. globulus* with an LC₅₀ value of 46.58 ppm, followed by *A. conyzoides* with LC₅₀ of 69.51 ppm. The efficacy of combinations of the EOs of the three plants at different ratios showed the combination of *A. conyzoides* with *C. citratus* at a 1:1 ratio to be the most effective with LC₅₀ value of 13.05 with a 95% confidence limit of 10.54–15.32. The composition of the EOs analyzed using GC–MS showed precocene II and β-caryophyllene as major compounds of EO of *A. conyzoides*. We further evaluated the efficacy of the combination of EO with a binary combination of synthetic insecticides and detected the highest efficacy in a combination of *A. conyzoides* with malathion–cypermethrin and malathion–temephos.

Conclusion Therefore, the EO of *A. conyzoides*, through synergistic action with organophosphate and pyrethroid synthetic insecticides, can effectively act against the vector of dengue, zika, and chikungunya. The EO of *A. conyzoides* or its major compounds can be explored for the development of mosquitocidal formulation using effective synthetic insecticides.

Keywords Dengue, Binary combination, Precocene, Caryophyllene, Botanical pesticides

Introduction

Mosquitoes have been playing a predominant role in the transmission of diseases such as malaria, dengue, yellow fever, filariasis, and chikungunya, which are among the

serious health issues in the world today (Nebbak et al., 2022). They serve as vectors to numerous pathogens and parasites, resulting in human illness and mortality, impacting the economic scenario of disease-endemic countries. Approximately 4 billion people worldwide are at risk of arthropod-borne diseases (French et al., 2025). Dengue is one such vector-borne disease, putting billions of people at risk (Dehghani & Kassiri, 2021). The spread and globalization of Dengue worldwide are attributed to

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the spread of *Aedes* vectors across the globe, followed by an increased rate of human travel (Pandiyan et al., 2019). *A. albopictus*, also known as the “Tiger mosquito,” ranks second in terms of dengue transmission in humans, following *A. aegypti* (Wong et al., 2025). First described by Skuse in the city of Calcutta, India, in 1854, it is native to Southeast Asia but has invaded many countries in the early part of the twentieth century (Gratz, 2004). It is an epidemiologically important vector for the transmission of viral pathogens, including the yellow fever virus, dengue fever, chikungunya, zika, and filarial nematodes such as *Dirofilaria immitis*. *A. albopictus* is known as the prime vector in the presence of a low population of *A. aegypti* and can serve as DENV reservoir species capable of both vertical and horizontal transmission (Garcia-Rejon et al., 2021).

Female *A. albopictus*, although anthropophilic, may feed on the blood of several other wild mammals and avian species (Paupy et al., 2010). The vaccine against these mosquito-borne diseases is not yet developed; hence, vector control is considered the best strategy to reduce dengue virus transmission. The interruption in the disease transmission cycle is possible through reducing man-mosquito contact and immature source reduction or suppression. The breeding habitat of the species includes water-holding containers such as tree holes, coconut shells, water jars, tires, pots, and cement tanks, and it can adapt to both semiurban and urban habitats (Borah & Bora, 2022; Borah & Bora, 2023). Application of synthetic insecticides, including organophosphates such as temephos and fenthion, and insect growth regulators such as diflubenzuron and methoprene in the breeding habitat constitute effective control methods for the larval mosquitoes. However, repeated use of these controlling agents has fostered several environmental and health concerns, including disruption of natural biological control systems, outbreaks of other insect species, widespread development of resistance, and undesirable effects on non-target organisms (Duarte et al., 2024). It is desirable to find compound(s) that can effectively control mosquitoes with minimal damage to the environment (Vivekanandhan et al., 2024a). Due to the severe effects of synthetic pesticides, botanical pesticides, including essential oils, are recorded as promising alternatives since they are effective, easily biodegradable, environmentally friendly, and have a comparatively easier regulatory framework for acceptance as pesticidal formulation (Vivekanandhan et al., 2019; Vivekanandhan et al., 2024b). Further, the combinations of essential oil or plant extract mixtures can provide specific additive or synergistic effects (Devi & Bora, 2017; Muturi et al., 2017; Vivekanandhan et al., 2018a), while the combination of essential oil with synthetic insecticide, showing

synergistic action, may reduce the use of synthetic insecticides. In light of the above facts, the present research work was undertaken to investigate the larvicidal activity of the essential oil of *A. conyzoides* (Asteraceae), *C. citratus* (Poaceae), and *E. globulus* (Myrtaceae) against the tiger mosquito, *A. albopictus*.

