



Evaluation of Fumigant Toxicity of *Eucalyptus globulus* Essential oil (Family: Myrtaceae) against the Mechanical Vector, American Cockroach, *Periplaneta Americana* (Linnaeus, 1758) (Blattodea: Blattidae)

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

The American cockroach, *Periplaneta americana* (Linnaeus, 1758), one of the largest and most resilient cockroach species, possess significant threats to public health and food safety due to its ability to transmit diseases and contaminate food. The widespread application of chemical pesticides to control cockroach infestations raises significant public health concerns due to its residual toxicity. The continued use of insecticides results in resistance development in cockroach

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populations which demands alternative pest management strategies wherein the chemical insecticides were completely restricted in human dwelling areas such as apartments, hospitals, restaurants and food preparation areas. The plant's diverse profile of bioactive compounds and secondary metabolites, coupled with its complex nature, renders it a promising natural resource for the development of safe alternative against cockroach management. The median lethal dosage LD₅₀ of *E. globulus* essential oil was found to be 16.74784 µL against the male American Cockroach, *P. americana* analysed using probit analysis. The present study was conducted to evaluate the fumigant toxicity of eucalyptus essential oil (*Eucalyptus globulus*) against the male American Cockroach, *Periplaneta americana* in a laboratory experiment. Five different doses of *E. globulus* essential oil 5, 10, 20, 30, 40 and 50 µL against male American Cockroach, *P. americana* were evaluated and compared to the untreated control (negative control). Results, indicated a dose dependent response with 100 per cent mortality achieved at 50 µL of eucalyptus essential oil followed by 40 (80%), 30 (60%), 20 (46.67%) and 5 (26.67) µL. The *Eucalyptus globulus* essential oil can be used as a potent fumigant against the male American Cockroach, *P. americana* which is an ecofriendly management of cockroach infestation.

Keywords: *Eucalyptus*; essential oil; fumigant toxicity; LD50, *P. americana*.

1. INTRODUCTION

The Domiciliary pest, *Periplaneta americana* (Linnaeus, 1758) commonly known as the American cockroach, belonging to the order Dictyoptera, is the most important economic pest globally, which was found to be a prolific mechanical vector of human intestinal parasites and various kinds of diseases such as cholera, dysentery, gastroenteritis, leptospirosis, typhoid fever etc., through its shed exoskeleton, pulverized dead cockroaches and faecal matter disseminating potent allergens (Pomes and Schal, 2020). Prolonged exposure to such allergen results in pulmonary health impact. Transmission of six allergens were reported and diagnosed in American cockroach namely Bla g 1: A protein found in cockroach saliva, faeces, and body parts, Bla g 1: A protein found in cockroach saliva, faeces, and body parts, Bla g 2: A protein present in cockroach hemolymph (blood-like fluid), Blag 3: A glutathione S-transferase enzyme, Bla g 4: A lipocalin protein, Bla g 5: A protein with unknown function and Bla g 6: A troponin C protein. These allergens can trigger respiratory issues, skin irritation, and gastrointestinal problems in sensitive individuals. Cockroach allergens are particularly concerning in urban areas, low-income households, and tropical regions. Exposure to cockroach allergens can occur through inhalation of airborne particles, skin contact with contaminated surfaces and ingestion of contaminated food or water (Arruda *et al.*, 2001).

American cockroaches were known for their destruction to commodities in which its mere presence can bring down reputation of a branded

facility, leading to loss of customer trust. In addition, loss of economic value of commodity is questionable. It is of great clinical importance in management of American cockroach as they cause negative health impact and were known to reside in manholes and drainage systems which is a hotspot of aggregation and platform of disease transmission to human dwelling areas. If control measures were not carried out it may abruptly increase in population causing nuisance and fatality.

Cockroaches generally prefer high temperature and humidity for optimum population growth, which is prevalent household pest in tropical countries like India where cockroach found to habitat and breed in drainage pipelines which consist of favourable warm humid environment facilitating access to kitchen and human occupied places through diverse entry points such as cracks, crevices and gaps in bathroom and kitchen fixtures. They chew cardboard, gums and wood furniture's. Cockroach exhibit thigmokinesis, enabling them to flatten and compress their elongated, oval-shaped bodies to navigate through narrow crevices and cracks (Arruda *t al.*, 2001).

