

Research Article

## Phytochemical analysis and larvicidal bio-efficacy of *Camellia sinensis* (L.) Kuntze leaf ethanol extracts against *Aedes aegypti* L.

### Pavitra Sharma

School of Life Sciences, Indira Gandhi National Open University, Maidan Garhi, New Delhi-110068, India; Deshbandhu College, University of Delhi, Kalkaji, New Delhi-110019, India

### Neera Kapoor

School of Life Sciences, Indira Gandhi National Open University, Maidan Garhi, New Delhi-110068, India

### Kamal Kumar Gupta\*

Deshbandhu College, University of Delhi, Kalkaji, New Delhi-110019, India

\*Corresponding author. E-mail: kgupta@db.du.ac.in

### Article Info

<https://doi.org/10.31018/jans.v18i1.7255>

Received: October 10, 2025

Revised: February 28, 2026

Accepted: March 4, 2026

### How to Cite

Sharma, P. *et al.* (2026). Phytochemical analysis and larvicidal bio-efficacy of *Camellia sinensis* (L.) Kuntze leaf ethanol extracts against *Aedes aegypti* L.. *Journal of Applied and Natural Science*, 18(1), 427 - 441. <https://doi.org/10.31018/jans.v18i1.7255>

### Abstract

The increasing resistance of *Aedes aegypti* to conventional chemical insecticides has intensified the search for eco-friendly alternatives in mosquito control. This study explores the larvicidal and developmental effects of *Camellia sinensis* (green tea) leaf extracts on *Ae. aegypti*, a major vector of dengue, Zika, and chikungunya viruses. Early 4<sup>th</sup> instar larvae of *Ae. aegypti* were treated with *C. sinensis* leaf ethanol extract (Cs-LEE) under controlled laboratory conditions. Key parameters, including larval and pupal mortality, developmental period, and adult emergence, were recorded. Results revealed a dose-dependent increase in larval and pupal mortality, significant delays in development, and reduced adult emergence in treated groups. Larval and pupal mortality was observed to be highest at 400 mg/L, 24.8% and 36.8% respectively. Development period of larvae and pupae increased to 5.57 days and 5.34 days at 400 mg/L. Similarly, adult emergence reduced to approximately 38% at 400 mg/L. Phytochemical screening confirmed the presence of bioactive substances such as catechins, alkaloids, and flavonoids, which are known to disrupt hormonal balance and interfere with enzymatic processes in insects. These findings highlight the potential of Cs-LEE as natural larvicidal agents. Their biodegradability, low toxicity to non-target organisms, and availability make them promising candidates for Integrated vector management (IVM) programs. Further research and field validation are necessary to develop safe, cost-effective botanical formulations for large-scale mosquito control.

**Keywords:** *Aedes aegypti*, *Camellia sinensis*, Development, Growth, Leaf ethanol extract (Cs-LEE), Phytochemicals, Survival

### INTRODUCTION

Mosquitoes are the vectors of many arboviruses that cause deadly diseases. In addition, they can cause allergic reactions including systemic sensitivity and local skin reactions. The most prevalent mosquito-borne illnesses are malaria, encephalitis, dengue fever, chikungunya, yellow fever, and the most severe, dengue hemorrhagic fever. *Aedes aegypti*, a day-feeding mosquito, is of significant epidemiological importance because it is the vector of dengue virus, yellow fever virus, Zika virus, and chikungunya. Dengue fever affects people worldwide and can cause symptoms ranging from a low-grade fever to a serious, potentially fatal hemorrhagic illness. According to the World Health Or-

ganization, approximately two fifth world populations are at risk of dengue fever. Over 7.6 million dengue cases, including 16,000 severe cases, and over 3,000 fatalities were reported to the WHO as of April 30, 2024 (World Health Organisation, 2024). The wide distribution and close association of *Ae. aegypti* with humans are a serious concern.

Over the past few years, controlling mosquitoes has been the main focus of numerous studies due to the substantial harm and harmful diseases they inflict. The target stages—egg, larva, pupa, and adult—determine the mosquito control strategies. These include employing chemical pesticides such as pyrethroids to target adult mosquitoes, synthetic insect growth regulators like methoprene and diflubenzuron (Milugo *et al.*,

2021), or plant-derived compounds as alternatives to kill mosquito larvae (Milugo *et al.*, 2021). Although the use of synthetic pesticides to control mosquito vectors has been very successful, it has led to several issues, including the rise of insecticide-resistant mosquitoes, environmental pollution, and a hazard to human health (Bellinato *et al.*, 2016; Choochote *et al.*, 2007). The knockdown resistance to pyrethroid noticed in all *Ae. aegypti* populations globally, is caused by changes in a voltage-gated sodium channel gene (Moyes, 2017). Moreover, the use of synthetic larvicides poses risks to ecosystems and human health due to their prolonged persistence in the environment. Additionally, pesticides disturb all natural ecosystems, especially soil and aquatic ecosystems, and their detrimental effects on non-target organisms are readily apparent (Ankit *et al.*, 2020; Oaya *et al.*, 2019). This situation has spurred the need for sustainable methods that use natural resources. Plant extracts have been used as safe alternatives to larvicides, as they contain a variety of phytochemicals that specifically kill mosquito larvae without endangering the environment or other living organisms (Hillary *et al.*, 2024). The use of plant-derived secondary metabolites, such as ocimenone, rotenone, and thymol, to reduce mosquito larvae is a cost-effective and environmentally friendly alternative to synthetic larvicides (Milugo *et al.*, 2021).

*Camellia sinensis* L. (Family: Theaceae), green tea, is an evergreen shrub or small tree found in tropical and subtropical regions. It is a great source of medicinal and pharmacologically active substances. It contains several bioactive compounds, with polyphenols accounting for one-third. Other compounds include alkaloids (caffeine, theobromine, and theophylline), flavonoids, anthocyanins, catechins, amino acids, proteins, carbohydrates, organic compounds, fluorides, minerals, and trace elements. The three flavonoids, kaempferol, quercetin, and myricetin, are abundant in green tea leaves (Das and Eun 2018, Xu *et al.* 2017, Lee *et al.* 2014, Manning and Roberts 2003). The extract from green tea leaves has the ability to repel and kill mosquitoes (Hassan *et al.*, 2020). Muema *et al.* (2016) assessed the effectiveness of the *C. sinensis* crude leaf extract and its fraction against *Anopheles arabiensis* and *Anopheles gambiae* larvae. They reported developmental disruptions in the larvae. Given the importance of plant extracts as a significant and safe substitute for synthetic pesticides, the current study was set out to evaluate the adverse effect of *C. sinensis* leaf ethanol extract (Cs-LEE) on larvae, pupae and adults of *Ae. aegypti* under laboratory conditions.

## MATERIAL AND METHODS

### Rearing of *Aedes aegypti*

Stock culture of *Ae. aegypti* was procured from the

National Institute of Malaria Research, New Delhi and established at the Insect Laboratory, Deshbandhu College, University of Delhi under control condition at temperature  $28 \pm 1$  °C, relative humidity  $70 \pm 5\%$  and a photoperiod of 12:12 (light/dark) (Shah and Gupta, 2025, Shazad *et al.*, 2024). Adult mosquitoes were reared in cloth cages. A small, moist cotton swab was placed on the top of each cage to provide water for adult mosquitoes, and 3-4 water-soaked, deseeded split raisins were placed in each cage as a food source. Periodic blood meals were provided to the adult females after 2 days of emergence to support egg maturation. The eggs were collected on Whatmann filter paper strips lining the enamel bowl (diameter 6 cm); the bowl was filled half with de-chlorinated tap water. For hatching, the eggs were transferred to a tray (25cm × 30cm × 5cm) containing de-chlorinated tap water. The larvae were fed a food mixture containing finely ground dog biscuits and yeast powder (3:1 w/w). Water was changed every other day to prevent the formation of scum on the surface. The pupae were separated into an enamel bowl and placed in cloth cages for adult emergence (Shah and Gupta, 2025; Sharma *et al.*, 2015).

