RESEARCH PAPER

Larvicidal, Pupicidal and Adulticidal Efficacy of Essential oils Against Musca Domestica L.: Molecular Docking Insights into Acetylcholine Esterase Inhibition

Deepa H N[1], Ramya Manjunath[*2a,b], Mousumi Mondal[3]

[1,2a]*Department of Biotechnology, School of Applied Sciences, REVA University, Bengaluru.*
[2b]*Centre for Biotechnology and Molecular Biology, REVA Research Centre, REVA University, Bengaluru-560064 Karnataka, India*
[3]*Aspartika Biotech Pvt Ltd, Bangalore.*

Received: 28th Feb, 2026; Revised: 6th March 2026; Accepted: 7th April, 2026; Available Online: 20th April, 2026

ABSTRACT
Houseflies (Musca domestica L.; Diptera: Muscidae) are anthropophilic pests of major public health concern owing to their ability to mechanically transmit various animal and human pathogens. In the present study, essential oils of eight medicinal and aromatic plants, Eucalyptus globulus, Piper nigrum, Cymbopogon citartus, Syzygium aromaticum, Zingiber officinale, Rosmarinus officinalis, Ocimum basilicum, and Curcuma longa, were investigated for their larvicidal, pupicidal and adulticidal properties, at different doses against M. domestica. Larvicidal activity with LC50 was observed in the range from 3.12 - 6.46 %, pupicidal activity between 6.41-11.05% and adulticidal activity in the range of 4.91 - 7.75%. S. aromaticum, E. globulus, and C. citartus essential oils showed to be most effective against larvae, pupae, and adults, while Z. officinale and O. basilicum oils R. officinalis and P. nigrum oils exhibited moderate activity. Furthermore, Drosophila melanogaster Acetyl Choline Esterase (AchE) crystal structure (PDB ID: 6XYU) was used as a template for docking studies to analyze the interactions of bioactive components of essential oils. Beta-caryophyllene in S. aromaticum oil has a strong binding affinity of −7.1 kcal/mol, whereas eugenol has a −6.2 kcal/mol affinity. Neral (−6.6 kcal/mol) and geraniol (−5.7 kcal/mol) from C. citartus; limonene (−6.1 kcal/mol) and 1,8-cineole (−5.9 kcal/mol) from E. globulus showed moderate binding. This study proposes plant-based essential oils as promising, environmentally sustainable options for combating houseflies, given growing concerns about insecticide resistance, ecological toxicity, and health risks associated with synthetic insecticides.
Keywords: *Essential oils, Musca domestica, Insecticidal Activity, LC50, Acetylcholinesterase, Molecular Docking, beta-Caryophyllene, Eugenol, Natural Pesticides.*
How to cite this article: Deepa HN, Manjunath R, Mondal M. Larvicidal, Pupicidal and Adulticidal Efficacy of Essential oils Against Musca Domestica L.: Molecular Docking Insights into Acetylcholine Esterase Inhibition. Int J Drug Deliv Technol. 2026;16(55s): 546-560. DOI: 10.25258/ijddt.16.55s.56
Source of support: Nil.
Conflict of interest: None

1. INTRODUCTION

Houseflies (Musca domestica L.) are the most significant global stubborn insect pests, which are fierce carriers of more than 1000 pathogenic organisms, from bacteria and viruses to protozoa and parasites1,2. They have become highly adapted to the human environment, including apartment landfills, animal shelters, food storage facilities and can reproduce on decaying materials3,4,5. These insect pests cause major health problems; hence, their control is essential for maintaining hygiene and quality of life1,4,6. Conventional pest management methods include chemical, mechanical, biological, and sanitary practices, with limitations. Chemical methods using organophosphates and pyrethroids leads to resistance over time, pollution, and toxicity to non-target organisms4,7. Mechanical control using UV light traps and sticky traps is effective at reducing fly numbers but demands continuous monitoring and maintenance, may fail under heavy infestations. Biological control using parasitic wasps, entomopathogenic fungi, and entomopathogenic nematodes is possible but slow-acting, time-consuming, and climate-dependent. Sanitation and waste disposal are largely preventable, but they must be practiced regularly and implemented consistently over the long term to be effective1. Insecticidal and fumigation methods provide short-term relief but do not completely eradicate fly populations due to resistance and larval survival5. The essential oils can be a matter of hope for pest management. Essential oils can interfere with the physiological and metabolic processes of insects, leading to mortality, and can be a promising substitute to synthetic insecticides8,9. The key components of essential oils possess insecticidal, repellent, growth-deterrent, larvicidal, and pupicidal properties. Additionally, they function as competitive inhibitors of acetylcholinesterase, the enzyme that catalyzes the hydrolysis of acetylcholine, a key neurotransmitter in the insect nervous system10,11. The inhibition of acetylcholinesterase disrupts the cholinergic system, which is essential for insect survival. The nine selected plants to derive essential oils have diverse applications – Curcuma longa, Cymbopogon citratus, Eucalyptus globulus, Ocimum basilicum, Rosmarinus officinalis and Syzygium aromaticum have been used as deodorisers, repellents, growth deterrents, and ovipositional deterrents, in addition to larvicidal and pesticidal properties12,13,14,15. Syzygium aromaticum oil has major active components such as Eugenol, Caryophyllene oxide, and caryophyllene. Eugenol acts as a neurotoxin by inhibiting enzyme activity, leading to nervous system failure and death of the insects and pests. According to the literature, Syzygium aromaticum oil exhibits 100% mortality in mosquitoes and stored-product pests due to its fumigant properties16. Similarly, Curcuma longa contains α-turmerone, β-turmerone, and Ar-turmerone, which disrupt insect metabolism and energy production and have effective repellent properties against agricultural pests such as aphids and weevils16,17,18. Cymbopogon citratus oil, extracted through hydro distillation, contains neral and geranial, possessing antimicrobial, anti-inflammatory, and mosquito-repellent activities 11. The multifunctionality of Cymbopogon citratus has made it very popular in the cosmetic, insecticide, and agricultural sectors. Piper nigrum essential oil is a potent insecticide with high levels of alpha-pinene, beta-pinene, carene, limonene, and beta-caryophyllene, and has been shown to have a strong insecticidal effect, especially against house flies8. Essential oils of Zingiber officinale is a mixture of camphene, eucalyptol, and linalool, which interfere with respiratory and the nervous system's function, are therefore very toxic to cockroaches and stored-product pests. Eucalyptus globulus oil include limonene, alpha-pinene, 1,8-cineole, p-cymene, and beta-pinene, which are widely used as insecticide. The impact of monoterpenes on flies has been widely reported19. The constituents of Ocimum basilicum have insecticidal, nematicide and fungistatic effects. Ocimum basilicum oil contains 1, 8- cineole, camphor and eugenol, indicating its larvicidal and pupicidal properties. Similarly, Rosmarinus officinalis oil, contains alpha-pinene, has exhibited fumigant toxicity to houseflies. The present study primarily focuses on assessing the biocontrol potential of various essential oils against the larval, pupal, and adult stages of M. domestica, and their effectiveness as a substitute for synthetic insecticides21. This study aims to establish environmentally friendly methods for controlling the housefly by understanding its chemical constitution and toxicity. The efficacy of these essential oils is ascertained by their physicochemical characteristics such as acid value, refractive index, yield density and ester value. They affect stability, solubility, and overall performance of the oils. To improve pest control, these properties can be used to optimize formulation and application techniques. The constituents of each essential oil are analyzed using GC-MS. Their larvicidal, pupicidal, and adulticidal effects are evaluated by exposing the houseflies to various concentrations of essential oils, and the mortality rates are recorded post application. The specific goal of this combined study was to investigated the potential if essential oils as eco-friendly substitutes for chemical pesticides.21,22,23. Further, employing molecular docking approach, the major compounds from essential oils with high LC50 values were used to determine the desired binding orientation and measurable affinity for the target enzyme, Acetylcholinesterase (AChE). Inhibition of AchE, a key enzyme of insect neurotransmission, by phenylpropanoids and terpenoids, the components of essential oils, is the mechanism of neurotoxicity, resulting in acetylcholine buildup, overstimulation of nerve impulses, and eventual paralysis24,25. To enable this crucial computational analysis, the M. domestica AChE structure was reliably generated by homology modelling, using the known Drosophila melanogaster AChE crystal structure as a template26,27,28. Focusing the docking analysis on the components of these three highly active oils, the study efficiently clarifies the molecular basis of the efficacy established against all housefly life stages, from larvae to adults.

2. MATERIALS AND METHODS

2.1. Plant material:

In the present study, 8 plants were used to extract active components via essential oil. The plants selected are Eucalyptus (Eucalyptus globulus), Black Pepper (Piper nigrum), Lemongrass (Cymbopogon citartus), Clove (Syzygium aromaticum), Ginger (Zingiber officinale), Rosemary (Rosmarinus officinalis), Sweet Basil (Ocimum basilicum), and Curcuma longa (Curcuma longa).