American cockroaches' harbours diverse microbes in their gut and faeces (Kakumanu *et al.*, 2018) which were known to trigger allergies, asthma in atopic children and respiratory issues through allergenic proteins. They secrete a mixture of xanthurenic acid, kiturenic acid which were known to have carcinogenic properties (Ling *et al.*, 2009).

The primary pest management strategies against American cockroach were conventional

insecticides, which were employed during the initial days but lot of concerns about their harmful side effects persist. Systemic pesticides like dichlorvos, chlorpyrifos, propoxur, and pyrethroids were applied repeatedly to control cockroach populations (Manzoor *et al.*, 2012). Varying levels of resistance in cockroach were documented to various chemical insecticides, such as organochlorine, organophosphorus, and carbamate compounds. The excessive usage of synthetic pesticides in urban environment against noxious pest has resulted in increased risk of pesticide resistance, enhanced pest resurgence and development of resistance/cross-resistance, toxicological implications to human health and increased environmental pollution. (Batish *et al.*, 2008, Kamaraj *et al.*, 2024). Moreover, their use is completely restricted in sensitive areas such as food preparation zones, restaurants, storage buildings, and apartments which results in, growing demand for safer alternatives to combat cockroach infestations. It is advisable to go for safer alternatives like essential oils as it found to have short period of residual activity combating potential issues of conventional insecticides such as indiscriminate usage, resurgence, resistance and biotype (Chang *et al.*, 2010 and Yeom *et al.*, 2018).

The essential oil (Eos) constitutes a complex mixture of secondary metabolites and can be a better alternative to synthetic chemical pesticides, thereby knocking down pest population, with novel mode of action, effective against resistant pests, reducing the resistance development in pests. The essential oils are highly target-specific, rapidly biodegradable and safer to non-target organisms. Essential oils, derived from plants offer a promising alternative to conventional insecticides which were known to exhibit insecticidal properties such as antifeedant and repellent effects which also inhibits cockroach reproduction (Isman, 2006 and Omara *et al.*, 2013).

The genus *Eucalyptus* (Myrtaceae family) originated in the eastern and southern regions of Australia, representing around 700 species known for its tall, evergreen and magnificent tree cultivated world-wide for various purposes such as gum, oil, pulp, timber and aesthetic value. The essential oil was extensively used in food, perfumery and pharmaceutical industry where it has wide spectrum of biological activity such as anti-microbial, fungal, insect repellent, herbicidal, acaricidal and nematocidal activities. The essential oil extracted through steam distillation

constitute complex mixture of monoterpenes (C10) and sesquiterpenes (C15), and a variety of aromatic phenols, oxides, ethers, alcohols, esters, aldehydes and ketones. Presence of volatile monoterpenes or essential oils in the plants provides an important defence strategy to the plants, particularly against insect pests and act as a signalling molecule. The eucalyptus essential oil and its novel compounds is significant in view of environment and toxicological implications combating the indiscriminate use of synthetic pesticides and overcoming the problem of increasing pest resistance (Batish *et al.*, 2008). The present study was mainly focussed on evaluating the fumigant toxicity of *Eucalyptus globulus* essential oil against the male American cockroach, *Periplaneta americana*.