### Preparation of *Camellia sinensis* leaf ethanol extract

Leaves of *C. sinensis* were dried using an incubator shaker at 37 °C. Subsequently, the dried leaves were ground into a fine powder using a pestle and mortar. The dried leaf powder (10 gm) was extracted with 200 mL of ethanol, in a conical flask. A conical flask was placed in an incubator shaker at 140 rpm for 72 h at room temperature. The extract was filtered using Whatman filter paper No. 1. The filtrate obtained was dried using rotary evaporator (Kumar *et al.*, 2023). A 10% stock solution was prepared in ethanol and stored at 4°C.

### Treatment of 4<sup>th</sup> instar larvae of *Aedes aegypti* with *Camellia sinensis* leaf ethanol extract

Early 4th instar larvae (L4) were selected and isolated from the stock culture for the experimental purpose. They were treated with four different concentrations of Cs-LEE ranging from 50 mg/L to 400 mg/L. The larvae were provided with food during bioassay (Sofi *et al.*, 2022, Sharma *et al.*, 2015). All observations were recorded every 24 hours until the adult emerged.

### Influence of *Camellia sinensis* leaf ethanol extract on survival, growth and development of *Aedes aegypti*

Influence of Cs-LEE on survival, growth and development of L4, pupation, and adult emergence of *Ae. aegypti* were recorded at 24-hour intervals until the adults emerged. The larval and pupal period indicated the

time duration of larval or pupal stage. Subsequently, they either die or moult to the next stage. The developmental period specifies the time a larva or pupa takes to moult into the next stage. Various growth and developmental indices, including the adult emergence index and larval and pupal growth indexes, were calculated.

Adult emergence index = Adult emergence  
Experimental) / Adult emergence (Control) Eq. 1

Larval growth index = Percent pupae formation /  
Time taken by L4 to develop into pupae Eq. 2

Pupal growth index = Percent adult formation /Time  
taken by pupae to develop into adult Eq. 3

### Statistical analysis

For each test concentration and the control, experiments were repeated 5 times with 25 larvae per replicate. The moribund and dead larvae in all replicates were combined and expressed as percentage mortality at each concentration. Mortality at the test concentration was corrected using Abbott's formula (Abbott, 1925).

Corrected percent mortality: (% treated mortality-%  
control mortality) X 100 / (100-% control mortality) )  
Eq. 4

The observed data was presented as mean  $\pm$  standard error. Descriptive statistics in MS Excel 2016 and SPSS version 19.0 software (SPSS, Chicago, IL, USA) was used to analyze all quantitative data. The statistically significant difference between groups was determined using a one-way ANOVA and Tukey's post hoc test.

### Liquid chromatography-mass spectrometry (LC-MS) analysis

The LC-MS analysis of Cs-LEE was carried out using an LCMS Q Exactive™ HF Hybrid Quadrupole Orbitrap Mass Spectrometer. After injecting 1.0  $\mu$ L of the sample, gradient elution was used to achieve separation at a flow rate of 0.4 mL/min for a total runtime of 25 minutes. A MicroTOF QIII Bruker Daltonics system was used for high-resolution mass spectrometry (HRMS), which provides precise mass measurements and resolves complex mixtures (Mamat *et al.*, 2020). In the ESI ion source, spray voltage in positive and negative ion modes was set to  $\pm 4$  kV, and the cone (skimmer) voltage was  $\pm 40$ –60 V for polar and  $\pm 25$ –30 V for non-polar components (Kelstrup *et al.*, 2018).

### Gas chromatography-mass spectrometry (GC-MS) analysis

Phytochemicals present in the Cs-LEE were analyzed by GC-MS. An aliquot of 2 mL was injected into a Fisons GC8000 series connected to a TSQ8000 MS (Triple Quadrupole) mass analyzer. For the chromatography, the DB5-MS column was used. The injection temperature was 230 °C. The helium flow was 1 mL/

min. A 5-minute delay, a 1-minute isocratic run, and a 5-minute increase in oven temperature to 310°C followed a 5-minute solvent delay at 70°C. The mass lab locate target approach was used to integrate the ion trace for the distinctive fragment of assigned peaks. Analysis and identification of the GC-MS mass spectrum were performed by comparing the data with existing software libraries, such as WILEY08, NIST08, and NIST08s (Hubschmann, 2025; Karakoti *et al.*, 2022; Adams, 2017).

## RESULTS

### Effect of *Camelia sinensis* leaf ethanol extract on the survival of L4 and pupae of *Aedes aegypti*

The Cs-LEE was reported to exert adverse effects on the survival and development of fourth instar larvae and pupae of *Ae. aegypti*. The effects were insignificant after 24 h of treatment. However, mortality was observed in L4 after 72 hours of treatment, with the highest mortality in the 400 mg/L Cs-LEE treatment. A dose-dependent increase in total mortality of L4 larvae in response to treatment with the Cs-LEE was observed (Table 1). The results were statistically significant at  $p < 0.001$ . Further, the pupae that emerged from treated L4 showed a direct relationship between mortality and treatment concentration. The results were significant at  $p < 0.001$  (Table 1).

### Effect of *Camelia sinensis* leaf ethanol extract on the inhibition of adult emergence

The influence of Cs-LEE on the inhibition of adult emergence (equation 1) is shown in Table 1. The percentage of inhibition of adult emergence was dose-dependent, increasing with increasing Cs-LEE concentration. The highest value (61.6%) was observed in the treatment at 400 mg/L.

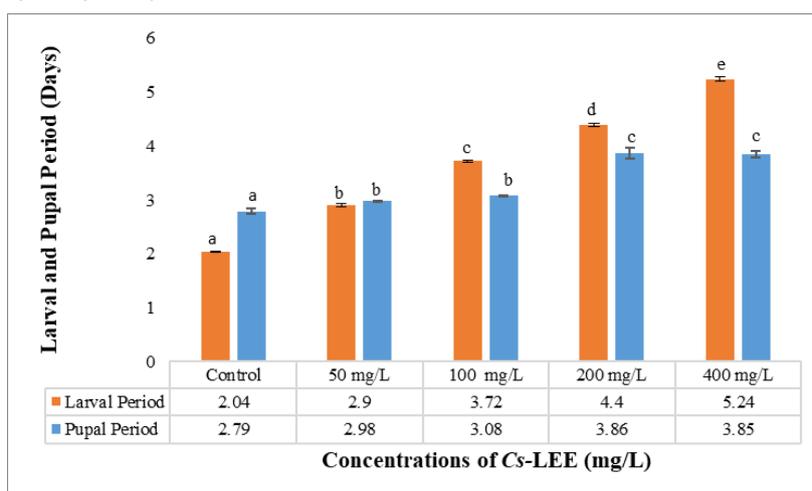
### Effect of *Camelia sinensis* leaf ethanol extract on the larval and pupal period

Treatment of Cs-LEE increased in the larval period of L4 of *Ae. aegypti*. It was observed that some of the L4 remained in the larval stage for an extended period, failed to pupate, and died. The larval period was calculated as the mean time duration the L4 remained in larval stage. Impact of Cs-LEE on the larval period of *Ae. aegypti* L4 is presented in Fig. 1. The results indicate a dose-dependent relationship between the concentration of the extract and the larval period. In the control group, the L4 pupated after 2.04 days. As the concentration of Cs-LEE increased, the larval period of the L4 increased steadily i.e. 2.9 days at 50 mg/L, 3.72 days at 100  $\mu$ g/mL, 4.4 days at 200 mg/L, and 5.24 days at 400 mg/L. The results were significant at  $p < 0.001$  (Fig. 1).