Materials and methods

Biological materials and extraction of essential oils

Eggs laid by wild *A. albopictus* were collected from the botanical garden, Dibrugarh University, Assam, India, using ovitraps. The collected eggs were allowed to hatch in trays containing tap water at 25–29 °C temperature and 80–90% relative humidity. The newly hatched larvae were fed with soya meal, and toxicity tests were conducted on fourth-instar larva. The eggs were also cultured parallelly to develop into adults. The pooled larvae were identified using the shape of pecten teeth on the eight abdominal segments and the comb scales which are tapering from one side, not forked as in *A. aegypti* (Tyagi et al., 2015). The adults were identified using morpho-taxonomic keys such as the presence of one broad white line in the middle of the thorax on the dorsal side, the absence of lyre-shaped marks, and black and white stripes in the legs (Rueda, 2004).

Fresh leaves of *C. citratus* (CC), *E. globulus* (EG), and *A. conyzoides* (AC) were collected early in the morning from the campus of Dibrugarh University, Assam, India, and the essential oils were extracted by hydro-distillation method using the Clevenger apparatus (Sarma et al., 2019). For extraction, freshly chopped leaves (250 g) of each plant were placed in a 5000-ml round-bottom flask containing water and heated using a heating mantle for 4 h at 40–60 °C. The deposited oil was collected in clean glass vials, dried over anhydrous sodium sulfate to remove excess water, and stored at 4 °C for further assay.

GC–MS analysis of essential oils

The essential oil (EO) of the plant with the highest synergistic action was subjected to gas chromatography coupled with high-resolution mass spectrometry (GC–HRMS) analysis at the Sophisticated Analytical Instrument Facility (SAIF), IIT Mumbai, for identification of chemical compounds. The analysis was done using the Agilent 7890 GC system. It was coupled with a JEOL AccuTOF GCV equipped with an EI/CI ionization source. The mass range was 35–800 amu, and the mass resolution was 5000. The sample oil was injected in splitless mode with the help of a headspace injector, and an HP5 capillary column was used. The maximum temperature was 280 °C. Compound identification was done by matching the mass spectra to the stored mass spectra of the NIST library as well as available literature data.

Larvicidal bioassay

The larvicidal bioassay was completed under laboratory conditions following the WHO protocol (2005). For the assay, 25 early fourth-instar larvae were introduced into 25 ml each of different concentrations (10, 15.6, 31.2, 62.5, 125, 250, 500, 1000 ppm) of the three different oils, individually maintaining three replications. 10,000 ppm stock solutions were prepared by adding 1 ml of the oils in 100 ml of distilled water. Serial dilutions were done from stock solutions for the preparation of the different concentrations; Tween 20 was used as an emulsifier. Commercially available neem oil formulation was used as a standard to compare the efficacy of the experimental oils. The formulation was a mixture of *Azadirachta* (00.03%), neem oil (90.57%), hydroxyl E1 (05.00%), and aroma (3.90%) purchased from Multiplex Agricare Pvt. Ltd. India. The mortality of the larvae was recorded at 24 h of treatment.

The larvicidal bioassay was also carried out using binary mixtures of the essential oils of *A. conyzoides*: *E. globulus* (AC: EG), *C. citratus*: *E. globulus* (CC: EG), and *C. citratus*: *A. conyzoides* (CC: AC) at the ratios of 9:1, 1:9, and 1:1, with a concentration of 1000 ppm against the larvae of *A. albopictus*, and the mortality percentage was recorded at an interval of 60 min for 180 min. The most effective ratio of each binary combination determined based on 100% lethal time was further evaluated for larvicidal activity by reducing the concentration to 500 ppm, 250 ppm, 125 ppm, 62.5 ppm, 31.2 ppm, 15 ppm and 7.5 ppm for determination of LC_{50} .

Similarly, LC_{50} of combinations of synthetic insecticides malathion (OP compound), temephos (OP compound), and cypermethrin (pyrethroid) were determined against *A. albopictus* larva. Then, for studying the compatibility and efficacy of the combination of EOs of *C. citratus*, *A. conyzoides*, and *E. globulus* with a binary combination of synthetic insecticides malathion, cypermethrin, and temephos, the LC_{50} concentrations of each were combined at the ratio of 1:1, and mortality percentage was recorded.