2. MATERIALS AND METHODS

2.1 Collection of American Cockroach, *P. americana* (L.)

The American cockroach populations which were collected from sewer manholes using a plastic container pierced with stick which is equal to the inner width of manhole. The plastic container was lowered down using a rope attached to the plastic cap. A small hole was provided near to the wooden stick which facilitates the entry of cockroach. The plastic container was placed with attractant such as sugar, biscuits and some vegetables which facilitates one time entry and were greased to avoid cockroach escape. The setup was left overnight and collected during the morning. The collected cockroaches were immediately taken to the lab and placed in separate glass tanks of size (45 x 30 cm) smeared with Vaseline to avoid escape of cockroach from the container, into which a newspaper placed at the bottom of the container to discard the dirt associated with it. To minimize potential contamination dead cockroaches were promptly removed with forceps. Cockroach populations were provided with egg cartons as shelter. The glass tank was covered with dark clothes to acclimatize the populations into normal condition. They were identified using taxonomic keys where morphological features representing 4 cm in length and reddish-brown tegmina with yellow marking on the pronotum. The dichotomous key was run in addition to the morphological feature and identified as *Periplaneta americana* which has distal segment of cercus elongated, length more than twice width; male with caudal tergite deeply notched;

distal portion of plate thin, projecting as hood over corresponding terminal sternite; median segment unspecialized.

2.2 Mass Culturing of American Cockroach, *P. americana* (L.)

The ootheca collected from populations were reared separately under laboratory condition with a photoperiod of 12:12 (L:D) hours and an ambient temperature of 24-30° C and 70-90 % relative humidity (RH). Dog food was provisioned since it was preferred by cockroach as it contains nutrients; hence it was supplied as food for nourishment (Tunaz *et al.*, 2009, Gaire *et al.*, 2017). Water was placed in a small plastic cup in which paper tissue wrapped around cotton eventually soaked in plastic cup containing water. It was done in order to avoid nymphs from drowning and aid in drinking water. The water and food replaced weekly to avoid microbial infestation. The cockroach populations and lab were well aerated to dilute the unpleasant odour. The tank was lined with chalk to avoid ants, mites, lizards and frogs attracted to the characteristic odour. Aluminium mesh was placed over the tank as it is easy to maintain and dilute the clumsiness during rearing. The mesh size was chosen in such a way that it prevents the egg parasitisation of ensign wasp.

2.3 Eucalyptus Essential Oil

The Eucalyptus essential oil purchased from Kazima Perfumers, Plot No, 309, Patparganj Area, Delhi-110092.

2.4 Chemical Analysis of *Eucalyptus globulus* Essential Oil by GC-MS

The essential oils were analyzed by using gas chromatography-mass spectrometry (GC-MS) to determine the chemical composition. The Clarus 680 GC was used in the analysis employed a fused silica column, packed with Elite-5MS (5%

biphenyl 95% dimethylpolysiloxane, 30 m × 0.25 mm ID × 250µm df) and the components were separated using Helium as carrier gas at a constant flow of 1 ml/min. The injector temperature was set at 260°C during the chromatographic run. The 1µL of extract sample injected into the instrument the oven temperature was as follows: 60°C (2 min); followed by 300 °C at the rate of 10°C min⁻¹; and 300 °C, where it was held for 6 min. The mass detector conditions were: transfer line temperature 240°C; ion source temperature 240°C; and ionization mode electron impact at 70 eV, a scan time 0.2 sec and scan interval of 0.1 sec. The fragments from 40 to 600 Da. The spectrums of the components were compared with the database of spectrum of known components stored in the GC-MS NIST (2008) library.

2.5 Fumigant Toxicity

The lab cockroaches were taken for fumigant toxicity study. The ethyl ether (solvent) was taken in piece of cotton and squeezed and placed over the corners of glass tank and covered with glass top for seducing the insects which facilitates handling of cockroach to respective treatment jars of size 25 x 15 cm. Five number of male cockroaches (4 months old) were released per replication and they were aerated with fast blowing air. A 25 ml sauce cup lid was poked with 25 holes using dissecting needle. The eucalyptus essential oil of doses 5, 10, 20, 30, 40 and 50 µl/L of air were pipetted out using micropipette and applied to the 1 cm² cotton, which were placed inside the sauce cup. For control group (Negative control), the insects were maintained without oil exposure (0 µl/L of air) (Elbeherly and Ibrahim, 2024). The mortality of test insects was assessed after 12th hour of treatment (Sharifard *et al.*, 2016). The test was slightly modified where sauce cup act as a barrier which prevents test insects interacting with the residue of essential oil.