**Table 1.** Effect of the Cs-LEE on the survival and development of the fourth instar larvae and pupae of *Aedes aegypti*

Test concentration	Total mortality of fourth instar larvae	Total mortality of pupae	% Inhibition of adult emergence
Control	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>
50 mg/L	4 <sup>b</sup>	5.80 <sup>b</sup> ± 1.02	9.60 <sup>b</sup> ± 0.97
100 mg/L	4 <sup>b</sup>	19.97 <sup>c</sup> ± 0.82	24 <sup>c</sup> ± 0.01
200 mg/L	11.20 <sup>c</sup> ± 0.80	21.57 <sup>c</sup> ± 2.10	32.80 <sup>d</sup> ± 1.40
400 mg/L	24.80 <sup>d</sup> ± 0.80	36.81 <sup>d</sup> ± 1.70	61.60 <sup>e</sup> ± 0.97
F value	376.25	117.87	679.61
p-value	<0.001	<0.001	<0.001

Average of five replicates; 25 individuals per replicate. Means followed by the same letters in a column are not significantly different at  $p < 0.05$  (ANOVA followed by Tukey's test)



**Fig. 1.** Effect of Cs-LEE on the larval and pupal period of *Aedes aegypti*. (Average of five replicates; 25 L4 per replicate. Early L4 were treated with Cs-LEE till they pupated. Means followed by the same letters in the column of same colour are not significantly different at  $p < 0.05$  (ANOVA followed by Tukey's test).

Similar trends were seen in the pupal period of the pupae developed from the treated L4. In many cases, the pupae remained in this stage and failed to emerge as adults. The results show dose-dependent impact on the pupal period (Fig. 1). In the control group, the mean pupal period was 2.79 days. In treatments at 50, 100, 200, and 400 mg/L, the pupal period increased to 2.98, 3.08, 3.86, and 3.85 days, respectively.

#### Effects of *Camelia sinensis* leaf ethanol extract on growth and development of L4 and pupae of *Aedes aegypti*

Cs-LEE showed a negative impact on the development period of fourth instar larvae (Fig. 2). The development period of fourth instar larvae increased with increasing concentrations of Cs-LEE. In control, L4 took 2.04 day to pupate. On the other hand, the fourth instar larvae moulted into pupae after 2.91 days, 3.74 days, 4.52 days, and 5.57 days in the treatments at the concentrations of 50 mg/L, 100 mg/L, 200 mg/L, and 400 mg/L of Cs-LEE, respectively. The larval period of fourth instar treated with Cs-LEE was significantly different from the control and among larvae treated at different doses ( $p < 0.05$ ).

Fig. 2 also presents the effect of varying concentrations of Cs-LEE on the developmental period of pupae of *Ae. aegypti*. The results demonstrate a concentration-dependent increase in the number of days the mosquito spends in the pupal stage. In the control group, the mean pupal duration was 2.79 days, representing the normal developmental time in the absence of treatment. At lower concentrations of 50 mg/L and 100 mg/L, only a slight increase in pupal duration was observed, with values of 3.00 days and 3.02 days, respectively. However, at higher concentrations, a notable increase in pupal duration was evident. At 200 mg/L, the mean pupal duration rose to 4.36 days, and at the highest treatment concentration (400 mg/L), it peaked at 5.34 days. The effect of Cs-LEE on the growth index of larvae and pupae showed a concentration-dependent decrease. In control groups, the growth index (GI) of fourth instar larvae was 49, whereas in the treatment with Cs-LEE at a concentration of 400 mg/L, it decreased to 13.46. The reduction in fourth larval growth index was also significant in the treatments at concentration at 50 mg/L, 100 mg/L and 200 mg/L with values 34.94, 25.69 and 19.68 respectively (Fig. 3). Similarly, growth index of pupae was 35.81 in case of control groups; it was lowest

(9.42) at 400 mg/L of Cs-LEE. A significant reduction in the growth index was also observed in the treatments at 50 mg/L, 100 mg/L and 200 mg/L (Fig 3).

Treatment with Cs-LEE also resulted in the formation of larva-pupa and pupa-adult intermediates, especially at concentrations of 200 mg/L and 400 mg/L. Morphologically, the larva-pupa intermediate stage exhibited features of both stages. They retained the elongated, cylindrical shape of the larva while beginning to show signs of pupal formation, such as the development of a curved body or darkened cuticle. In some cases, the transition was incomplete, leading to partially developed pupae with visible larval traits. These malformed forms are often non-functional and die before fully entering the pupal stage (Fig. 4a).

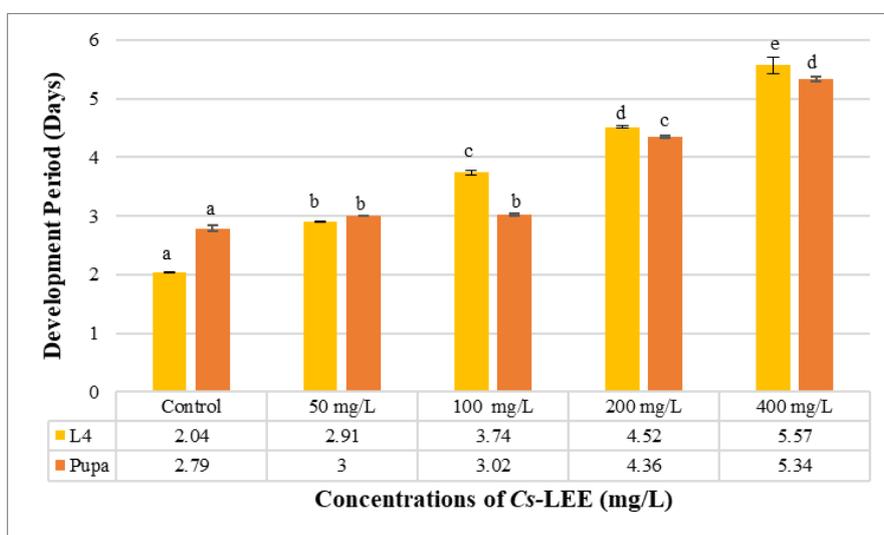
The pupa-adult intermediate stage showed incomplete

or defective adult structures. Their wings were crumpled or unexpanded, legs were malformed, and parts of the body such as the head or thorax remained partially enclosed within the exuviae (Fig.4b). No such intermediates were observed in control groups as well as at 50 mg/L and 100 mg/L.

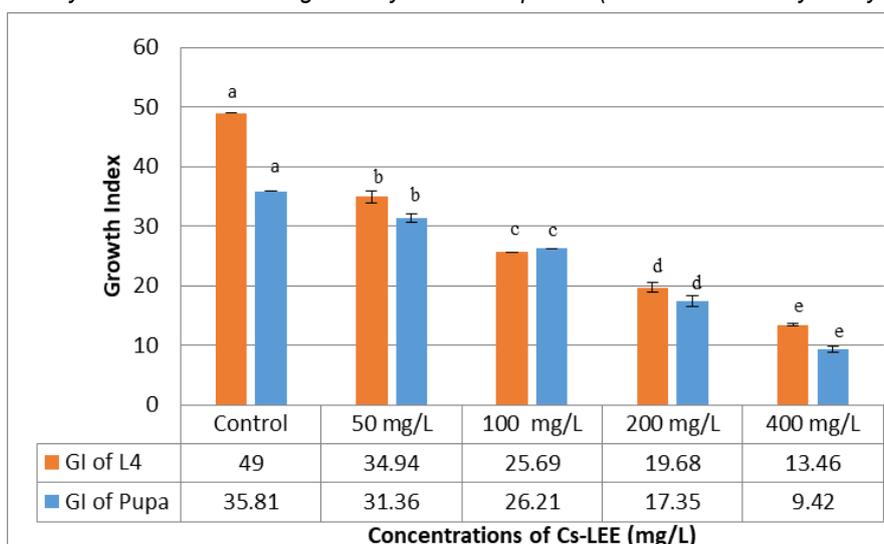
Further, adult mortality at the time of moulting was also observed mainly due to failure to detach exuviae at concentrations of 200 mg/L and 400 mg/L of Cs-LEE (Fig. 4c).