2.2. Extraction of Essential Oils

Essential oils from the selected aromatic plants were extracted by hydro-distillation using a Clevenger – type apparatus. 100 g of shade dried and finely powdered plant materials of the mentioned plants were subjected to hydro-distillation for 3 hours to achieve maximum essential oil recovery. The extracted oils were collected separately, weighed and the yield was calculated as percentage (w/v). Residual traces of essential oils were recovered using hexane and anhydrous sodium sulphate was added to eliminate residual moisture from the extracts23,29. The oils was stored in amber-colored vials and refrigerated until further analysis.

2.3. Quality analysis of the Essential oil extracts

2.3.1. Evaluation of physicochemical parameters: The physicochemical parameters of the extracted essential oil were evaluated on the basis of color, appearance, specific gravity, acid value, and Ester value as per the AOAC, 1990 &AOAC, 2000 methods.

2.3.2. Chemo profiling of oil components through GC-MS: GC-MS analysis was used to ascertain the chemical composition. The column was initially heated to 60° C for 5 min, then raised to 240° C at a rate of 5° C/min. The helium gas was the carrier gas employed at a split ratio of 1:30 and a flow rate of 1 mL/min. The analysis was performed using GC-MS solution\library\nist17. Finally, component identification was performed by comparing their respective retention times and mass spectra with data from the NIST 17.1 library and the available literature23,32,33.

2.4. Rearing of Musca domestica: Housefly larvae were collected from a poultry farm in Basavapattana village, Shidlagatta Taluk, Chikkaballapur District. The collected larvae were transferred to containers and covered with cotton cloth to allow ventilation. The larvae were fed to a nutrient-rich substrate consisting of poultry feed (wheat bran, yeast, and water) and were placed in a laboratory condition at a temperature of (27.5±2.0) °C and a relative humidity of (72.5±2.0) %, maintained under a photoperiod of 12:12 hours of light and darkness. After five days, the larvae converted into pupae, which were separated from the container and placed separately. The pupae transformed into adult moths after four to five days. The laid eggs were placed on a sawdust soaked with milk medium, to keep them moist and encourage hatching. All stages of the housefly were maintained continuously for the experiments8,23,34,35,36.

2.5. Effect of essential oil against Musca domestica larvae: The third instar larvae of Musca domestica were collected and placed in sterile containers, with 20 larvae, for each concentration. The concentrations of each essential oil prepared by serial dilution range from 25% to 1.56%. It is recommended to use Tween 20 as an emulsifier, diluted according to the WHO guidelines, with minor adjustments. The essential oil was uniformly exposed to housefly larvae, pupae, and adults using the residue film method to assess its activity. Tween 20 and distilled water served as negative controls and cypermethrin was positive control. Both the test groups and the control group were fed on a diet of wheat bran, yeast, and water. Mortality rates were recorded 24 hours post-treatment, alongside behavioral changes and sub-lethal effects that indicated physiological stress 36,37,38.

2.5.2. Effect of Essential Oil against Musca domestica Pupa: The 2 to 3-day-old pupa was selected for pupicidal bioassays in a small container with required aeration. The housefly pupae were subjected to different concentrations of each oil, ranging from 25% to 1.56%, along with controls. The pupicidal effect was studied by the residue film method with slight modifications. LC50 values were determined using different concentrations of the selected essential oils. The diluted oils were transferred to cotton swabs and placed into plastic containers containing pupae. These methods were performed at 28° C and a relative humidity of 65-70%. If the pupa emerged as an adult in more than 10% of cases during the experiment, the experiment was considered void and repeated. The observation and effectiveness of the essential oils were recorded on the 6th day of exposure. The percentage inhibition of pupal emergence was measured 39.

2.5.3 Effect of Essential Oil against Musca domestica Adult Flies: The adulticidal assay was carried out in 200 ml bottles; 20 adult flies were placed in each bottle. Each cotton swab is treated with various concentrations of 8 essential oils, within the range of 25% to 1.56%. The bioassay was carried out in triplicates. The bottles were then closed and kept at 28 ± 2 °C. The death rates were measured 24h after treatment40,41.

2.5.4. Statistical Analysis: Statistical analysis was done using Prism 8.4.2. Probit analysis was performed to ascertain the LC50, at $p < 0.05$. A one-way ANOVA was performed to evaluate the differences among treatments, followed by Tukey's Honestly Significant Difference (HSD) post-hoc test for multiple comparisons.

2.5.5 In-silico study of the MdAChE inhibitory potentials of bioactive components from selected essential oils.

The 3D structure of Musca domestica Acetylcholinesterase (MdAChE, UniProt Accession No. Q8MXC4) has been predicted by comparative homology modelling on the SWISS-MODEL template modelling server. The high-resolution crystal structure of Drosophila melanogaster AChE in its Tacrine-bound form (PDB Code: 6XYY, 2.7 Å resolution) has been used as a template for this purpose. The template model is important because it involves a ligand-bound structure, ensuring that it preserves the details of the AChE binding pocket in its catalytically active state. Based on sequence alignment, the 3D structural coordinates of the model template, with insertions and deletions modelled by energy-minimised loops, were transferred to MdAChE. Finally, to interpret MdAChE structure, it was modelled by including its functional structure with respect to its template model, which illustrates that it has been modelled into its functional homodimer structure. To validate this model, it has been analyzed using GMQE, QMEAN, and QSQE scores, focusing on the overall model and its quaternary structure. Later, this model underwent various validations to assess its stereochemistry, focusing on Ramachandran favored model structures, Ramachandran outliers, bad bond structures, and bad angle structures 42. To pick the best model with overall high GMQE scores, Ramachandran favored Structure Analysis was preferred, to minimize bad structures. Molecular docking has been carried out using the tools CB Dock 2, Blind docking, and Discovery Studio 2025 for visualization25,26,28,43.

3. RESULTS

3.1. Essential oil content (%) (v/w) of aromatic plants: The essential oil content from the eight plants obtained through hydro-distillation is mentioned in the Table 1

Table 1: Extraction of Essential oil by the hydro-distillation method emphasizing the duration of extraction and the yield percentage of oils.

Sl. No	Plant Name	Scientific Name	Part used	Duration of extraction (Hrs.)	Oil Content (%)
1	Black pepper	Piper nigrum	Dried seeds	5	4.52
2	Clove	Syzygium aromaticum	Leaves	3	9.16
3	Eucalyptus	Eucalyptus globulus	Leaves	3	2.11
4	Ginger	Zingiber officinale	Dried Rhizome	5	1.2
5	Lemongrass	Cymbopogon citratus	Aerial Part	3	1.14
6	Rosmary	Rosmarinus officinalis	Leaves	3	0.96
7	Sweet Basil	Ocimum basilicum	Whole plant	3	1.12
8	Turmeric	Curcuma longa	Dried Rhizome	5	2.67

3.2. Quality analysis of essential oils and extract by physio-chemical parameters: The essential oil and oleoresin extracted were evaluated according to the physio-chemical parameters such as appearance, specific gravity (Gravimetric method), ester value, saponification value, and Peroxide value as per the AOAC 1999 methods. The results are mentioned in Table 2.

Table 2: Physicochemical properties of extracted essential oils

Sl. No	Plant Name	Appearance	Specific Gravity at 30°C	RI	Acid value	Ester value
1	Piper nigrum	Bright Yellow	0.90	1.48	0.78	55.11
2	Syzygium aromaticum	Light brown	1.038	1.52	2.03	23.4
3	Eucalyptus globulus	Bright yellow	0.9060	1.46	2.01	10.01
4	Zingiber officinale,	Pale yellow	0.989	1.49	1.07	131.28
5	Cymbopogon citartus	Light Yellow	0.892	1.48	4.48	14.02
6	Rosmarinus officinalis	Colorless	0.904	1.46	0.98	60,550
7	Ocimum basilicum	Light Yellow	0.933	1.67	3.31	24.9
8	Curcuma longa	Bright yellow	0.977	1.44	1.79	27.88

3.3. Chemo profiling of the Oil components through GC-MS: The chemo profiling of the extracted essential oil was checked by GCMS. The major active components of the extracts were determined. Piper nigrum is rich in alpha-pinene (6.36%), beta-pinene (8.33%),3-Carene (2.10%), Limonene (5.70%), Beta-caryophyllene (14.79%). Syzygium aromaticum oil is rich in Eugenol (83.47%), Beta-Caryophyllene (12.06%). Eucalyptus globulus has Eucalyptol and 1,8-cineole. Zingiber officinale, is rich in Zingiberene (30.76%), alpha.-Farnese (16.43%), alpha-pinene (2..3%), Camphene (5.49%), β-Sesquiphellandrene (13.14), Cymbopogon citratus has Niral (22.99%) and Geraniol (3.82%), α-Citra(40.94%), Rosmarinus officinalis is rich in α-Pinene(14.84%), Camphene (9.47%), β-Pinene(5.91%), Eucalyptol(21.85%), Ocimum basilicum is rich Linalool (25.93%), camphor (61.39%), while Curcuma longa Ar-Turmerone (45.209%), α-Turmerone (6.003%), β-Turmerone (1.199%).