Table 1. Physical properties of *Eucalyptus globulus*

| S. No | Physical Properties | Description/ Values |
|-------|----------------------------------|--|
| 1 | Appearance | Colourless |
| 2 | Odour | Characteristic |
| 3 | Solubility | Soluble in alcohols and fixed oils; Insoluble in water |
| 4 | Extraction method | Steam Distillation |
| 5 | Plant part used for distillation | Leaf |
| 6 | Refractive Index | 1.440-1.480 |
| 7 | Optical Rotation | 0° to +10° |
| 8 | Specific gravity | 0.898-0.920 |

2.6 Assessment of Mortality

Mortality of cockroach was made sure by the following steps:

- a. By exposure of light and observing their movements.
- b. By touching their legs with a forceps and noticing their movements.

After exposure to light and touching with forceps, if there were no movement then the cockroaches were considered dead (Manzoor *et al.*, 2012).

2.7 Statistical Analysis

The recorded data were compiled and tabulated for statistical analysis. Data were analysed using ICAR-WASP 2.0 software (Jangam and Thali, 2004). The data were analysed in a Completely Randomized Block Design (CRBD) by "F" test for significance as described by Panse and Sukhatme (1967). Percentage mortality data were subjected to arcsine square root transformation before analysis to stabilize variance. Analysis of Variance (ANOVA) was performed to determine significant differences between treatments. Critical difference values of the experiment were computed using Duncan's Multiple Range Test (Gomez and Gomez, 1984).

3. RESULTS AND DISCUSSION

The fumigant toxicity results revealed that 50 µl was found to have complete mortality against the male American cockroach, *Periplaneta americana* followed by followed by 40 (80%), 30 (60%), 20 (46.67%), 10 (33.33 %) and 5 (26.67%) µL (Table 2.). The toxicity of Eucalyptus globulus essential oil against the male American cockroach was evaluated through Probit analysis (Table 3). The results revealed a median lethal dosage (LD₅₀) of 16.74784 µl, with upper and lower confidence limits of 28.09616 µl and 9.98322 µl, respectively. The Probit curve exhibited a slope (b) of 1.484 and an intercept (a) of 3.187, indicating a significant dose-response relationship. The correlation coefficient (R²) of 0.877 and non-significant Chi-test (χ^2 Sig = 0.903) confirmed the goodness-of-fit of the model. These findings suggest that Eucalyptus globulus essential oil is a potent insecticide against the American cockroach, with potential

applications in pest management strategies. Rapid knockdown effect, hyperactivity, convulsion and paralysis were observed on *Periplaneta americana* on exposure to *Eucalyptus globulus* EO. The American cockroach when exposed to fumigant toxicity of *Eucalyptus globulus* EO repels them away and test insects were found to climb the top of the plastic container, after 1-2 hours, the test insect was found to be restless, agitated, disoriented, loss of coordination with reduced movement and activity. Increased grooming behaviour typically noticed to avoid the toxicity conferred by volatile interaction on antenna. The intermediate symptoms (2-4 hours) were muscle weakness, tremors with abdominal contractions, regurgitation, difficult walking and standing. During a period of 4-6 hours, paralysis (immobility), loss of antennal function and increased respiration rate among the test insect were noticed. At the end of 12 h, due to respiratory failure, dehydration and neurological collapse, the test insect was completely dead. The vapor phase of eucalyptus essential oil interfered with respiratory function and caused oxidative stress in American cockroach. Similar results were reported in Sharififard *et al.* (2016), where eucalyptus at 50 µL taken in acetone exhibited complete mortality due to fumigant toxicity against a total number of thirteen, 3rd and 4th instar nymphs of *Supella longipalpa* after a recovery period of 24 h. The per cent mortality due to contact toxicity of *Eucalyptus globulus* EO against *Supella longipalpa* was found to be 100 per cent for doses from 5-30 per cent when acetone was taken as solvent and has per cent mortality of 36.2 per cent for 2.5 per cent concentration. Alzogaray *et al.* (2011) reported that 50 µL essential oil and its compounds of different eucalyptus species namely *Eucalyptus sieoxydon*, *Eucalyptus viminalis*, *Eucalyptus dunni*, *Eucalyptus grandis*, *Eucalyptus globulus globulus*, *Eucalyptus cinerea*, *Eucalyptus globulus maidenii*, *Eucalyptus gunnii*, *Eucalyptus camaldulensis*, *Eucalyptus tereticornis* and hybrids such as *Eucalyptus grandis* x *Eucalyptus tereticornis*, *Eucalyptus grandis* x *Eucalyptus camaldulensis* has knockdown time KT₅₀ between 0.965 to 2.69 hours (62, 64.1, 64.5, 68.7, 74.5, 76.2, 81, 84, 112.5, 140, 161, 57.9, 63.8 minutes) whereas KT₅₀ of monoterpene compound of eucalyptus such as α-pinene and 1,8 cineole was found to have 38.8 and 55.3 minutes against group of twenty first instar nymphs of *B. germanica*. Yeom *et al.* (2013) reported that plant essentials oils from eucalyptus species namely *Eucalyptus*