**Phytochemical analysis of Cs-LEE**

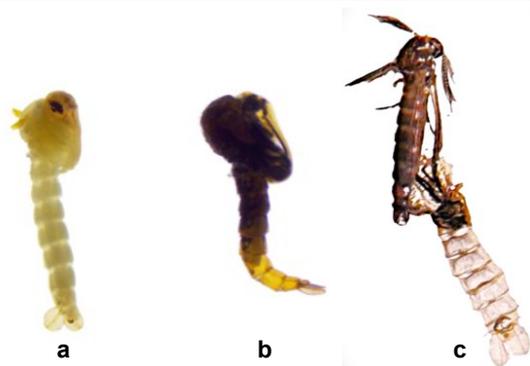
LC-MS analysis of Cs-LEE is presented in Fig. 5 and 6. The LC-MS chromatogram of the Cs-LEE sample showed a direct relationship between the mass-to-charge (m/z) ratio and the relative abundance of ions.



**Fig. 2.** Developmental period of fourth instar larvae and pupae of *Aedes aegypti* at different concentration of Cs-LEE. Average of five replicates; 25 L4 per replicate; The L4 were exposed to Cs-LEE continuously till they moulted to pupae. The bars of same colour represented by same letter are not significantly different at  $p < 0.05$  (ANOVA followed by Tukey's test).



**Fig. 3.** Growth index of fourth instar larvae and pupae of *Aedes aegypti* at different concentrations of Cs-LEE; Average of five replicates; 25 L4 per replicate; The L4 were exposed to Cs-LEE continuously till they moulted to pupae. The bars of same colour represented by same letter are not significantly different at  $p < 0.05$  (ANOVA followed by Tukey's test).



**Fig. 4.** Developmental anomalies observed in the *Aedes aegypti* treated with Cs-LEE: a. Larva-pupa intermediate, b. pupa-adult intermediate, c. Adult failed to detach its exuviae

The molecular mass of the ions generated from the sample is shown on the x-axis as  $m/z$  ratios, and the relative abundance of these ions is shown on the y-axis, emphasizing the intensity or concentration of the species in the sample. The compounds that were ionized during the mass spectrometry study are shown by the peaks. In negative ionization mode, the ion at  $m/z$  441.1231 had the highest intensity, indicating that this fragment was the most abundant species detected in the sample. This peak stands out as one of the most significant due to its high relative abundance.

At  $m/z$  883.2780, 915.2726 and 833.6112, other notable peaks were detected, signifying distinct fragments or molecular ions produced from the chemicals in the Cs-LEE. One of the prominent peaks with high relative abundance in the positive mode was the ion at  $m/z$  195.1541, which had the highest intensity and indicated that this fragment was the most prevalent in the sample. Other prominent peaks were found at  $m/z$  304.4118, 593.5130, and 786.9247, which indicate different fragments or molecule ions generated from the compounds in the Cs-LEE. The peaks represent the molecular fragments of the substances contained in the sample. The peaks of greater strength indicated higher quantities of those particular substances. By comparing the  $m/z$  values in this spectrum with databases of known compounds, the chemical structures of the molecules were determined. LC-MS analysis of Cs-LEE representing the major components present in the extract and their biological activities are presented in Table 3. The LC-MS analysis of Cs-LEE revealed 430 and 470 distinct chemicals in the raw datasets for negative and positive modes, respectively. Based on their molecular weights and retention durations, these chemicals were identified with great accuracy. Numerous bioactive molecules from several chemical classes, such as Patulin, Fluconazole, Osmundalin, Kaempferol Kaempferol/ Kaempferol 3-sophoroside 7-rhamnoside, Quercetin 3- (glucosyl-(1->4)-rhamnoside) 7-rutinoside,

Astragalin 7- rhamnoside, Protocatechuic Acid, Fluconazole, Citrusin D, Luteolin 6-C-glucoside 8-C-arabinoside/ carlinoside/ Carlina oxide were analyzed. Each of these compounds has a wide variety of biological functions.

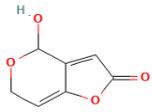
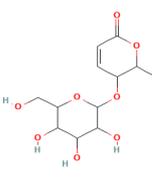
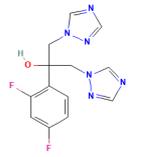
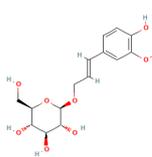
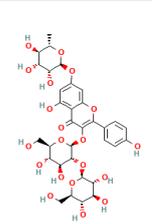
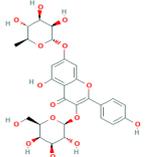
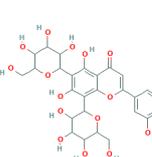
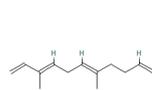
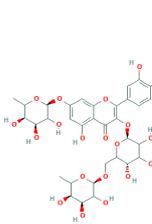
GC-MS chromatogram of Cs-LEE is presented in Fig. 7. GC-MS analysis of Cs-LEE representing the major components present in the extract and their biological activities are presented in Table 3. The GC-MS profile of the Cs-LEE sample reveals a rich composition of bioactive molecules with proven or potential insecticidal effects. The total number of peaks detected was 52, with a total ion count (TIC) of 91,697,860. Major compound was found to be Caffeine (1,3,7-trimethyl-3,7-dihydro-1h-purine-2,6-dione) (peak #25) with dominant peak area 38.32%. Top 10 major compounds (Area %) were Caffeine (1,3,7-Trimethyl-3,7-dihydro-1H-purine-2,6-dione) with 38.32% area, Hydroquinone, TMS derivative with 19.59 percent area, Palmitic Acid, Trimethylsilyl derivative (TMS) with area% of 5.54, Octadecatrein (9Trimethylsilyl (3E,6E,9E)-3,6,9-octadecatrien) has area% of 4.76, 3,5-Dihydroxybenzoic acid, 3TMS derivative with 3.44 area%, Adipic acid, TMS derivative found to have 2.91 area %, Phytol, TMS derivative has 2.59 area %, 1,3,5-Benzetriol, 2TMS derivative has 2.58%, Pyrogallol, 3TMS derivative with 1.50 area % and 1,3,5-Benzetriol, 3TMS derivative has area % of 1.31.

## DISCUSSION

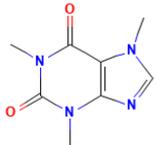
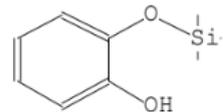
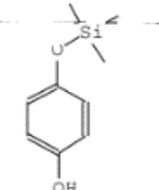
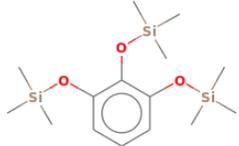
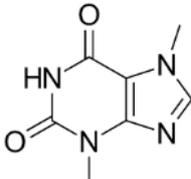
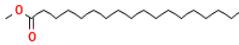
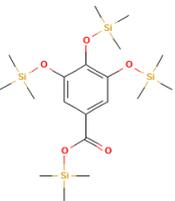
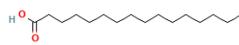
Across the world, one of the biggest threats to public health is mosquito-borne illness. It can be managed by applying repellent to prevent mosquito bites and by killing larvae and adult mosquitoes. As using synthetic insecticides pose many serious issues, plant extracts are being considered as possible natural sources of mosquito control (Sengul Demirak and Canpolat, 2022). In this study, the larvicidal potential of the ethanol extract of *Camellia sinensis* leaves against fourth-instar larvae of the *Ae. aegypti* mosquito was assessed. The present study shows that Cs-LEE affected the survival and development of fourth instar larvae (Fig. 2), with mortality observed to be highest at 400 mg/L (Table 1). A direct correlation between exposure duration and larval mortality, as well as between the concentration of green tea leaf extract to which the larvae were exposed and their mortality, was observed in early studies (Hassan *et al.*, 2020). In another study, green tea oil was found to be effective against larvae and adults of *Culex* mosquitoes (Radwan *et al.*, 2022). Green tea leaf extract has also shown larvicidal activity against *Anopheles arabiensis* and *Anopheles gambiae*; larval exposure to crude extract at 250 ppm and 500 ppm for 24 h resulted in larval mortality over 90 % in *A.*



**Table 2.** LC-MS profiling of Cs-LEE identified its principal phytochemical components along with their insecticidal and insect growth-regulating activities