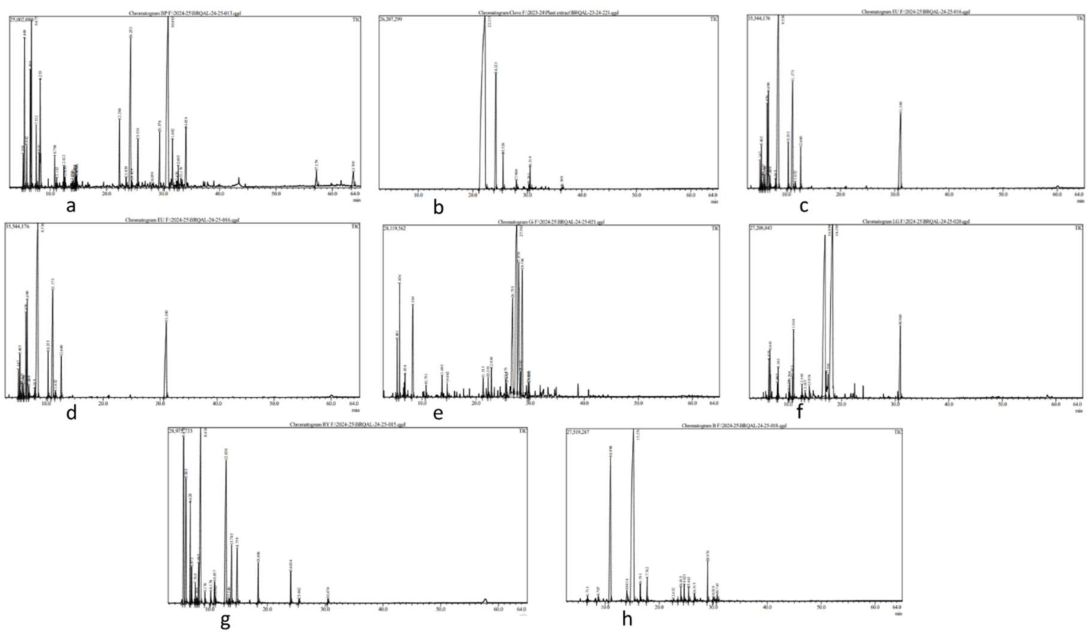


Fig. 1: Chromatogram of the hydro-distilled essential oil extract of a) Piper nigrum, b) Syzygium aromaticum, c) Eucalyptus globulus, d) Zingiber officinale, e) Cymbopogon citratus, f) Rosmarinus officinalis, g) Ocimum basilicum, h) Curcuma longa, using GC-MS. The chromatographic profile indicates distinct peaks at various retention intervals, reveals the presence of several volatile phytoconstituents, while tiny peaks indicate trace components. The chromatogram was obtained under standardized GC-MS operating conditions and used for the qualitative characterization of phytochemical components present in essential oils extract.

Table 3: Major Active components identified from GCMS Analysis

Component Identified	P. nigrum	S. aromaticum	E. globulus	Z. officinale	C. citratus	R. officinalis	O. basilicum	C. longa
α-Pinene	6.36	-	2.59	2.30	-	14.84	-	-
β-Pinene	8.33	-	-	-	-	5.91	-	-
3-Carene	2.10	-	-	-	-	-	-	-
Limonene	5.70	-	-	-	-	-	-	-
Sabinene	5.42							
β-Caryophyllene	14.79	12.06	-	-	-	-	1.04	-
Eugenol	-	83.47	-	-	-	-	-	-
Eucalyptol	-	-	62.41	8.34	-	21.85	-	-
Zingiberene	-	-	-	30.76	-	-	-	-
α-Farnesene	-	-	-	16.43	-	-	-	-
Camphene	-	-	13.72	5.49	-	9.47	-	-
Camphor			3.35					
β-Sesquiphellandrene	-	-	-	13.14	-	-	-	-
Neral	-	-	-	-	33.97	-	1.12	-
Geraniol	-	-	-	-	40.94	-	-	-
α-Citral	-	-	-	-		-	1.59	-
Linalool	-	-	-	-		-	25.93	-
Ar-Turmerone	-	-	-	-	-	-	-	45.21
α-Turmerone	-	-	-	-	-	-	-	6.00
β-Turmerone	-	-	-	-	-	-	-	1.20
Anethole	-	-	-	-	-	-	61.39	-

3.4. Rearing of Housefly (Musca Domestica): The entire life cycle, from egg to adult, can be completed in about 10-15 days under optimal conditions, such as warm temperatures and adequate food supply. The rapid reproduction rate and short generation time contribute to the housefly's ability to thrive in various environments. Figure 2 demonstrates the standard laboratory rearing protocol and developmental progression of M. domestica.

a. Rearing of *Musca domestica* ***b. Prepared diet*** ***c. Eggs deposited on the***

d. Larvae of M. domestica **e. Pupal stage** **f. eclosed adult flies**

Fig. 2: Rearing of house flies and observation of different developmental stages in the life cycle of Musca domestica. (a). Adult houseflies maintained in insect rearing bottles under standard laboratory conditions ((27.5±2.0 °C and a relative humidity of 72.5±2.0 %, maintained under a photoperiod of 12:12 hours of light-dark photoperiod). (b). Media used for the growth of house flies. (c). Freshly laid eggs of M. domestica, appearing as creamy-white clusters on the oviposition medium. (d). Larvae forms of M. domestica. (e). Pupal stage showing reddish brown to dark brown puparia under optimized conditions. (f). Emergence of newly eclosed adult flies from pupae, demonstrating successful completion of metamorphosis.

3.5. Toxicity Assays: The eight plant essential oils were examined against the larvae of Musca domestica under controlled conditions at different concentration ranges from (25%- 1.56%). The tested oils exhibited dose-dependent mortality, with higher concentrations associated with increased mortality, and this effect was observed at 24h exposure.

3.5.1. Larvicidal activity: Table 4 summarises the larvicidal potential of the eight essential oils against third-instar M. domestica, the mortality rates of larvae exposed to different concentrations are shown. Three replicates (R1, R2, and R3) are provided for each concentration. All the eight essential oils showed 50-60% to 90-100% mortality at 12.5% and 25% concentration. Syzygium aromaticum exhibited the highest toxicity with the lowest LC_{50} (3.12%), followed by Eucalyptus globulus (LC_{50} - 4.10%) and Cymbopogon citratus (LC_{50} - 3.57%) at 24h of exposure. The ranks of essential oils as per their larvicidal effectiveness were as follows: Syzygium aromaticum > Cymbopogon citartus >Eucalyptus globulus > Rosmarinus officinalis > Curcuma longa > Zingiber officinale > Piper nigrum > Ocimum basilicum. The differences in larvicidal potency can be credited to variations in chemical composition and the relative abundance of bioactive compounds in each oil. At all tested concentrations, cypermethrin exhibited strong larvicidal action against Musca domestica larvae, leading to 100% mortality (100.0±0.0%) with an LC50 value of less than 1%. On the contrary, Tween 20 and distilled water exhibited no larvicidal effect. M. domestica displayed morphological flaws and larval mortality at all tested doses of essential oils. Considerable structural damage included inflated and burnt integuments in dead larvae. The presence of anomalous morphology was used to identify deformed larvae after exposure to the treatments. The data were statistical analysis using one-way ANOVA and appropriate post hoc tultiple comparison tests at $p < 0.05$ level of significance. Lower LC50 values suggest more potent larvicidal action of the treatments. The experimental validation was verified by using positive and negative controls. Figure 3 represents the dose-response curve illustrating the larvicidal potential of different plant derived essential oils against larvae at different concentrations. A concentration-dependent increase in larval mortality was observed for all tested concentrations, depicting greater larvicidal efficacy with increasing concentration.

Table 4: Larvicidal activity of different treatments against larvae expressed as mortality percentage (Mean ± SD), LC 50 values and 95% confidence intervals.