To understand the role of the major compounds of the most effective EO, their efficacy was studied against the larvae.

Statistical analysis

Lethal concentration 50 (LC_{50}) and 90 (LC_{90}) were determined through regression equation, and a 95% confidence limit (CI 95) was calculated using Probit analysis (Finney, 1971) using SPSS 13.0. When the mortality percentage in control reached 1–20%, the observed mortality was corrected by Abbott's formula (Abbott, 1925).

Table 1 Efficacy of three essential oils, *A. conyzoides*, *E. globulus*, and *C. citratus*, against the larvae of *A. albopictus*

Essential Oils	LC_{50} (ppm)	LC_{90} (ppm)
<i>Ageratum conyzoides</i> (AC)	69.51	156.5
<i>Eucalyptus globulus</i> (EG)	46.58	98.03
<i>Cymbopogon citratus</i> (CC)	124.09	250.3
<i>Azadirachta indica</i> (AI)	95.67	195.68

Table 2 Efficacy of binary combinations of essential oils, *A. conyzoides*: *E. globulus* (AC: EG), *C. citratus*: *E. globulus* (CC: EG), and *A. conyzoides*: *C. citratus* (AC: CC) on percent mortality of larvae of *A. albopictus* after different hrs of treatment

EO Combination	% Mortality at 60 min	% Mortality at 120 min	% Mortality at 180 min
AC: EG (9:1)	84.0	100	100.0
CC: EG (9:1)	65.33	89.33	100.0
AC: CC (1:1)	100.0	100.0	100.0

Results

Larvicidal activity

Among the essential oils tested, *E. globulus* EO illustrated the highest larvicidal action against the test larvae showing the lowest LC_{50} value of 46.58 ppm followed by *A. conyzoides* EO with LC_{50} of 69.51 ppm and *C. citratus* illustrating the lowest larvicidal activity with LC_{50} of 124.09 ppm (Table 1).

Binary combinations using each of the three essential oils were prepared and evaluated against *A. albopictus* larvae at 1000 ppm concentration to find out the effective ratio of the binary mixtures based on the minimum time required for 100% mortality. The combinations of *A. conyzoides*, *E. globulus* at 9:1 ratio, *C. citratus*, *E. globulus* at 9:1 ratio, and *A. conyzoides*, *C. citratus* at 1:1 ratio were found to be the most effective (Table 2). In case of the three effective essential oil combinations, *A. conyzoides*, *C. citratus* (1:1) showed the maximum efficacy with the lowest LC_{50} value of 13.05 ppm and LC_{90} of 62.94 ppm, followed by *A. conyzoides*: *E. globulus* (9:1) with LC_{50} of 28.21 ppm and LC_{90} of 62.39 ppm and *C. citratus*: *E. globulus* (9:1) showing the minimum efficacy with LC_{50} of 98.99 ppm and LC_{90} of 223.79 ppm. The combination LC_{50} values were found to be lower than the individual LC_{50} values, indicating synergistic activity (Table 3).

Efficacy study of combinations prepared using LC_{50} values of EOs with synthetic insecticides showed the combination of *A. conyzoides* EO with malathion–cypermethrin (1:1) to cause the highest larvicidal mortality of 100%

Table 3 Comparison of LC₅₀ (ppm) values of individual and effective combinations of the EOs of *A. conyzoides* (AC), *E. globulus* (EG), and *C. citratus* (CC) against the larvae of *A. albopictus* to show synergistic action

Oil A	Oil B	LC ₅₀ of individual oil (ppm)		Binary ratio	Combined LC ₅₀ (ppm)
		Oil A	Oil B		
AC	EG	69.51	46.58	9:1	28.21
CC	EG	124.09	46.58	9:1	98.99
AC	CC	69.51	124.09	1:1	13.05

followed by *A. conyzoides* EO with malathion–temephos (1:1) causing larval mortality of 90.67% (Table 4).

GC–HRMS analysis

The GC–HRMS analysis of the most effective essential oil was carried out at SAIF, IIT, and Mumbai, and the results were compared with the NIST library. In the EO of *A. conyzoides* EO, five major compounds were identified, with area peaks ranging from 2.51 to 38.71% (Fig. 1 and Tables 5, 6).