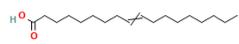
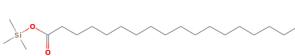
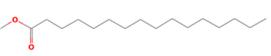
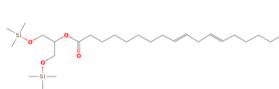
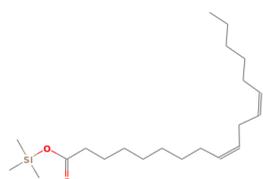
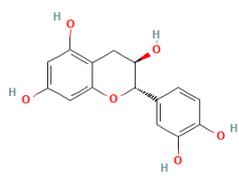
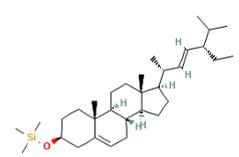
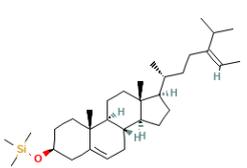
S. No.	Name of phyto-chemical	Molecular formula	Molecular weight (g/mol)	Chemical structure	Biological activity	References
1.	Patulin	C <sub>7</sub> H <sub>6</sub> O <sub>4</sub>	154.12		Insecticidal, larvicidal	Reiss, 1975
3.	Osmundalin	C <sub>12</sub> H <sub>18</sub> O <sub>8</sub>	290.27		Insecticidal	Mitai <i>et al.</i> , 2024 Numata <i>et al.</i> , 1990
4.	Fluconazole	C <sub>13</sub> H <sub>12</sub> F <sub>2</sub> N <sub>6</sub> O	306.27		Insecticidal	Kačaniová <i>et al.</i> , 2025
5.	Citrusin D	C <sub>16</sub> H <sub>22</sub> O <sub>8</sub>	342.34		Repellent, anti-feedant	Mursiti <i>et al.</i> , 2019
6.	Kaempferol/ Kaempferol 3-sophoroside 7-rhamnoside	C <sub>33</sub> H <sub>40</sub> O <sub>20</sub>	756.7		Insecticidal	Periferakis <i>et al.</i> , 2022
7.	Astragalin 7- rhamno- side (kaempferol 3-O-glucoside 7-O-rhamnoside)	C <sub>27</sub> H <sub>30</sub> O <sub>15</sub>	594.5		Insecticidal	Riddick, 2024
8.	Luteolin 6-C-glucoside 8-C-arabinoside/ carlinoside/ Carlina oxide	C <sub>27</sub> H <sub>30</sub> O <sub>16</sub>	610.5		Insecticidal, anti-oxidant, antibacterial, antifungal,	Sowa <i>et al.</i> , 2023
9.	Sinensal-alpha/ Alphaisinesal/ (E,E,E)-2,6,10- Trimethyldodeca- 2,6,9,11-tetraen-1-ol	C <sub>15</sub> H <sub>22</sub> O	218.33		Insecticidal, anti-microbial	Tzi Bun Ng <i>et al.</i> , 2016
10.	Quercetin 3- (glucosyl-(1->4)- rhamnoside) 7- rutinoside	C <sub>33</sub> H <sub>40</sub> O <sub>20</sub>	756.7		Insecticidal	Gao <i>et al.</i> , 2022

**Table 3.** GC-MS profiling of *Cs*-LEE identified its principal phytochemical components along with their insecticidal and insect growth-regulating activities

S. No.	Name of phyto-chemical	Molecular formula	Molecular weight (g/mol)	Chemical structure	Biological activity	References
1.	Caffeine (1,3,7-Trimethyl-3,7-dihydro-1H-purine-2,6-dione)	C <sub>8</sub> H <sub>10</sub> N <sub>4</sub> O <sub>2</sub>	194.19		Larvicidal activity against mosquitoes	Vilvest <i>et al.</i> , 2025
2.	Catechol	C <sub>9</sub> H <sub>14</sub> OSi	182		Larvicidal activity against mosquitoes	Tang <i>et al.</i> , 2023
3.	Hydroquinone	C <sub>9</sub> H <sub>14</sub> O <sub>2</sub> Si	182		Insect growth inhibition	Céspedes <i>et al.</i> , 2004
4.	Pyrogallol	C <sub>15</sub> H <sub>30</sub> O <sub>3</sub> Si <sub>3</sub>	342		Disruptive effect on insect development	Chauhan and Sohal 2018
5.	Theobromine	C <sub>7</sub> H <sub>8</sub> N <sub>4</sub> O <sub>2</sub>	180		Insecticidal	Bourdarias <i>et al.</i> , 2025
6.	Octadecanoic acid, Methyl ester	C <sub>19</sub> H <sub>38</sub> O <sub>2</sub>	298		Insecticidal, antifeedant	Arunthirumeni <i>et al.</i> , 2023 Khanday and Sharma 2021
7.	Benzoic acid, 3,4,5-tris(trimethylsiloxy)	C <sub>19</sub> H <sub>38</sub> O <sub>5</sub> Si <sub>4</sub>	458		Insecticidal Antimicrobial	Ousman <i>et al.</i> , 2025 Salinas-Sánchez <i>et al.</i> , 2021
8.	Palmitic acid	C <sub>19</sub> H <sub>40</sub> O <sub>2</sub> Si	328		Insecticidal	Perumalsamy <i>et al.</i> , 2015 Salinas-Sánchez <i>et al.</i> , 2021
9.	Linoleic acid	C <sub>21</sub> H <sub>40</sub> O <sub>2</sub> Si	352		Insecticidal	Salinas-Sánchez <i>et al.</i> , 2021

Contd.....

**Table 3.** Contd.....

10.	9-Octadecenoic acid	C <sub>21</sub> H <sub>42</sub> O <sub>2</sub> Si	354		Insecticidal	Shaalan <i>et al.</i> , 2025
11.	Stearic acid	C <sub>21</sub> H <sub>44</sub> O <sub>2</sub> Si	356		Insecticidal	Sabit <i>et al.</i> , 2025
12.	Hexadecanoic acid	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	270		Insecticidal	Mokhtar <i>et al.</i> , 2021
13.	2-linoleoylglycerol	C <sub>27</sub> H <sub>54</sub> O <sub>4</sub> Si <sub>2</sub>	498		Insect growth inhibitor	Gu, <i>et al.</i> , 2025
14.	9,12-octadecadienoic acid	C <sub>21</sub> H <sub>40</sub> O <sub>2</sub> Si	352		Insecticidal	Farag <i>et al.</i> , 2021
15.	9-octadecenoic acid	C <sub>21</sub> H <sub>42</sub> O <sub>2</sub> Si	354		Insect repellent	Viswakethu <i>et al.</i> , 2025
16.	Catechine	C <sub>30</sub> H <sub>54</sub> O <sub>6</sub> Si <sub>5</sub>	650		Insecticidal	Ruttanaphan <i>et al.</i> , 2023
17.	Stigmasterol	C <sub>32</sub> H <sub>56</sub> O <sub>Si</sub>	484		Insecticidal antimicrobial	Anagued <i>et al.</i> , 2024
18.	Silane	C <sub>32</sub> H <sub>56</sub> O <sub>Si</sub>	484		Insecticidal	Narendrakumar <i>et al.</i> , 2021

*gambiae* and 75% in *A. arabiensis*. Exposure at a concentration of 100 ppm resulted in mortality rates of < 50 % in both species. However, growth disruption effects and developmental abnormality such as larval-pupal intermediates were reported (Muema *et al.*, 2016). The results were somewhat similar to our reports on *Ae. aegypti*, which indicate high mortality at high concentrations and growth-suppressive and developmental-disruptive activity of Cs-LEE at low concentrations. The essential oils present in *Camellia sinensis*, and *Citrus medica* had shown high larvicidal activity against *Ae. albopictus* (Sheng *et al.*, 2020). Furthermore, the caf-

feine and other chemical components present in green tea aqueous extracts make it an efficient larvicide against *Drosophila prosaltans* (Zabar *et al.*, 2013).