polybractea, *Eucalyptus smithii*, *Eucalyptus radiata*, *Eucalyptus dives* and *Eucalyptus globulus* found to have complete mortality when tested for fumigant toxicity at 15 mg and 7.5 mg/L of air against male German cockroach whereas for female German cockroach, complete mortality was found to be at 15mg/L of air after 48h. In case of contact toxicity, complete mortality was recorded at 2mg / L of air for both male and female German cockroach which is clearly evident that eucalyptus essential oil serves as a fumigant and contact toxicity for German cockroaches. The LC₅₀ (ppm) values of *Eucalyptus globulus* essential oil was found to be 25.7 mg/cm³ for *Periplaneta americana*, 14.3 mg/cm³ for *Blattella germanica* and 15.28 mg/cm³ for *Supella longipalpa* whereas knockdown effect was found to be 100 percentage for 10 per cent per cubic centimetre after 1 hour, in which per cent mortality after recovery period was found to be 94±1.7, 94.2±4.1 and 95.2±3.6 after 24 h (Zibae and Khorram, 2015). The repellancy rate of Eucalyptus EO was found to be 85.00±4.08 against German cockroach (Chooluck *et al.*, 2019). The lethal dose of Eucalyptus EO was found to be 10.54 per cent and mixture of eucalyptus with rosemary (1:1) EO recorded 3.23 per cent against German cockroach (Asgari *et al.*, 2023). The contact toxicity of *Eucalyptus globulus* EO was found to be 47.58 µL/ml at 24 h exposure compared to *Citrus limon*, *Laurus nobilis*, *Mentha piperita*, and *Ocimum tenuiflorum* (Kamaraj *et al.*, 2024). Fumigant toxicity of Eucalyptus EO in enclosed spaces such as inaccessible cockroach hiding places (crevices or electrical or plumbing ducts and cabinet voids) could be useful for controlling the cockroach populations (Asgari *et al.*, 2023).

3.1 Chemical Constituents of *Eucalyptus globulus* Essential Oil

The chemical composition was determined by GC-MS is presented in Table 3. Five components representing 6-Methy-3-Trifluoromethyl-2,3-Dihydro-Benzo [1,4] Oxathine 4-Oxide representing 62.529 per cent of the total detected constituents in *Eucalyptus globulus* essential oil presented in Table 3. The other components were present in total amount of less than 37.471 % which constitute 1R. Alpha. Pinene (1.512 %), Benzoic acid, 4-(Aminosulfonyl)- (3.655 %), Dispiro[2.1.2.4]Undecane, 8-Methylene- (4.254