	MORTALITY%, SD OF LARVAE					
Treatment	**25%**	**12.5%**	**6.25%**	**3.13%**	**1.56%**	**LC50 (%) 95% CI (LHL-UHL)**
C. longa	100 ± 0	91.67 ± 2.89	56.67 ± 2.89	16.67 ± 2.89	3.33 ± 2.89	5.87 (5.556 -6.237)
C. citratus	100 ± 0	95 ± 5	76.67 ± 2.89	41.67 ± 2.89	11.67 ± 2.89	3.57 (3.129 -4.072)
E. globulus	100 ± 0	86.67 ± 5.77	56.67 ± 7.64	51.67 ± 2.89	35 ± 5	4.10 (3.210 -4.941)
O. basilicum	100 ± 0	88.33 ± 2.89	50 ± 5	23.33 ± 5.77	2.89 ± 0	6.461 (5.816 -7.212)
P.nigrum	100 ± 0	88.33 ± 2.89	50 ± 5	23.33 ± 5.77	6.67 ± 2.89	6.46 (5.922 -7.077)
R. officinalis	100 ± 0	96.67 ± 2.89	76.67 ± 2.89	53.33 ± 2.89	18.33 ± 2.89	4.13 (3.769-4.529)
S. aromaticum	100 ± 0	100 ± 0	83.33 ± 5	51.67 ± 5	30 ± 2.89	3.12 (2.916-3.332)
Z. officinale	100 ± 0	96.67 ± 2.89	51.67 ± 2.89	21.67 ± 2.89	6.67 ± 2.89	6.12 (5.860 -6.415)
Negative Control	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	NA
Positive Control	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	<1.0

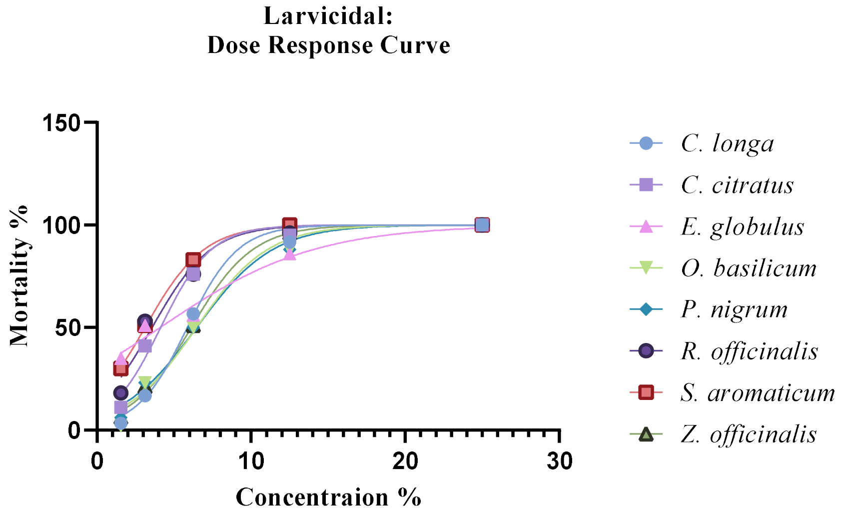


Fig 3: Dose–Response curve showing dose-dependent inhibition of essential oils against Musca domestica larvae.

Dose-response graphs showing the ability of essential oils to kill larvae. Five concentrations (25.00–1.56%) were selected to measure mortality (%), with each point representing the mean ± SD of three replicates. In GraphPad Prism, nonlinear regression was used for fit curves.

3.5.2: Pupicidal activity: The pupicidal activity of the essential oils against M.domestica pupae mortality% is presented in Table 5. All the 8 eight essential oils at 25%-12.5% concentration affected high mortality rates, ranging from (100.0%-50%) with LC50 values and LC90 values ranging from (6.41% - 11.05%), respectively. In pupicidal assays, Syzygium aromaticum showed the highest effectiveness with the lowest LC_{50} (6.41%), followed by Eucalyptus globulus (LC_{50}: 6.40%) and Cymbopogon citratus (LC_{50}: 7.60%) at 10 th day. Intermediate toxicity was observed for Piper nigrum (LC_{50}: 8.71%), Zingiber officinale (LC_{50}:8.78) and Ocimum basilicum (LC_{50}: 8.76), while lower activity was noted in Curcuma longa (LC_{50}: 11.05) and Rosmarinus officinalis (10.24%). The ranks of eight essential oils as per their effectiveness as pupicidal agents were as follows: Syzygium aromaticum > Eucalyptus globulus > Cymbopogon citratus> Ocimum basilicum > Piper nigrum > Zingiber officinale. Rosmarinus officinalis > Curcuma longa. Cypermethrin (positive control) was extremely toxic at all tested doses, with an LC50 value of less than 1% and mortality rate of 100 ± 0%, while distilled water & tween 20 did not show any pupicidal activity The pupicidal activity of the oils followed a similar trend to their larvicidal activity, but higher concentrations were required to achieve comparable mortality rates. The data were statistically analysed using one-way ANOVA at $p < 0.05$. Lower LC50 values suggest more potent pupicidal action of the treatments. The experimental validation was verified by using positive and negative controls. Figure 4 represents the dose-response curve illustrating the pupicidal activity of different essential oils against pupae at different concentrations. A concentration-dependent increase in pupal mortality was observed for all tested concentrations, depicting greater toxicity with increasing concentration.

Table 5: The mean mortality% and LC50 values of M. domestica pupa to eight essential oils, cypermethrin and a negative control (water and Tween 20) at 10 th day (%)

Treatment	Mortality%, SD of Pupa					LC50 (%) 95% CI (LHL-UHL)
	25.00%	12.50%	6.25%	3.13%	1.56%	
C. longa	100 ± 0	58.33±2.89	26.67±2.89	5.00±0.00	1.67±2.89	11.05 9.079 - 13.51
C. citratus	100 ± 0	83.33±2.89	41.67±18.9	13.33±7.64	1.67±2.89	7.60 6.024 - 9.426
E. globulus	100 ± 0	86.67±2.89	51.67±2.89	23.33±2.89	6.67±2.89	6.40 5.119 - 8.069
O. basilicum	100 ± 0	73.33±2.89	38.33±2.89	13.33±2.89	0.00±0.00	8.76 6.401 - 11.53
P.nigrum	100 ± 0	73.33±2.89	38.33±2.89	13.33±2.89	1.67±2.89	8.71 6.609 -11.20
R. officinalis	100 ± 0	65.00±5.00	28.33±2.89	6.67±2.89	0.00±0.00	10.24 8.371 -12.45
S. aromaticum	100 ± 0	93.33±2.89	48.33±2.89	16.67±2.89	0.00±0.00	6.41 5.516 -7.695

Z. officinale	100 ± 0	76.67±2.89	33.33±2.89	11.67±2.89	0.00±0.00	8.78 7.155 -10.53
Negative Control	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	NA
Positive Control	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	<1.0

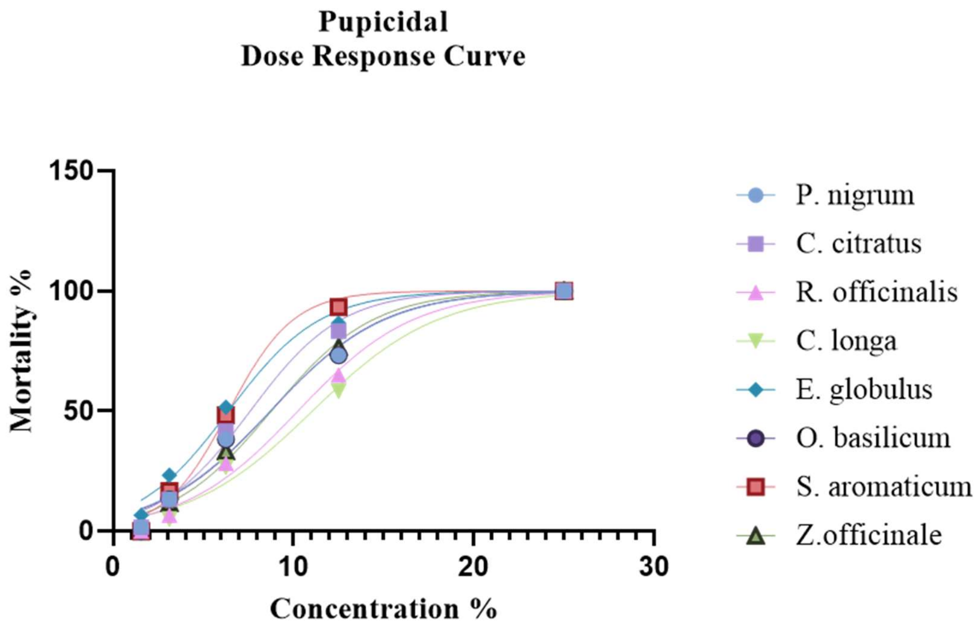


Fig 4: Dose–Response Curve Showing Dose-Dependent Inhibition of Essential Oils against Musca domestica pupa.

Dose-response graphs showing the ability of essential oils to kill the pupa. Five concentrations (25.00–1.56%) were selected to measure mortality (%), with each point representing the mean ± SD of three replicates. In GraphPad Prism, nonlinear regression was used for fit curves.