The *C. sinensis* leaves ethanol extract increased the development period of fourth instar larvae and pupae of *Ae. Aegypti* (Fig. 2). These findings concur with those of Hassan *et al.* (2020), who found that the developmental period of both *Culex pipiens* larvae and pupae was significantly affected after exposure to the *C. sinensis* leaf extract. Dieng *et al.* (2016) reported a longer larval development period and lower pupation and adult emergence rates in *Ae. albopictus* when ex-

posed to sublethal dosages of *C. sinensis* leaf extract. Therefore, it was hypothesized that this extract might have disrupted some crucial hormone-regulated developmental processes. Lopez *et al.* (2015) have documented effect of green tea polyphenolic extracts on the larval stage of *Drosophila melanogaster*.

The *C. sinensis* leaf extract leaves resulted in the formation of larva-pupa and pupa-adult intermediates. With increasing extract concentrations, the percentage of intermediates increased. Dead larva-pupa transitional stages and pupa-adult intermediates are shown in Fig. 4a and 4b respectively. The larva-pupa intermediates possessed a larval abdomen and a pupa's head. Moreover, adults that could not get detached from exuviae were also observed (Fig. 4c). These findings suggest that the *C. sinensis* leaf extract disrupted metamorphosis, possibly by altering hormone regulation or interfering with chitin synthesis during moulting (Saxena and Yadav, 1983; Mwangi and Rembold, 1988). Plant extracts have been shown to cause morphological abnormalities in mosquito larvae in a number of earlier investigations. Benzene extracts of *Argemone mexicana*, *Jatropha curcus*, *Pergularia extensa* and *Withania somnifera* have been shown to influence *Culex quinquefasciatus* larval morphology, including larval-pupal intermediates (Karmegam *et al.*, 1997). Khater and Shalaby (2008) reported similar findings about aberrant moulting in *Culex pipiens* after exposure to fenugreek oils (*Trigonella foenum-grecum*), earth almond (*Cyperus esculentus*), mustard (*Brassica campestris*), olibanum (*Boswellia serrata*), rocket (*Eruca sativa*), and parsley (*Carum ptroselinum*). Consequently, the larvae failed to develop normally; the deformed larvae, pupae, and adults formed. The study may be further carried out to evaluate the effects of sublethal doses of Cs-LEE on reproductive fitness, including oviposition behaviour, egg viability, and hatching, for effective vector management.

The Cs-LEE has a significant effect on the growth index of both the L4 and pupa of *Ae. aegypti*, it dropped significantly in a dose-dependent manner (Fig. 3). The reduced growth index was correlated with decline in adult emergence. This suggested that although the Cs-LEE was less toxic to L4 of *Ae. aegypti* during initial exposure, it reduced fitness by impairing growth and adult emergence. These findings concur with those of Shaalan *et al.* (2005) and Saxena *et al.* (1993). It is well known that plant extracts have a variety of effects, such as neurotoxic, inhibition of detoxifying enzymes, disruption of larval growth, and midgut injury in insects. Green tea extract's varied chemical composition points to a multifaceted impact on mosquito physiology (Sharawi, 2023). An intriguing technique for determining the types and quantities of active ingredients in the plant extract is LC-MS and GC-MS. These methods were used to identify bioactive components present in the ethanolic

extract of *C. sinensis* leaves. Some of the important chemicals reported in green tea included polyphenols such as catechins (proanthocyanidins, epigallocatechin), flavonoids (quercetin and kaempferol), alkaloids (caffeine), saponins, and certain fatty acids (palmitic acid, linoleic acid) (Table 2 and 3) These chemicals have been reported to have insecticidal or deterring effects.

Proanthocyanidins, the polyphenolic compounds are condensed tannins. These are found in plants and help them to defend against fungal invasions and insect herbivory (War *et al.*, 2012). Proanthocyanidins have been reported to hampered larval survival and adult emergence of *Anopheles arabiensis* and *Anopheles gambiae* (Muema *et al.*, 2016). These compounds may also decrease nutrient assimilation by lowering the bioaccessibility and digestion of amino acids, thereby hampering the growth of larval and pupal stages of *Ae. aegypti* as reflected by the lower growth indices in the present studies (Frazier *et al.*, 2010). Further, these have been shown to inhibit the activity of ecdysone 20-monooxygenase in a variety of dipterans, including mosquitoes (Dieng *et al.*, 2016). Ecdysone is an essential hormone for insect moulting. The observed longer larval and pupal durations and abnormalities are consistent with ecdysial failure and incorrect development may be caused by interference with ecdysone biosynthesis or metabolism (Dieng *et al.*, 2016, Qasim *et al.*, 2020, Hassan *et al.*, 2020). In insects, cell proliferation and DNA replication occur during the post-embryonic stages before growth and morphogenesis (Lee *et al.*, 2003). Green tea polyphenols have anticarcinogenic effects and therefore inhibit growth, induce apoptosis, and arrest the cell cycle in insects (Gupta *et al.*, 2000; Khan *et al.*, 2006).

Quercetin and Kaempferol the two hydroxyflavones were reported in the Cs-LEE. These compounds have been reported to cause cell cycle arrest by blocking tyrosine phosphorylation of CDC25A during the G2/M phase and/or triggering apoptosis (Alianni *et al.*, 2001). Further, alkaloids and methylxanthines reported in the Cs-LEE may reduce nutrient intake and lead to starvation, resulting in impaired larval growth (Dieng *et al.*, 2016; Procópio *et al.*, 2015). Caffeine has been reported to disrupt the development of mosquito larvae (Laranja *et al.*, 2003). Moreover, larvicidal and pupicidal properties of Caffeine have been reported against *Ae. aegypti* and *Culex quinquefasciatus* (Valvest *et al.*, 2025). Saponins reported in the Cs-LEE can bind to digestive enzymes and indirectly affect beneficial bacteria in the insect's digestive system, further hindering digestion and nutrient absorption (Qasim *et al.*, 2020). Moreover, saponins harm midgut epithelial cells both structurally and physiologically (Cui *et al.*, 2019). Larval death may results from this disruption of digestive enzyme biosynthesis and nutrition absorption (Cui *et al.*,

2019). Furthermore, the toxicity of fatty acids, such as Palmitic acid and Linoleic acid, against mosquito larvae has also been reported earlier (Perumalsamy *et al.*, 2015; de Melo *et al.*, 2018). The findings indicate that AChE is the main target site of these fatty acids, leading to accumulation of acetylcholine at nerve endings, continuous overstimulation of cholinergic receptors, and ultimately rapid paralysis and death (Perumalsamy *et al.*, 2015).

A variety of bioactive phytochemicals, including flavonoids, phenolics, alkaloids, fatty acids, and saponins, may collaborate to confer green tea extract its efficacy rather than any one acting alone. Together, these substances affect several vital physiological functions in mosquitoes, including the modulation of hormones (ecdysone), the digestion and absorption of nutrients, and the integrity of the cuticle and midgut. Compared to pesticides with a single mode of action, this multi-target strategy makes it more difficult for mosquitoes to build resistance (Corzo-Gómez *et al.*, 2024). Due to the inherent difficulty of developing resistance to multiple simultaneous attacks, this comprehensive targeting of several biological pathways explains why green tea extract is beneficial across the diverse life stages of *Ae. aegypti* and implies a potential for robust, long-term efficacy. The present work focuses on the growth-suppressive and development-disruptive effects of Cs-LEE against *Ae. aegypti* larvae under laboratory studies. However, in the natural environment, factors such as variable water quality and the presence of natural predators may affect the extract's efficacy. Therefore, further studies in semi-field and field conditions can assess the efficacy of the extract in real-world scenarios.

## Conclusion

The present study reported that Cs-LEE had adverse effects on the survival, growth, and development of *Ae. aegypti* larvae and pupae. Consequently, both larvae and pupae showed increased mortality and a prolonged developmental period. The Cs-LEE was also shown to have growth-suppressive activity, as reflected by a decreased growth index in the treated larvae; the abortive moulting and formation of larval-pupal intermediates indicate developmental-disruptive activities of the extract. The phytochemical analysis of the Cs-LEE revealed the presence of a variety of active ingredients including flavonoids, catechins, caffeine and polyphenols in high concentrations. Many of these compounds have JH-mimic activities; therefore, they interfere with insect hormones and contribute to growth-suppressive and developmental-disruptive activities. Present study revealed presence of JH mimic compounds in the Cs-LEE. Further, the effects related to increased JH levels were reported in the larvae treated with Cs-LEE. There-

fore the research work can be extended to find JH titers in treated larvae using GC-MS/MS. Studies can also be planned to evaluate the synergistic potential of Cs-LEE with other botanical extracts to make it more effective.