%), Benzoic acid, 4-Nitroso- (28.051 %). The compound 6-Methy-3-Trifluoromethyl-2,3-Dihydro-Benzo[1,4] Oxathine 4-Oxide constituting 62.529 per cent may be the characteristic feature to exhibit fumigant toxicity in American cockroach, *Periplaneta americana*. Among the chemical constituents 1R-alpha-pinene which is a configurational enantiomer of alpha-pinene (monoterpene), main secondary metabolite of many conifer-derived essential oils (Allenspach *et al.*, 2020; Baser and Buchbauer, 2016), juniper (Baser and Buchbauer, 2016, Dosoky *et al.*, 2019 and Fahed *et al.*, 2017) and cannabis where its enantiomer has been reported in black pepper, *Piper nigrum* (Asadi, 2022). The entourage effect produced by *Cannabis sativa* L which is primarily composed of α-Pinene constituents (Russo, 2011) and turpentine is mainly composed of α-Pinene (Baser and Buchbauer, 2016 and Zhu *et al.*, 2017) which is prominently used in cosmetics and beverages. α-Pinene ((1R,5R)-2,6,6-trimethylbicyclo[3.1.1]hept-2-ene) is a bicyclic hydrocarbon consisting of two isoprene units resulting in the sum formula of C₁₀H₁₆. α-Pinene with its volatile and hydrophobic properties has a fresh pine scent and woody flavour (Vespermann *et al.*, 2017). The insecticidal activity was reported by Burcul *et al.* (2020) which has a promising cholinesterase inhibitory activity). The contact toxicity LC₅₀ of α-pinene was found to be 4.133 ppm for *Sitophilus zeamais* (Langsi *et al.*, 2020). The toxicity activity of *E. camaldulensis* EO is attributed specially to 1,8-cineole and α-pinene as major constituents (Negahban and Moharramipour, 2007; Alzogaray *et al.*, 2011). α-pinene have contact and fumigant toxicity in stored product pests. These monoterpenes (typically volatile, and rather lipophilic compounds) were present in the pine needle essential oils in significant amounts and thus the toxicity of the essential oils may be attributed to the high total concentration of monoterpenes in the oil. Omara *et al.*, 2018; Papachristos *et al.*, 2004; Stamopoulos *et al.*, 2007). The mortality observed in fumigant toxicity assay could be as a result of volatile constituents entering the cuticle of the insects or due to nerve impulse inhibition of acetylcholine impulse which leads to paralysis and death of the insects (Abdelgaleil *et al.*, 2016). α-Pinene has the potential in repellent activity against *Periplaneta americana*. Therefore, it has the potential to repel *Blattella germanica* as both species are come from the same order (Blattodea) and family (Blattidae) (Nour *et al.*, 2017).

Table 2. Fumigant toxicity of *Eucalyptus globus* essential oil against the male American cockroach, *P. americana* (L.) at five different doses recorded after 12 h interval

| S. No | Treatment Dose ($\mu\text{L/L}$ of air) | Mortality Mean (%) 12 th HAT |
|-------|--|---|
| 1 | Eucalyptus EO - 5 μL | 26.67 (30.79) ^d |
| 2 | Eucalyptus EO - 10 μL | 33.33 (35.01) ^d |
| 3 | Eucalyptus EO - 20 μL | 46.67 (43.08) ^c |
| 4 | Eucalyptus EO - 30 μL | 60.00 (50.77) ^c |
| 5 | Eucalyptus EO - 40 μL | 80.00 (63.44) ^b |
| 6 | Eucalyptus EO - 50 μL | 100.00 (89.71) ^a |
| 8 | Untreated control (Negative control) | 0.00 (0.29) ^e |
| | Standard error of the difference, SE.d | 3.77 |
| | Critical Difference, CD (P=0.05) | 7.53 |

Mean of three replications; Values in parentheses are arcsine transformed values; Transformed values with same alphabets in superscript do not differ significantly ($p \geq 0.05$, by one-way ANOVA and Duncan's multiple range test)

Table 3. Probit analysis parameters of *Eucalyptus globus* essential oil toxicity against the male American cockroach, *P. americana* (L.)

a. Curve fitting parameters

| | |
|---|------------------------|
| Median lethal dosage, LD ₅₀ | 16.74784 μl |
| Upper confidence limit | 28.09616 μl |
| Lower confidence limit | 9.98322 μl |
| Y | 1.48x+31872 |
| Slope (b) | 1.484 |
| intercept (a) | 3.187 |
| Standard deviation, SD | 0.115 |
| Degree of Freedom, df | 3 |
| Correlation coefficient, R ² | 0.877 |
| Chi-test (χ^2) Sig | 0.903 |

b. Probit curve parameters

| | |
|---|-------|
| Slope (b) | 1.456 |
| intercept (a) | 3.217 |
| Correlation coefficient, R ² | 0.830 |