3.5.3. Adulticidal activity: Adulticidal activity of the essential oil against 2-day-old adult houseflies is presented in Table 6. Adulticidal activity indicated that Syzygium aromaticum was the most potent oil with the lowest LC_{50} (4.91%), followed by Cymbopogon citratus (5.63) and Eucalyptus globulus (LC_{50}: 5.45). moderate toxicity was observed Piper nigrum (LC_{50}: 7.05) Ocimum basilicum (LC_{50}: 7.16) and Rosmarinus officinalis (LC_{50}: 7.12) while Curcuma longa (LC_{50}: 7.64). exhibited comparatively lower effects. The least adulticidal activity was observed for Zingiber officinale (LC_{50}: 7.75). All oils caused 100% mortality at the highest concentration (25%), with decreasing mortality observed at lower concentrations. The ranks of eight essential oils according to their effectiveness against female house flies are as follows: Syzygium aromaticum > Eucalyptus globulus > Cymbopogon citartus > Piper nigrum >Rosmarinus officinalis >Ocimum basilicum > Curcuma longa >Zingiber officinale. Distilled water and tewwn 20 did not exhibit any adulticidal action, while cypermethrin was extremely toxic to house flies at all tested concentrations, with a a mortality rate of 100 ± 0.0 % and an LC50 value of less than 1.0%. To identify significant variations between the concentrations and treatments, the data was statistically analysed using one-way ANOVA and suitable post hoc multiple comparison tests. A significance level of $p < 0.05$ was established. Lower LC50 values suggest more potent adulticidal action of the treatments. The experimental validation was verified by using positive and negative controls. Figure 5 represents the dose-response curve illustrating the adulticidal activity of all essential oils against adult flies at different concentrations. A dose-dependent increase in mortality was observed for all tested concentrations, depicting greater toxicity with increasing concentration.

Table 6: The mortality rate and LC50 values of M. domestica Adults to eight essential oils, cypermethrin and a negative control (water and Tween 20) at 24h of exposure

	Mortality%, SD of Adult fly					
Treatment	**25%**	**12.50%**	**6.25%**	**3.13%**	**1.56%**	**LC50 LHL-UHL**
C. longa	95.00 ± 5.00	90.00 ± 5.00	45.00 ± 5.00	13.33 ± 2.89	6.67 ± 2.89	6.89 5.926 - 8.065
C. citratus	100.00 ± 0.00	83.33 ± 2.89	61.67 ± 2.89	26.67 ± 2.89	8.33 ± 2.89	5.67 3.871 - 8.704
E. globulus	100.00 ± 0.00	96.67 ± 2.89	51.67 ± 2.89	38.33 ± 2.89	16.67 ±2.89	5.46

						3.927 - 7.265
O. basilicum	100.00 ± 0.00	88.33 ± 2.89	41.67 ± 2.89	6.67 ± 2.89	0.00 ± 0.00	7.16 5.882 - 9.051
P.nigrum	100.00 ± 0.00	95.00 ± 5.00	38.33 ± 2.89	20.00 ± 5.00	1.67 ± 2.89	7.05 5.860 - 8.530
R. officinalis	100.00 ± 0.00	88.33 ± 2.89	40.00 ± 5.00	26.67 ± 2.89	3.33 ± 2.89	7.12 5.387 - 9.293
S. aromaticum	100.00 ± 0.00	88.33 ± 2.89	70.00 ± 5.00	26.67 ± 2.89	8.33 ± 2.89	4.91 3.815 - 6.519
Z. officinale	100.00 ± 0.00	78.33 ± 2.89	41.67 ± 2.89	23.33 ± 2.89	6.67 ± 2.89	7.75 6.214 - 9.532

The ANOVA results for each essential oil treatment show significant differences in adult mortality across the different concentration levels of each essential oil. The p-value for each essential oil is p <0.001, which is less than the regularly used significance level of 0.05, indicating that the differences in mortality between the groups are statistically significant for each essential oil tested.

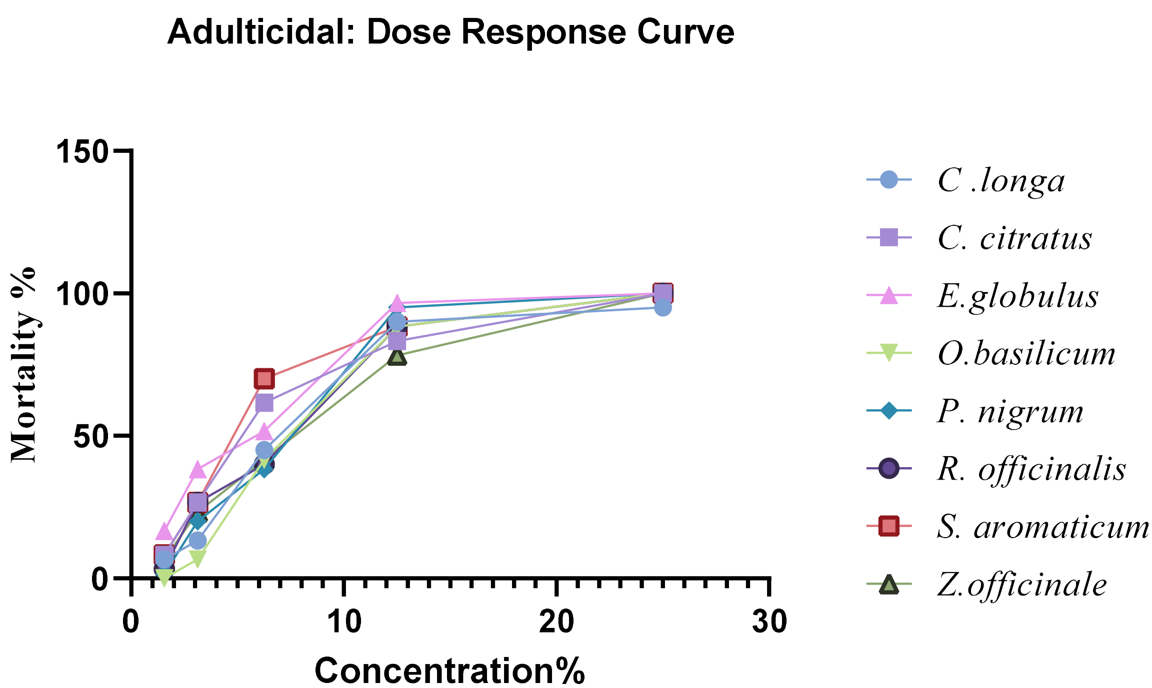


Fig 5: Dose–Response Curve Showing Dose-Dependent Inhibition of essential Oils against Musca domestica adult fly.

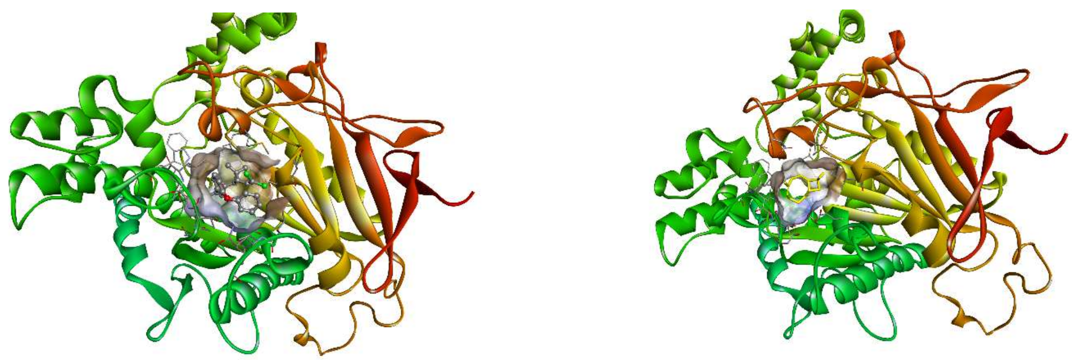
Dose-response graphs showing the ability of essential oils to kill the adult flies. Five concentrations (25.00–1.56%) were selected to measure mortality (%), with each point representing the mean ± SD of three replicates. In GraphPad Prism, nonlinear regression was used for fit curves.

3.5.4 Molecular docking studies of active components on the target protein

Molecular docking analysis demonstrated that several bioactive constituents from the selected essential oils exhibited favorable binding interactions with the homology-modelled Musca domestica acetylcholinesterase. Compounds from Syzygium aromaticum, Eucalyptus globulus, and Cymbopogon citartus oils showed comparatively stronger binding affinities, indicating their potential to interact effectively with the enzyme active site. The synthetic insecticide used as a positive control displayed the strongest interaction, validating the docking approach. Overall, the binding patterns found warrant considering acetylcholinesterase inhibition as a possible molecular mechanism underlying the insecticidal effect of the tested essential oils.

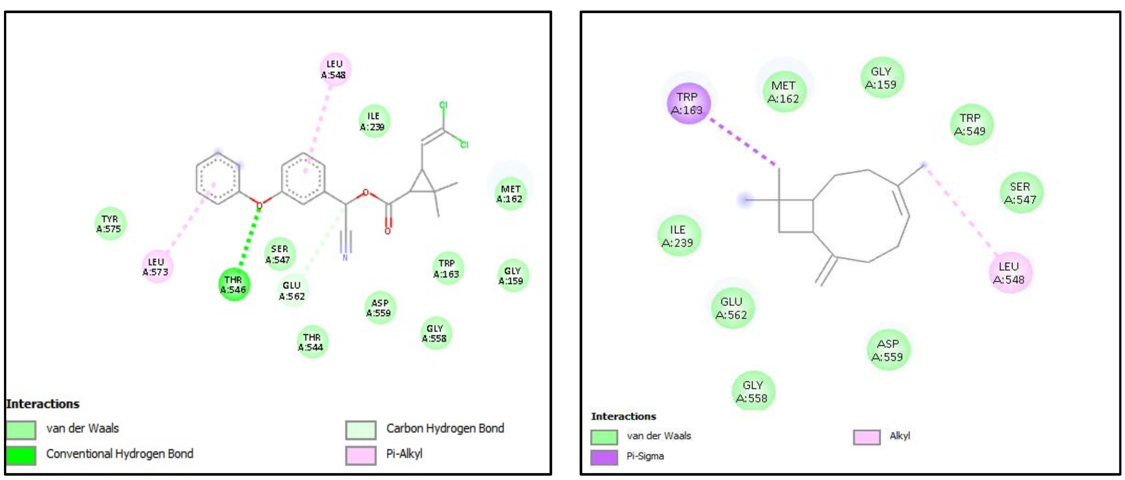
Table 7: Molecular docking studies of selected essential oils showing major phytochemical components, corresponding PubChem numbers, and docking scores against the target protein receptor. Docking scores represent the binding affinity pf the ligand toward the receptor, where more negative values indicate stronger and more stable protein-ligand interactions.