Efficacy of caryophyllene and precocene

As *A. conyzoides*, EO showed the best synergistic activity when combined with the malathion–cypermethrin combination; two major compounds, β-cis-caryophyllene and precocene II, were assayed against the larvae for toxicity studies. The LC₅₀ of phytocompounds β-cis-caryophyllene was 2497.43 ppm with a 95% confidence limit of 1863.707–3271.68, and that of precocene II was 46.22 ppm with a 95% confidence limit of 38.15–54.94 (Table 7).

Discussion

Owing to the worldwide risk of mosquito vector-transmitted diseases and their development of resistance against insecticides, there has been a spike in research for the development of effective and eco-friendly mosquito-cidal products of plant origin. Hence, botanical metabolites such as plant essential oils and extracts are being studied as alternatives to synthetic insecticides (Vivekanandhan et al. 2018b). In this study, we explored the mosquito larvicidal potential of essential oils from *A. conyzoides*, *C. citratus*, and *E. globulus*, along with their binary combinations with recommended synthetic

Table 4 Efficacy of combinations of essential oils (LC₅₀) with binary combination of synthetic insecticides (LC₅₀) on larvae of *A. albopictus* after 24-h treatment

Combinations (1:1)				Percent Mortality (EO + Insecticides)
EO	LC ₅₀ (ppm)	Insecticides (1:1)	LC ₅₀ (ppm)	
<i>Cymbopogon citratus</i>	124.09	Malathion–Cypermethrin	0.279	54.67
		Malathion–Temephos	0.057	45.33
<i>Ageratum conyzoides</i>	69.51	Malathion–Cypermethrin	0.279	100
		Malathion–Temephos	0.057	90.67
<i>Eucalyptus globulus</i>	46.58	Malathion–Cypermethrin	0.279	44
		Malathion–Temephos	0.057	80

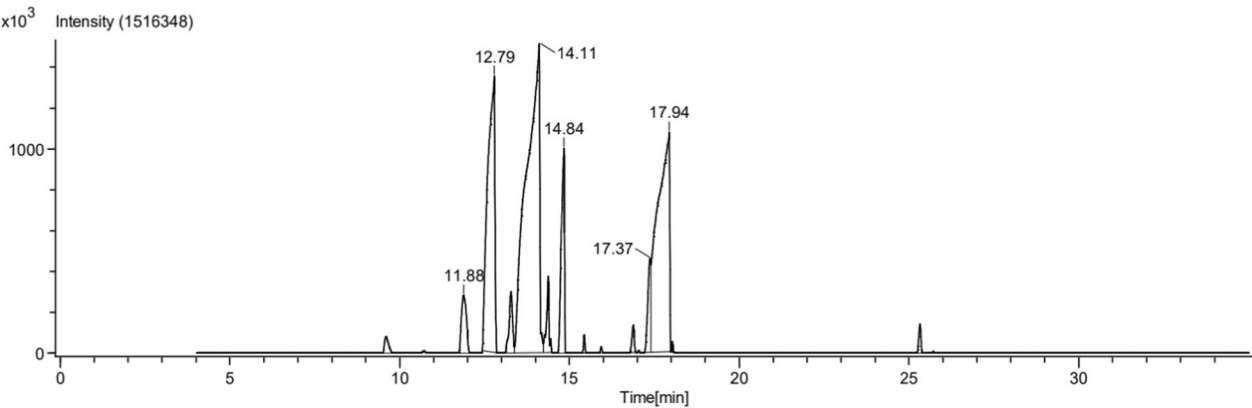
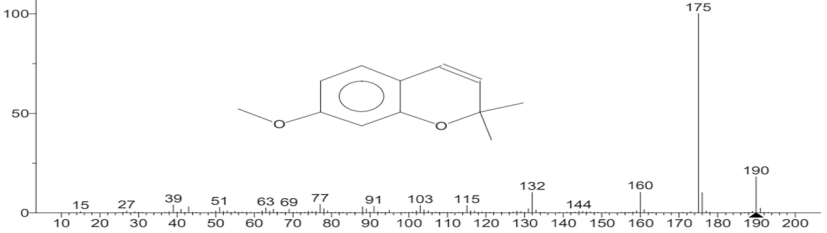
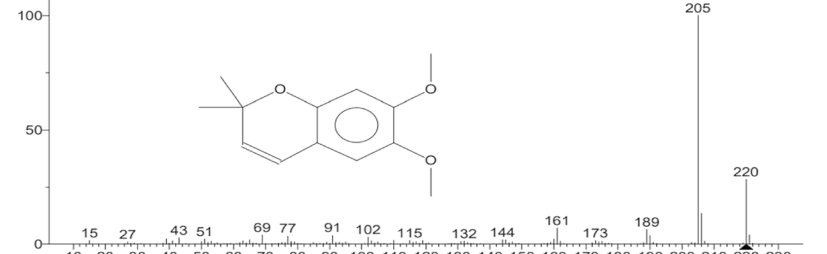
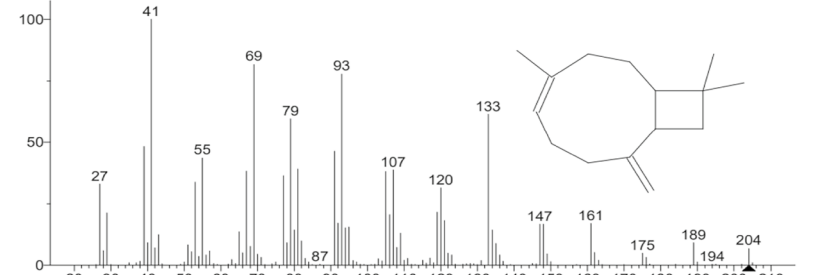