Fig. 1. Experimental setup of fumigant toxicity of *Eucalyptus globulus* essential oil against male American cockroach

Table 4. Chemical constitution of *Eucalyptus globus* essential oil by GC-MS and their relative proportion in pure essential oil

| S.No | Retention Time | Constituent | Molecular Weight | Formula | Peak Area % |
|------|----------------|--|------------------|--|-------------|
| 1 | 3.229 | 1R.Alpha.Pinene | 136 | C ₁₀ H ₁₆ | 1.512 |
| 2 | 4.249 | 6-Methy-3-Trifluoromethyl-2,3-Dihydro-Benzo [1,4] Oxathine 4-Oxide | 250 | C ₁₀ H ₉ O ₂ F ₃ S | 62.529 |
| 3 | 4.794 | Benzoic acid, 4-(Aminosulfonyl)- | 201 | C ₇ H ₇ O ₄ NS | 3.655 |
| 4 | 9.656 | Dispiro[2.1.2.4]Undecane, 8-Methylene- | 162 | C ₁₂ H ₁₈ | 4.254 |
| 5 | 12.342 | Benzoic acid, 4-Nitroso- | 151 | C ₇ H ₅ O ₃ N | 28.051 |

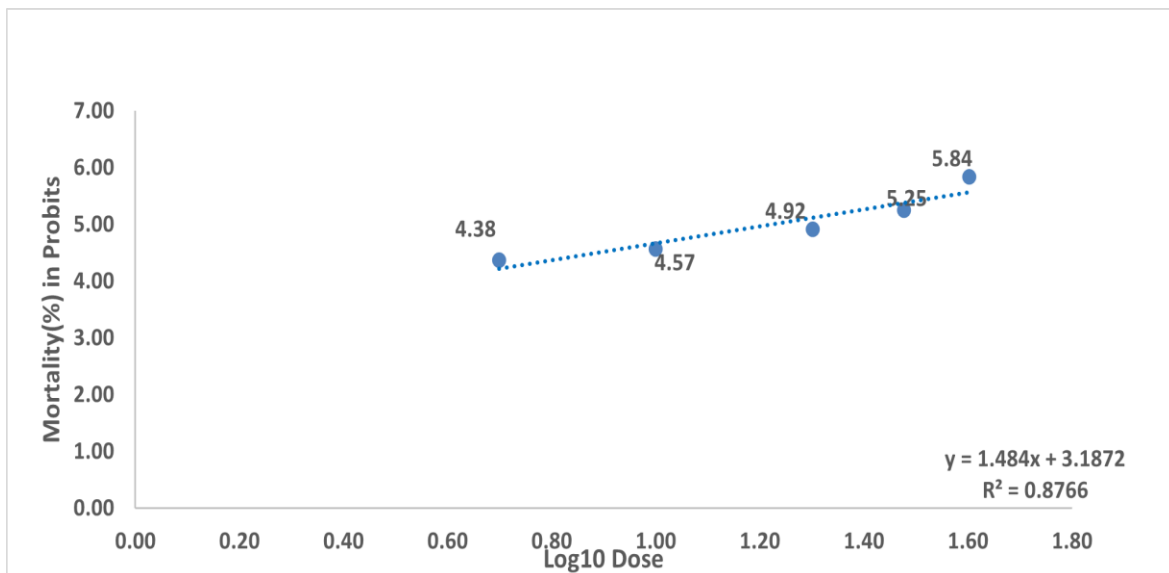


Fig. 2. Relationship between fumigant toxicity (LD₅₀) of *Eucalyptus globus* essential oil against the male American cockroach, *P. americana* (L.). Each point represent mortality due to six different doses (5,10, 20, 30, 40 and 50 µl) recorded after 12 h interval