Sl. No	Essential oil	Major Active Components	PubChem: ID	Docking Score (Kcal/Mol)
1	Syzygium aromaticum	Eugenol	3314	6.2
		Beta-Caryophyllene	5281515	7.1
2	Eucalyptus globulus	1,8-Cineole (Eucalyptol),	2758	5.9
		alpha-Pinene	6654	5.3
		p-Cymene	7463	6.0
		Limonene	22311	6.1
3	Cymbopogon citartus	Neral	643779	6.6
		Gerinial	638011	5.7
4	Positive Control	Cypermethrin	2912	8.3



6A 6B

Fig 6: Protein-ligand interaction analysis of protein Musca domestica AChE with Cypermethrin (6A) and Beta-Caryophyllene(6B). Three-dimensional molecular docking visualization of the protein-ligand complex showing the interaction of the ligand within the active-site cavity of the target protein.



7A 7B

Fig 7: Two-dimensional molecular interaction illustrating the binding interactions between docked ligan and the active-sites of the target protein. Key interacting residues – THR A:546, SER A:547, LEU A:548, LEU A:573, TYR A:575, ILE A:239, MET A:162, TRP A:163, ASP A:559, GLU A:562, and GLY residues contribute to the stabilized protein-ligand complex. 7A: 2D Visualisation of bond formation between standard ligand cypermethrin and the target protein 7B: 2D Visualisation of bond formation between ligand Beta-Caryophyllene

4. DISCUSSION

The effectiveness of eight essential oils - Piper nigrum, Syzygium aromaticum, Eucalyptus globulus, Curcuma longa, Rosmarinus officinalis, Ocimum basilicum, Zingiber officinale and Cymbopogon citartus - in controlling housefly larvae, pupa and adult stages was investigated. Previous research has shown that essential oils from several plants can have significant larvicidal, and pupicidal, activities against M. domestica. This study focuses on screening various plants and herbs for controlling houseflies through essential oils extracted using different methods22,44. Existing protocols to mitigate M. domestica menace, such as chemical and mechanical traps, have significant disadvantages, including poor effectiveness, non-biodegradability, and toxicity to humans and animals45,46. GC-MS analysis revealed that Piper nigrum essential oil, rich in alpha-pinene (6.36%), Beta pinene (8.33%),3-Carene (2.10%), Limonene

(5.70%), Beta-caryophyllene (14.79%). The chemical constituents identified in the present study were consistent with those previously reported in the related studies47,48. Cymbopogon citartus essential oil, extracted through hydro-distillation, contains components such as Niral (22.99%) and Geraniol (3.82%), which have antimicrobial, anti-inflammatory, and mosquito repellent activities12. Ar-Turmerone (45.209%), α-Turmerone (6.003%), β-Turmerone (1.199%) from Curcuma longa, have shown larvicidal and adulticidal activity against houseflies. The insecticidal mode of action of these compounds against Musca domestica is largely neurophysiological and metabolic. Turmerones disrupt normal insect nervous function by interfering with acetylcholinesterase activity and neurotransmission, resulting in disturbances in motility, paralysis, and death. Besides, the lipophilic properties of turmerones facilitate penetration through the insect cuticle, leading to membrane destabilisation and respiratory stress49. Eucalyptus globulus is rich in eucalyptol and 1,8-cineole, which contribute to its long-established insecticidal use. The presence of monoterpenes and repellent activity against flies has been documented extensively50. Zingiber officinale is rich in Zingiberene (30.76) alpha.-Farnese (16.43%), alpha-pinene (2..3%), Camphene (5.49%), β-Sesquiphellandrene (13.14%), Cymbopogon citratus were, Niral (22.99%) and Geraniol (3.82%), α-Citra(40.94%), Rosmarinus officinalis is rich in α-Pinene(14.84%),Camphene (9.47%), β-Pinene(5.91%),Eucalyptol(21.85%), Ocimum basilicum is rich Linalool (25.93%), camphor (61.39%), constituents possess insecticidal, nematicidal, and fungistatic while Curcuma longa Ar-Turmerone (45.209%), α-Turmerone (6.003%), β-Turmerone (1.199%). Syzygium aromaticum oil, primarily composed of eugenol and caryophyllene, has demonstrated potent larvicidal and pupicidal activity based on toxicity studies, which determined the dose-dependent toxicity of multiple oils against Musca domestica. Syzygium aromaticum, Cymbopogon citratus, and Eucalyptus globulus were strategically selected for in-depth studies of molecular mechanisms due to their superior lethal concentrations (LC50 and LC90), as shown in Tables 4, 5, 6, and Figures 3, 4, 5. The values were close to those reported in the research papers50,52,53. The current investigation demonstrates that the selected essential oils exhibit significant bio-efficacy against the housefly across all developmental stages. The larvicidal, pupicidal, and adulticidal activities were significantly higher than the negative control, indicating strong dose-dependent toxicity. The pupicidal effects of essential oils can be associated to their ability to penetrate the pupal cuticle and physiological processes during metamorphosis. The lower susceptibility of pupae compared to larvae may be due to the puparium's protective nature and reduced metabolic activity during this stage. The larvicidal, pupicidal and adulticidal effects were slightly lower than those of Cypermethrin. Cypermethrin is widely used in agriculture, veterinary medicine, and public health for insect control47. However, the World Health Organisation classifies it as moderately hazardous to humans22,48. The formulations emphasise the need for safer, less environmentally harmful substitutes, including essential oils, particularly for long-term pest management, whereas alcohol-based formulations achieve complete dissolution, exhibit relatively higher toxicity, result in fast and immediate effects, are highly volatile, and are usually more suitable for laboratory use or quick-action treatments, water-based formulations (using surfactants like Tween 20) form emulsions, are safer with lower toxicity, provide slower but prolonged action, show greater stability with less evaporation, and are more suitable for large-scale field applications. Docking studies were executed by CB Dock2 using the blind docking method. The three-dimensional structure of Musca domestica Acetylcholinesterase (MdAChE) has not been deposited in the Protein Data Bank (PDB); it must be modelled based on the UniProt sequence Q8MXC4. The crystal structure of Drosophila melanogaster AChE has been used to model MdAChE through homology modelling. This model has been checked by calculating its Root Mean Square Deviation (RMSD) against the PDB structure 1QON, yielding an RMSD of 0.414 Å28. Ligand chemical structures were obtained from the PubChem database. The insecticidal efficiency of the studied essential oils (EOs), against the common housefly (Musca domestica), is based on a synergistic neurotoxic action26. Contrary to currently used synthetic agents, where the targeted binding site is solely considered and explored, the EOs display the "cocktail effect," affecting several different biological processes, enzymatic inhibition (MdAChE) - most particularly beta-Caryophyllene (−7.1 kcal/mol), together with Neral (−6.6 kcal/mol), act strongly against Acetylcholinesterase. Upon binding to the active site and the hydrophobic pocket of the target MdAChE, they inhibit acetylcholine hydrolysis, thereby inducing the irreversible muscular paralysis secondary to continuous nervous excitation; receptor disruption - the EOs act against several different types of biological targets, particularly against the more specialised ones, present and/or biologically essential, in the context of the target insects' biology, including octopamine binding sites targeted by Eugenol, and GABA-gated ion channels, targeted by Neral. This is relevant in addition to the impact on nervous hyperexcitability, and failure of protein/nucleic acid synthesis. Furthermore, against the physical and respiratory disruptions, the EOs' mobile VOC (volatile chemical components) ′ volatility, particularly exhibited by the Monoterpene, "1,8-Cineol" and Limonene, similarly behaves towards the spiracles, while beta-Caryophyllene and p-Cymene display surfactant properties increasing the fluidity of the Cuticular Membrane, and thereby facilitating the quick influx of the toxic substances. "Broad spectrum" target compound action, and consequently the reduced probability of the development of "metabolic resistance" in the "common housefly," particularly since the synthetic compound Cypermethrin, displaying the highest binding affinity of -8.3 kcal/mol25,26,28.