## ACKNOWLEDGEMENTS

The authors acknowledge Prof. Rajendra Kumar Pandey, Principal, Deshbandhu College, University of Delhi, Delhi, India, and Dr. Himmat Singh, Scientist E, ICMR-NIMR, Dwarka, Delhi, India, for providing all the necessary facilities and support during the research work.

## Conflict of interest

The authors declare that they have no conflict of interest.

## REFERENCES

1. Aligiannis, N., Mitaku, S., Mitrocotsa, D. & Leclerc, S. (2001). Flavonoids as cyclin-dependent kinase inhibitors: inhibition of cdc 25 phosphatase activity by flavonoids belonging to the quercetin and kaempferol series. *Planta Med.*, 67:468–70.
2. Anagued Haman, E., Moumbon, V. P., Fadimatou, S. A., Momeni, J. & Ngameni, B. (2024). A new sphingoid derivative from *Acacia hockii* De Wild (Fabaceae) with antimicrobial and insecticidal properties. *Physical Sciences Reviews*, 9(3), 1641-1654.
3. Ankit, Saha, L., Kishor, V. & Bauddh, K. (2020). Impacts of synthetic pesticides on soil health and non-targeted flora and fauna. *Ecological and Practical Applications for Sustainable Agriculture*, 65-88.7.
4. Arunthirumeni, M., Vinitha, G. & Shivakumar, M. S. (2023). Antifeedant and larvicidal activity of bioactive compounds isolated from entomopathogenic fungi *Penicillium* sp. for the control of agricultural and medically important insect pest (*Spodoptera litura* and *Culex quinquefasciatus*). *Parasitology International*, 92, 102688.
5. Bellinato, D. F., Viana-Medeiros, P. F., Araújo, S. C., Martins, A. J., Lima, J. B. P. & Valle, D. (2016). Resistance status to the insecticides temephos, deltamethrin, and diflubenzuron in Brazilian *Aedes aegypti* populations. *BioMed Research International*, 2016(1), 8603263.
6. Bourdarias, M., Landschoot, S., Eeckhout, M. & Hesta, M. (2025). Influence of theobromine in feed on larval growth and survival in *Tenebrio molitor*. *PLoS One*, 20(7), e0328354.
7. Céspedes, C. L., Torres, P., Marín, J. C., Arciniegas, A., de Vivar, A. R., Pérez-Castorena, A. L. & Aranda, E. (2004). Insect growth inhibition by tocotrienols and hydroquinones from *Roldana barba-johannis*. *Phytochemistry*, 65(13), 1963-1975.
8. Chauhan, N. S. & Sohal, S. K. (2018). Disruptive effect of pyrogallol on development of *Spodoptera litura* (Fab.) larvae. *Journal of Biopesticides*, 11(1), 7-13.
9. Choochote, W., Chaithong, U., Kamsuk, K., Jitpakdi, A., Tippawangkosol, P., Tuetun, B., ... & Pitasawat, B.

- (2007). Repellent activity of selected essential oils against *Aedes aegypti*. *Fitoterapia*, 78(5), 359-364.
10. Corzo-Gómez, J. C., Espinosa-Juárez, J. V., Ovando-Zambrano, J. C., Briones-Aranda, A., Cruz-Salomón, A. & Esquinca-Avilés, H. A. (2024). A review of botanical extracts with repellent and insecticidal activity and their suitability for managing mosquito-borne disease risk in Mexico. *Pathogens*, 13(9), 737.
  11. Cui, C., Yang, Y., Zhao, T., Zou, K., Peng, C., Cai, H., ... & Hou, R. (2019). Insecticidal activity and insecticidal mechanism of total saponins from *Camellia Oleifera*. *Molecules*, 24(24), 4518.
  12. Das, P. R. & Eun, J. B. (2018). A comparative study of ultra-sonication and agitation extraction techniques on bioactive metabolites of green tea extract. *Food Chem.*, 2018, 253, 22– 29, DOI: 10.1016/j.foodchem.2018.01.080
  13. de Melo, A. R., Garcia, I. J. P., Serrão, J. E., Santos, H. L., dos Santos Lima, L. A. R. & Alves, S. N. (2018). Toxicity of different fatty acids and methyl esters on *Culex quinquefasciatus* larvae. *Ecotoxicology and Environmental Safety*, 154, 1-5.
  14. Dieng, H., Tan Yusop, N. S. B., Kamal, N. N. B., Ahmad, A. H., Ghani, I. A., Abang, F., ... & Noweg, G. T. (2016). Exposure of a dengue vector to tea and its waste: survival, developmental consequences, and significance for pest management. *Journal of Agricultural and Food Chemistry*, 64(18), 3485-3491.
  15. Farag, S. M., Essa, E. E., Alharbi, S. A., Alfarraj, S. & El-Hassan, G. A. (2021). Agro-waste derived compounds (flax and black seed peels): Toxicological effect against the West Nile virus vector, *Culex pipiens* L. with special reference to GC-MS analysis. *Saudi Journal of Biological Sciences*, 28(9), 5261-5267.
  16. Frazier, R. A., Deaville, E. R., Green, R. J., Stringano, E., Willoughby, I. & Plant, J., et al. (2010). Interactions of tea tannins and condensed tannins with proteins. *J Pharm Biomed Anal.*, 51:490–5.
  17. Gao, Y. L., Pan, Z. Y., Meng, X., Yuan, Y. F., Li, H. Y. & Chen, M. (2022). The effect of quercetin on the growth, development, nutrition utilization, and detoxification enzymes in *Hyphantria cunea* Drury (Lepidoptera: Arctidae). *Forests*, 13(11), 1945.
  18. Gu, C., Wang, M., Lin, Y., Zhang, Y., Khan, A., Song, Y. & Zeng, R. (2025). Metabolomic profiling reveals the anti-herbivore mechanism of rice (*Oryza sativa*) induced by silicon. *Arthropod-Plant Interactions*, 19(1), 6.
  19. Gupta, S., Ahmad, N., Nieminen, A-L. & Mukhtar, H. (2000). Growth inhibition, cell-cycle dysregulation, and induction of apoptosis by green tea constituent (-)-epigallocatechin-3-gallate in androgen-sensitive and androgen-insensitive human prostate carcinoma cells. *Toxicol Appl. Pharmacol.*, 164:82–90.
  20. Hassan, M. I., Atwa, W. A., Moselhy, W. A. & Mahmoud, D. A. (2020). Efficacy of the green tea, *Camellia sinensis* leaves extract on some biological activities of *Culex pipiens* and the detection of its phytochemical constituents. *Egyptian Academic Journal of Biological Sciences, F. Toxicology & Pest Control*, 12(1), 59-70.
  21. Hillary, V. E., Ceasar, S. A. & Ignacimuthu, S. (2024). Efficacy of plant products in controlling disease vector mosquitoes, a review. *Entomologia Experimentalis et Applicata*, 172(3), 195-214.
  22. Hubschmann, H. J. (2025). *Handbook of GC-MS: fundamentals and applications*. John Wiley & Sons.
  23. Kačániová, M., Vukic, M., Čmiková, N., Bianchi, A., Garzoli, S., Saad, R. B., ... & Vukovic, N. (2025). Exploring the bioactive potential of *Pinus mugo* Turra essential oil: volatile composition, antioxidant, antimicrobial, antibiofilm and insecticidal activities. *Flavour and Fragrance Journal*, 40(2), 349-364.
  24. Karakoti, H., Mahawer, S. K., Tewari, M., Kumar, R., Prakash, O., de Oliveira, M. S. & Rawat, D. S. (2022). Phytochemical profile, in vitro bioactivity evaluation, in silico molecular docking and ADMET study of essential oils of three *Vitex* species grown in Tarai Region of Uttarakhand. *Antioxidants*, 11(10), 1911
  25. Karmegam, N., Sakthivadivel, M., Anuradha, V. & Daniel, T. (1997). Indigenous-plant extracts as larvicidal agents against *Culex quinquefasciatus* Say. *Bioresource Technology*, 59(2-3), 137-140.
  26. Kelstrup, C. D., Bekker-Jensen, D. B., Arrey, T. N., Hogrebe, A., Harder, A. & Olsen, J. V. (2018). Performance evaluation of the Q exactive HF-X for shotgun proteomics. *Journal of Proteome Research*, 17(1), 727-738.
  27. Khan, N., Afaq, F., Saleem, M., Ahmad, N. & Mukhtar, H. (2006). Targeting multiple signaling pathways by green tea polyphenol (-)-epigallocatechin-3-gallate. *Cancer Res.*, 66:2500–5.
  28. Khanday, S. & Sharma, G. D. (2021). GC-MS analysis and antifeedant activity of *Azardiachta indica*-leaf extract. *Stechnolock Plant Biol. Res.*, 1, 1-15.
  29. Khater, H. F. & Shalaby, A. A. S. (2008). Potential of biologically active plant oils to control mosquito larvae (*Culex pipiens*, Diptera: Culicidae) from an Egyptian locality. *Revista do Instituto de Medicina Tropical de Sao Paulo*, 50, 107-112.
  30. Kumar, D., Singh, B., Kumar, G., Shakya, R., Vikram, K., Rani, A. & Singh, H. (2023). Phyto-fabrication and characterization of *Alternanthera sessilis* leaf extract-mediated silver nanoparticles and evaluation of larvicidal potential. *Biomass Conversion and Biorefinery*, 15(2), 2329-2344.
  31. Laranja, A. T., Manzatto, A. J. & Campos Bicudo, H. E. M. (2003). Effects of caffeine and used coffee grounds on biological features of *Aedes aegypti* (Diptera, Culicidae) and their possible use in alternative control. *Genet. Mol. Biol.*, 26(4):419–29.
  32. Lee, L. A. & Orr-Weaver, T. L. (2003). Regulation of cell cycles in *Drosophila* development: intrinsic and extrinsic cues. *Annu. Rev. Genet.*, 37:545–78.
  33. Lee, L. S., Kim, S. H., Kim, Y. B. & Kim, Y. C. (2014). Quantitative analysis of major constituents in green tea with different plucking periods and their antioxidant activity. *Molecules*, 19 (7), 9173– 9186, DOI: 10.3390/molecules19079173)
  34. Lopez, T. E., Pham, H. M., Barbour, J., Tran, P., Van Nguyen, B., Hogan, S. P., ... & Jafari, M. (2016). The impact of green tea polyphenols on development and reproduction in *Drosophila melanogaster*. *Journal of Functional Foods*, 20, 556-566.
  35. Mamat, S. F., Azizan, K. A., Baharum, S. N., Noor, N. M. & Aizat, W. M. (2020). GC-MS and LC-MS analyses re-