Fig. 1 GC–HRMS chromatogram of *A. conyzoides* essential oil

Table 5 GC–HRMS chromatogram of major compounds of *A. conyzoides* essential oil

Major Compounds	Chromatogram
14.11 Precocene I	
17.94 Precocene II	
12.79 β-cis-caryophyllene	

insecticides, malathion, temephos, and cypermethrin against the dengue vector, *A. albopictus*.

In the present study, the essential oil of *E. globulus* showed the highest larvicidal efficacy against the larvae of *A. albopictus* with an LC_{50} value of 46.58 ppm, followed by the EO of *A. conyzoides*, which is comparable to results found by other researchers. In a recent study, *E. grandis* EO exhibited an LC_{50} value of 23.667 ppm against *A. aegypti* (Vivekanandhan et al., 2023). In a study from Pakistan using *E. camaldulensis*, LC_{50} was found to be 49.8 ppm, which is close to the result of the current study (Bilal et al., 2012). In similar toxicity studies against *A. aegypti*, *E. grandis* demonstrated a low LC_{50} value of 32.4 ppm (Lucia et al., 2007). Another study from Columbia showed *E. citriodora* exhibiting an LC_{50} value of 71.22 ppm (Vera et al., 2014). The percentage of oil compounds and oil yield vary with the plant species, topography and growth phase of the plant (Mohanty et al., 2018). Therefore, variations in toxicity values may be attributed to differences in the composition of the

essential oils based on the plant species, ecology, and topography of the location. Our study shows that the EO of *A. conyzoides* and *E. globulus* is more efficacious than that of already popularly used EO of *A. indica* against *A. albopictus*. Earlier studies indicated the EO of *A. conyzoides* to be effective against other Dipteran species (Bora & Khanikor, 2011) and *E. globulus* against *Aedes* vectors (Kaura et al., 2019).

In the current study, GC–HRMS analysis of the extracted essential oils revealed major compounds of *A. conyzoides* to be precocene I (38.71%), precocene II (27.20%) and β-cis-caryophyllene (18.69%). Precocene I (52.18%) was found to be the major compound in the same species collected from Maharashtra (Patil et al., 2010), and along with precocene I (61.7%), precocene II (23.5%) was the major compound in samples from Nepal (Satyal et al., 2018). In the sample of China, precocene II constituted the major portion (45.75%) (Liu & Liu, 2014). The efficacy of the essential oil of the plants considered in the study may be attributed to the presence of

Table 6 Chemical constituents of *A. conyzoides* essential oil

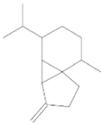
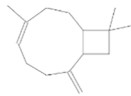
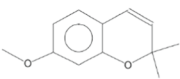
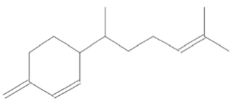
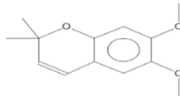
Structure	Compound name	RT	% Area	MF	RMF	Reference
	β -Cubebene	11.88	2.51	533	922	NIST: 9256 CAS: 13744-15-5
	β -cis-caryophyllene	12.79	18.69	725	958	NIST: 249403 CAS: 118-65-0
	Precocene I	14.11	38.71	952	960	NIST: 108083 CAS: 17598-02-6
	β -Sesquiphellandrene	14.84	6.02	703	958	NIST: 249522 CAS: 20307-83-9
	Precocene II	17.94	27.20	889	928	NIST: 235552 CAS: 644-06-4