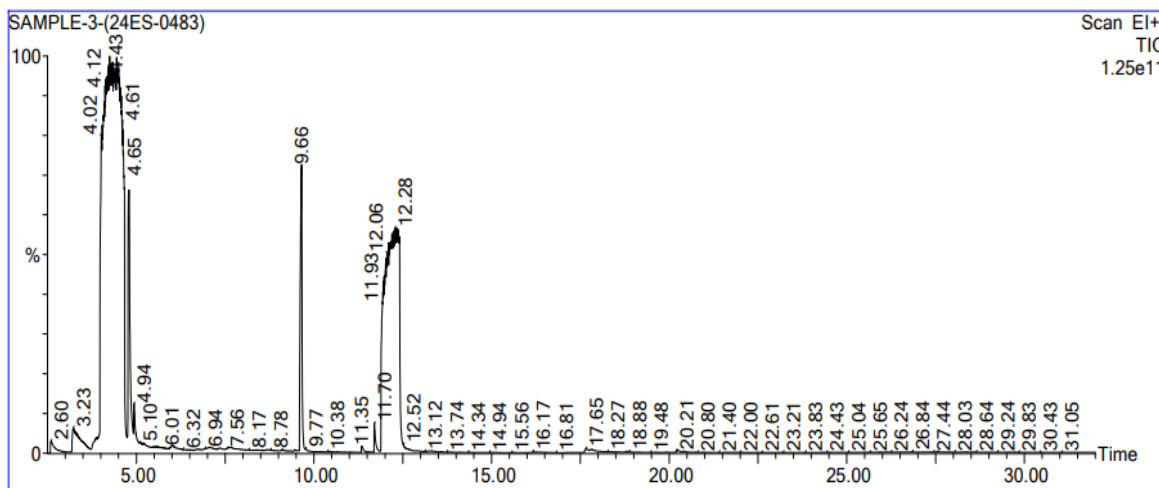


Fig. 3. Chromatogram of total components in the essential oil of *Eucalyptus globus*

4. CONCLUSION

The dose-response relationship between *Eucalyptus globulus* essential oil against male American cockroach mortality was evaluated and median lethal dosage LD₅₀ accurately modelled using Probit analysis. The median lethal dosage (LD₅₀) was determined to be 16.74784 µl, with corresponding mortality rates of 80%, 60%, 46.67%, 33.33%, and 26.67% at doses of 50, 40, 30, 20, and 10 µl, respectively. Notably, a dose as low as 5 µl still achieved 26.67% mortality. These findings suggest that *Eucalyptus globulus* essential oil is a potent insecticide against male American cockroaches. For practical applications, a recommended dose of 20-30 µl is proposed against male American cockroaches, which has mortality efficacy between 33.33-46.67 per cent with potential economic and environmental considerations. The chemical constituent 6-Methy-3-Trifluoromethyl-2,3-Dihydro-Benzo[1,4] Oxathine 4-Oxide representing conferred fumigant toxicity against American cockroaches. Evaluation of individual chemical compounds of *Eucalyptus globulus* EO and its combination could bring valuable insight of fumigant toxicity against American cockroaches. It could ideally elucidate the exact compound conferring toxicity and also helps in identifying the compounds which would act either synergistic or antagonistic, that would derive ideal and enriched chemical composition against American cockroach. The eucalyptus essential oil is an excellent source of fumigant and contact toxicity where it serves as a potential repellent agent against cockroach where its hidden infestation and hard-to-reach areas can be easily eliminated. It can be used to treat in food preparation area to deter cockroach infestations. The essential oil and its components will support the design and development of innovative pest control strategies. It can be potentially applied and far effective in areas where chemical pesticides were completely restricted in food preparation areas such as pantry in restaurants, hotels, apartments, hospitals etc., Safe concerns against human and its potential risk of residual impact were completely met out by *Eucalyptus globulus* essential oil where its mixture of compounds act as a prominent fumigant and contact toxicity and repellent activity against wide range of pests. Further research is warranted to explore synergistic effects, optimal formulation, and field efficacy.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models

(ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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