5. CONCLUSION

This study demonstrates the insecticidal properties of eight essential oils on various life stages of the Musca domestica. The differences in the performance of the various essential oils highlight the importance of specific chemical components in determining their insecticidal effects. Syzygium aromaticum, Eucalyptus globulus and Cymbopogon citartus oils had the greatest larvicidal, pupicidal, and adulticidal. There is moderate activity in Rosmarinus officinalis, Piper nigrum, Ocimum basilicum oil, Zingiber officinale, and Curcuma longa. Extensive research is required to evaluate the safety and effectiveness of these oils in the field and to develop appropriate formulations for use there. This research emphasizes the applicability of the essential oils as natural substitutes for chemical insecticides. Among the benefits of essential oils, include their lesser toxicity to non-target species, lower risk of resistance, and environmental sustainability. The advantages are meant to overcome significant challenges associated with conventional chemical insecticides, such as environmental contamination, health hazards, and insect resistance. By leveraging safer, more sustainable pest management methods, essential oils can serve as natural insecticides. This recommends the utilization of essential oils in pest management programs as a viable solution to the ever-increasing demand for organic and environmentally friendly products. They are extremely efficient and have good safety profiles; hence, they can serve as effective substitutes for conventional chemical insecticides, thereby making pest management practices more sustainable and health conscious. The findings lead to further research and the application of essential oils in various conditions to help pests rely on more friendly, environmentally friendly, and sustainable solutions.

REFERENCES

1. Chintalchere, J. M, Mudasir A D, Radhakrishna S P. Evaluation of some essential oils against the larvae of House Fly, Musca domestica by using residual Film method. Adv Biotech & Micro. 2018; 9(1): 555752.https://doi.org/10.19080/AIBM.2018.09.555752

2. Kumar, P, Mishra, S, Malik, A, Satya, S. Biocontrol potential of essential oil monoterpenes against housefly, Musca domestica (Diptera: Muscidae). Ecotoxicol Environ Saf. 2014; 100(1): 1–6. https://doi.org/10.1016/j.ecoenv.2013.11.013

3. Khan, H A A. Toxicity, repellent and oviposition deterrent effects of select essential oils against the house fly Musca domestica. J Asia Pac Entomol. 2021; 24(1): 15–20. https://doi.org/10.1016/j.aspen.2020.10.002

4. Khater, H F, Geden, C J. Efficacy and repellency of some essential oils and their blends against larval and adult house flies, Musca domestica L. (Diptera: Muscidae). J Vector Ecol. 2019; 44(2). https://doi.org/10.1111/jvec.12357

5. Tesfaye, B, Tefera, T. Extraction of Essential Oil from Azadirachta indica Seed by Using Soxhlet Extraction Methods. Int J Adv Eng Manag Sci. 2017; 3(6): 646–650. https://doi.org/10.24001/ijaems.3.6.5

6. Gul, S, Rehman, F U, Kamran Taj, M, Gul, S, Khan, M A, Taj, I, Azam, S, Khan, S. The house flies Musca domestica as mechanical vector and its management to control in quetta city, balochistan. PJMLS. 2024; 7(3): 437–448. https://doi.org/10.31580/pjmls.v7i3.3034

7. Vandeweyer, D, De Smet, J, Van Looveren, N, Van Campenhout, L. Biological contaminants in insects as food and feed. J Insects Food Feed. 2021; 7(5): 807–822. https://doi.org/10.3920/JIFF2020.0060

8. Chintalchere, J M, Dar, M A, Raut, K D, Pandit, R S. Bioefficacy of Cymbopogon citartus and Tea Tree Essential Oils Against House Fly, Musca domestica. Proc Natl Acad Sci India B Biol Sci. 2021; 91(2): 307–318. https://doi.org/10.1007/s40011-020-01220-z

9. Gallon, C, Martello, R H, Cozzer, G, Rezende, C A L, Calisto, J F F, Floss, P A, Oliveira, J V, Rezende, R S, Dal Magro, J, Albeny-Simões, D. Chemistry matters: biological activity of Eucalyptus globulus essential oils on mosquito larval mortality. Entomol Exp Appl. 2020; 168(5): 407–415. https://doi.org/10.1111/eea.12908

10. Mohamed, D, Darwish, A, Aboelela, H, Baz, M, Moharam, A. Biocontrol efficacy of some essential oils as larvicides and inhibitors of the emergence of adult Musca domestica. Parasitol United J. 2024; 17(2): 105–111. https://doi.org/10.21608/puj.2024.295018.1251

11. Tyagi, V, Dey, P, Patel, R, Hazarika, H, Goswami, D, Chattopadhyay, P. Chemical composition and bioefficacy for larvicidal and pupicidal activity of essential oils against two mosquito species. Int J Mosq Res. 2017; 4(4): 112–118.

12. Hamdeni, I, Boukhris-Bouhachem, S, Louhaichi, M, Boulila, A, Amri, I, Coque, J J R, Hamrouni, L. Synergistic and Additive Interactions in Essential Oils Obtained from Combined Plant Materials: Enhanced Control of Insect Pests. 2025. https://doi.org/10.20944/preprints202511.1533.v1

13. Liu, J, Zhang, M, Fu, W J, Hu, J F, Dai, G H. Efficacy of bioactive compounds from Curcuma longa L. against mosquito larvae. J Appl Entomol. 2018; 142(8): 792–799. https://doi.org/10.1111/jen.12527

14. Budiman, B, Hamidah, H, Afifah, N, Munir, M, Miswan, M, Tasya, Z. Plant-Based Bioinsecticide for Controlling Houseflies (Musca domestica): Efficacy of Leaf Extracts. J Public Health Pharm. 2025; 5(3): 487–493. https://doi.org/10.56338/jphp.v5i3.6237

15. Soonwera, M, Moungthipmalai, T, Puwanard, C, Sittichok, S, Sinthusiri, J, Passara, H. Adulticidal synergy of two plant essential oils and their major constituents against the housefly Musca domestica and bioassay on non-target species. Heliyon. 2024; 10(5). https://doi.org/10.1016/j.heliyon.2024.e26910

16. Chaubey, M K. Essential oils as green pesticides of stored grain insects. Eur J Biol Res. 2019; 9(4): 202–244. https://doi.org/10.5281/zenodo.3528366

17. Suwannayod S., Sukontason K.L., Pitasawat B., Junkum A., Limsopatham K., Jones M.K., Somboon P., Leksomboon R., Chareonviriyaphap T., Tawatsin A., et al. Synergistic Toxicity of Plant Essential Oils Combined with Pyrethroid Insecticides against Blow Flies and the House Fly. Insects. 2019; 10:178. https://doi.org/10.3390/insects10060178

18. Pavela R. Essential Oils for the Development of Eco-Friendly Mosquito Larvicides: A Review. Ind. Crop. Prod. 2015; 76:174–187. https://doi.org/10.1016/j.indcrop.2015.06.050

19. Chantawee, A, Soonwera, M. Larvicidal, pupicidal and oviposition deterrent activities of essential oils from Umbelliferae plants against house fly Musca domestica. Asian Pac J Trop Med. 2018; 11(11): 621–629. https://doi.org/10.4103/1995-7645.246338

20. Kökdener, M. Larvicidal and pupicidal effects of some essential oils against Musca domestica Linnaeus, 1758 (Diptera:Muscidae). Int J Agric Environ Food Sci. 2023; 7(1): 234–243. https://doi.org/10.31015/jaefs.2023.1.28

21. Senthoorraja, R, Subaharan, K, Manjunath, S, Pragadheesh, V S, Bakthavatsalam, N, Mohan, M G, Senthil-Nathan, S, Basavarajappa, S. Electrophysiological, behavioural and biochemical effect of Ocimum basilicum oil and its constituents methyl chavicol and linalool on Musca domestica L. Environ Sci Pollut Res. 2021; 28(36): 50565–50578. https://doi.org/10.1007/s11356-021-14282-x

22. Chantawee, A, Soonwera, M. Larvicidal, pupicidal and oviposition deterrent activities of essential oils from Umbelliferae plants against house fly Musca domestica. Asian Pac J Trop Med. 2018; 11(11): 621–629. https://doi.org/10.4103/1995-7645.246338

23. Oyarce, G A, Loyola, P, Iubini-Aravena, M, Romero, A, Rodríguez-Maciel, J C, Becerra, J, Silva-Aguayo, G. Adulticidal and repellent activity of essential oils from three cultivated aromatic plants against Musca domestica L. Insects. 2025; 16(5). https://doi.org/10.3390/insects16050542

24. Mansour, S A, Bakr, R F A, Hamouda, L S, Mohamed, R I. Adulticidal activity of some botanical extracts, commercial insecticides and their binary mixtures against the housefly, Musca domestica L. Egypt Acad J Biolog Sci. 2012; 5(1): 151–167.