Table 7 Efficacy of β -cis-caryophyllene and precocene II, against the larvae of *A. albopictus*

Compounds	LC ₅₀ (ppm)	95% Confidence limit
β -cis-caryophyllene	2497.43	1863.707–3271.68
Precocene II	46.22	38.15–54.94

a major compound or their combinations. A major compound detected in the EO of *A. conyzoides*, caryophyllene, was earlier reported to be effective against other dipteran species (Khanikor & Bora, 2011). The other major compound, precocene, a chromene, is known for its anti-juvenile hormone and insect growth regulatory properties. Chromenes are reported to be effective larvicide against *Culex pipiens* (El-Sayed et al 2024).

In the present study, the binary combinations of *A. conyzoides*/*C. citratus* (1:1) showed remarkable efficacy with the lowest LC₅₀ values of 13.05 ppm. The combination demonstrated synergistic action against the larvae after 24-h treatment under laboratory conditions, as the LC₅₀ of the combined EOs was less than the LC₅₀ of the individual oils. Further, in combinations of EO with synthetic insecticides, the LC50 values further decreased, indicating synergistic compatibility among the EO and the synthetic insecticides used. In such combinations,

major constituents of the EOs may play a vital role to enhance the efficacy. To understand if only the major compounds are responsible for the efficacy of the EO, the efficacy of precocene II and β -caryophyllene was studied. The results showed precocene to be more effective with an LC₅₀ value lower than that of the individual EO of *A. conyzoides*. Hence, precocene may be attributed to the efficacy of the EO of *A. conyzoides* against the larvae of *A. albopictus*.

The major advantage of using combinations of plant-based essential oils and synthetic insecticides is the reduction in the use of synthetic insecticides, thereby reducing the possibility of resistance development in target mosquito vectors (Pavela & Benelli, 2016; Vivekanandhan et al., 2018c). In the current study, malathion was invariably used in every combination with essential oil because, in the state of Assam, it is used for fogging regularly to control mosquitoes and is recommended by NVBDCP for use in India. Similarly, cypermethrin is highly used in agricultural fields and temephos as mosquito larvicides. Although the dengue vectors are susceptible to malathion, continuous use of pyrethroids is already known to cause progression of resistance development in different mosquito species. Hence, presently, EOs have been portrayed to be an alternative to pyrethroids in the

case of pyrethroid-resistant mosquitoes in which the pyrethroids act by interfering with the voltage-gated sodium channel, and EOs act in many different ways, including interference with cholinergic, octopaminergic and GABA systems (Rattan, 2010; Gnankine & Bassole, 2017). Combination of chemicals having different mechanisms of action will induce less selection pressure for resistance development. Further, EOs can also act as carriers of synthetic insecticides. Hence, the EO of *A. conyzoides* or its major compounds can be explored for the development of insecticidal formulation using effective synthetic insecticides.

Conclusion

The study shows the synergistic effects of the combinations of essential oil (EO) of *A. conyzoides* with organophosphate and pyrethroid synthetic insecticides against *A. albopictus*. Precocene and caryophyllene are the major compounds of the EO of *A. conyzoides*. The EO of *A. conyzoides*, or its major compounds, can be explored for the development of mosquitocidal formulation using effective synthetic insecticides.

Abbreviations

CC	<i>C. citratus</i>
EG	<i>E. globulus</i>
AC	<i>A. conyzoides</i>
EO	Essential oil
LC ₅₀	Lethal concentration ₅₀

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Author contributions

D.B. conceptualized and supervised this study and wrote and edited the manuscript. S.C., K.P., I.S., and S.B. performed the pilot study; J.A., A.P., and B.K. confirmed the results of pilot study; H.B. generated initial data designing the experiment. D.B., J.A., A.P., and B.K. analyzed the results. All authors read and approved the final manuscript.

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Dibrugarh University.

Data availability

No datasets were generated or analyzed during the current study.

Declarations

Ethical approval and consent to participate

Not applicable.

Human or animal rights

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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