25. Shehata, A Z I, Elhawary, E A, Mokhtar, M M, Waheeb, H O, Fares, M, Emam, D E M, Bakr, N A, Sadek, A M, El-Tabakh, M A M. Molecular docking and insecticidal activity of Pyrus communis L. extracts against disease vector, Musca domestica L. (Diptera: Muscidae). Egypt J Basic Appl Sci. 2023; 10(1): 800–811. https://doi.org/10.1080/2314808X.2023.2283268

26. Awad, M, Alfuhaid, N A, Amer, A, Hassan, N N, Moustafa, M A M. Towards sustainable pest management: toxicity, biochemical effects and molecular docking analysis of Ocimum basilicum essential oil on Agrotis ipsilon and Spodoptera littoralis. Neotrop Entomol. 2024; 53(3): 669–681. https://doi.org/10.1007/s13744-024-01137-6

27. Duque, J E, Urbina, D L, Vesga, L C, Ortiz-Rodríguez, L A, Vanegas, T S, Stashenko, E E, Mendez-Sanchez, S C. Insecticidal activity of essential oils from American native plants against Aedes aegypti: an introduction to their possible mechanism of action. Sci Rep. 2023; 13(1). https://doi.org/10.1038/s41598-023-30046-8

28. Johnson, T O, Ojo, O A, Ikiriko, S, Ogunkua, J, Akinyemi, G O, Rotimi, D E, Oche, J R, Adegboyega, A E. Biochemical evaluation and molecular docking assessment of Cymbopogon citratus as a natural source of acetylcholinesterase-targeting insecticides. Biochem Biophys Rep. 2021; 28. https://doi.org/10.1016/j.bbrep.2021.101175

29. Ekesiobi, A O. Evaluation of the aqueous leaf extract of Ocimum gratissimum against larvae of Musca domestica.

IPS J Drug Discov Res Rev. 2025; 3(1): 15–22. https://doi.org/10.54117/ijddrr.v3i1.26

30. Rovida, A, Endo, K M, Polli, A D, Bulla, L, Scudeler, E L, Jas, A, Conte, H. Use of Azadirachta indica (Azadirachta indica A. Juss) oil in the control of Musca domestica L. in poultry breeding farms. J World's Poult Res. 2015; 5(4).

31. Soyelu, O J, Oluwamakinde, B A, Okonji, R E. Effect of Azadirachta indica, Siam weed and Vetiver oils on physiological reactions and fitness of house fly, Musca domestica L. J Appl Sci Environ Manag. 2020; 24(3): 519–523. https://doi.org/10.4314/jasem.v24i3.20

32. Nhaca, I A A, Chissico, H M, Massango, P A, Muiambo, H F, Focke, W W, Munyemana, F, et al. Evaluation of larvicidal activity of selected plant extracts and essential oil against Musca domestica and Anopheles arabiensis. Int J Med Plants Nat Prod. 2020; 6(4): 9–19. https://doi.org/10.20431/2454-7999.0604002

33. Palacios, S M, Bertoni, A, Rossi, Y, Santander, R, Urzúa, A. Efficacy of essential oils from edible plants as insecticides against the house fly, Musca domestica L. Molecules. 2009; 14(5): 1938–1947. https://doi.org/10.3390/molecules14051938

34. Chauhan, N, Malik, A, Sharma, S. Repellency potential of essential oils against housefly, Musca domestica L. Environ Sci Pollut Res. 2018; 25(5): 4707–4714. https://doi.org/10.1007/s11356-017-0363-x

35. Khan, H A A. Toxicity, repellent and oviposition deterrent effects of select essential oils against the house fly Musca domestica. J Asia Pac Entomol. 2021; 24(1): 15–20. https://doi.org/10.1016/j.aspen.2020.10.002

36. Kumar, P, Mishra, S, Malik, A, Satya, S. Biocontrol potential of essential oil monoterpenes against housefly, Musca domestica (Diptera: Muscidae). Ecotoxicol Environ Saf. 2014; 100(1): 1–6. https://doi.org/10.1016/j.ecoenv.2013.11.013

37. Chintalchere, J M, Dar, M A, Pandit, R S. Evaluation of some essential oils against the larvae of house fly, Musca domestica by using residual film method. Adv Biotech Microbiol. 2018; 9(1). https://doi.org/10.19080/AIBM.2018.09.555752

38. Kaur, R, Singh, J. Toxicity, monitoring and biodegradation of cypermethrin insecticide: a review. Nat Environ Pollut Technol. 2021; 20(5): 1997–2005. https://doi.org/10.46488/NEPT.2021.V20I05.016

39. Chintalchere, J M, Dar, M A, Pandit, R S. Biocontrol efficacy of bay essential oil against housefly, Musca domestica (Diptera: Muscidae). J Basic Appl Zool. 2020; 81(1). https://doi.org/10.1186/s41936-020-0138-7

40. Sarma, R., Adhikari, K., Mahanta, S. et al. Combinations of Plant Essential Oil Based Terpene Compounds as Larvicidal and Adulticidal Agent against Aedes aegypti (Diptera: Culicidae). Sci Rep 9, 9471 (2019). https://doi.org/10.1038/s41598-019-45908-3

41. Mohamed, D, Darwish, A, Aboelela, H, Baz, M, Moharam, A. Biocontrol efficacy of some essential oils as larvicides and inhibitors of the emergence of adult Musca domestica. Parasitol United J. 2024; 17(2): 105–111. https://doi.org/10.21608/puj.2024.295018.1251

42. Marimuthu, P, Lee, Y J, Kim, B H, Seo, S S. In silico approaches to evaluate the molecular properties of organophosphate compounds to inhibit acetylcholinesterase activity in housefly. J Biomol Struct Dyn. 2019; 37(2): 307–320. https://doi.org/10.1080/07391102.2018.1426046

43. Liu, Y, Yang, X, Gan, J, Chen, S, Xiao, Z X, Cao, Y. CB-Dock2: improved protein-ligand blind docking by integrating cavity detection, docking and homologous template fitting. Nucleic Acids Res. 2022; 50(W1): W159–W164. https://doi.org/10.1093/nar/gkac394

44. Soonwera, M, Moungthipmalai, T, Puwanard, C, Sittichok, S, Sinthusiri, J, Passara, H. Adulticidal synergy of two plant essential oils and their major constituents against the housefly Musca domestica and bioassay on non-target species. Heliyon. 2024; 10(5). https://doi.org/10.1016/j.heliyon.2024.e26910

45. Geden, C J, Nayduch, D, Scott, J G, Burgess, E R, Gerry, A C, Kaufman, P E, Thomson, J, Pickens, V, Machtinger, E T. House fly (Diptera: Muscidae): biology, pest status, current management prospects and

research needs. J Integr Pest Manag. 2021; 12(1). https://doi.org/10.1093/jipm/pmaa021

46. Said-Al, H A H, Said-Al Ahl, H A H, Hikal, W M, Tkachenko, K G. Essential oils with potential as insecticidal agents: a review. Int J Environ Plann Manag. 2017; 3(4): 23–33.

47. Weluwanarak, T, Changbunjong, T, Leesombun, A, Boonmasawai, S, Sungpradit, S. Effects of Piper nigrum L. fruit essential oil toxicity against stable fly (Diptera: Muscidae). Plants. 2023; 12(5). https://doi.org/10.3390/plants12051043

48. Rajkumar, V.; Gunasekaran, C.; Dharmaraj, J.; Chinnaraj, P.; Paul, C.A.; Kanithachristy, I. Structural characterization of chitosan nanoparticle loaded with Piper nigrum essential oil for biological efficacy against the stored grain pest control. Pestic. Biochem. Physiol. 2020; 166, 104566. https://doi.org/10.1016/j.pestbp.2020.104566

49. Hazarika, H, Tyagi, V, Krishnatreyya, H, Islam, J, Boruah, D, Kishor, S, Chattopadhyay, P, Zaman, K. Essential oil based controlled-release non-toxic evaporating tablet provides effective repellency against Musca domestica. Acta Trop. 2020. https://doi.org/10.1016/j.actatropica.2020.105620

50. Mansour, S A, Bakr, R F A, Hamouda, L S, Mohamed, R I. Adulticidal activity of some botanical extracts, commercial insecticides and their binary mixtures against the housefly, Musca domestica L. Egypt Acad J Biolog Sci. 2012; 5(1): 151–167.

51. Kumar, P., Mishra, S., Malik, A., & Satya, S. (2011). Repellent, larvicidal and pupicidal properties of essential oils against Musca domestica. Medical and Veterinary Entomology, 25(4): 403–412.

52. Marimuthu, P., Lee, Y. J., Kim, B., & Seo, S. S. (2019). In silico approaches to evaluate the molecular properties of organophosphate compounds to inhibit acetylcholinesterase activity in housefly. Journal of Biomolecular Structure and Dynamics, 37(2), 307-320.

53. Elmaidomy, A. H., Mohamed, E. M., El-Dien, R. T. M., Abou-Zied, H. A., Mostafa, A. A., Taha, R. H., ... & Youssif, K. A. (2026). Chemical composition and insecticidal activity of essential oils from Citrus limon, Citrus aurantium, and Citrus margarita against Musca domestica. Scientific Reports, 16(1), 12858