- veal the distribution of primary and secondary metabolites in mangosteen (*Garcinia mangostana* Linn.) fruit during ripening. *Scientia Horticulturae*, 262, 109004.
36. Manning, J. & Roberts, J. C. (2003). Analysis of catechin content of commercial green tea products. *J. Herb. Pharmacother.*, 3 (3), 19– 32, DOI: 10.1080/J157v03n03\_03
  37. Milugo, T. K., Tchouassi, D. P., Kavishe, R. A., Dinglasan, R. R. & Torto, B. (2021). Naturally occurring compounds with larvicidal activity against malaria mosquitoes. *Frontiers in Tropical Diseases*, 2, 718804.
  38. Mitoi, E. M., Aldea, F., Helepciuc, F. E., Ciocan, A. G., Frum, A., Popescu, D. I., ... & Soare, L. C. (2024). The Production of useful phenol compounds with antioxidant potential in gametophytes and sporophytes from In-vitro cultures in four ornamental ferns species. *Horticulturae*, 10(8), 799.
  39. Mokhtar, M. M., Du, Z. & Cheng, F. (2021). Insecticidal efficacy and chemical composition of *Balanites aegyptiaca* (L.) Delile seed oils against *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae). *Chilean Journal of Agricultural Research*, 81(1), 102-108.
  40. Moyes, C. L., Vontas, J., Martins, A. J., Ng, L. C., Koou, S. Y., Dusfour, I., ... & Weetman, D. (2017). Contemporary status of insecticide resistance in the major *Aedes* vectors of arboviruses infecting humans. *PLoS Neglected Tropical Diseases*, 11(7), e0005625.
  41. Muema, J.M., Bargul, J.L., Nyanjom, S.G., Mutunga, J.M. & Njeru, S.N. (2016). Potential of *Camellia sinensis* proanthocyanidins-rich fraction for controlling malaria mosquito populations through disruption of larval development. *Parasites Vectors*, 9, 1–10.
  42. Mursiti, S., Lestari, N. A., Febriana, Z., Rosanti, Y. M. & Ningsih, T. W. (2019). The activity of d-limonene from sweet orange peel (*Citrus Sinensis* L.) extract as a natural insecticide controller of bedbugs (Cimex cimicidae). *Oriental Journal of Chemistry*, 35(4), 1420.
  43. Mwangi, R. W. & Rembold, H. (1988). Growth-inhibiting and larvicidal effects of *Melia volkensii* extracts on *Aedes aegypti* larvae. *Entomologia Experimentalis et Applicata*, 46(2), 103-108.
  44. Narendrakumar, G. & Karthick Raja Namasivayam, S. (2021). Surface-modified nanosilica–chitinase (SiNp-Chs)-doped nano enzyme conjugate and its synergistic pesticidal activity with plant extracts against armyworm *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae). *IET Nanobiotechnology*, 15(1), 117-134.
  45. Ng, T. B., Bekhit, A. E. D. A., Fang, E. F., Li, X., Lu, Q., Guo, H. & Wong, J. H. (2016). Grapefruit (*Citrus paradisi*) oils. In *Essential Oils in Food Preservation, Flavor and Safety* (pp. 463-470). Academic Press.
  46. Numata, A., Takahashi, C., Fujiki, R., Kitano, E., Kitajima, A. & Takemura, T. (1990). Plant constituents biologically active to insects. VI. antifeedants for Larvae of the yellow butterfly, *Eurema hecabe*, *Eurema mandarina*, in *Osmunda Japonica*. (2). *Chem. Pharm. Bull.*, 38, 2862–2865.
  47. Oaya, C. S., Malgwi, A. M., Degri, M. M. & Samaila, A. E. (2019). Impact of synthetic pesticides utilization on humans and the environment: an overview. *Agricultural Science & Technology* (1313-8820), 11(4).
  48. Ousman, B. M., Mennane, Z., Boussaoudi, I., Otchom, B. B. & Saoud, Y. (2025). First investigation of phytochemical screening, HPLC-MS characterization, and antibacterial activity of *Azadirachta indica* A. Juss (Mim or Neem) leaf extracts, grown in the republic of Chad. *Natural Product Communications*, 20(2), 1934578X251322360.
  49. Adams, P. R. (2017). Identification of essential oil components by gas chromatography/mass spectrometry.
  50. Periferakis, A., Periferakis, K., Badarau, I. A., Petran, E. M., Popa, D. C., Caruntu, A., ... & Costache, D. O. (2022). Kaempferol: antimicrobial properties, sources, clinical, and traditional applications. *International Journal of Molecular Sciences*, 23(23), 15054.
  51. Perumalsamy, H., Jang, M. J., Kim, J. R., Kadarkarai, M. & Ahn, Y. J. (2015). Larvicidal activity and possible mode of action of four flavonoids and two fatty acids identified in *Milletia pinnata* seed toward three mosquito species. *Parasites & Vectors*, 8(1), 237.
  52. Procópio, T. F., Fernandes, K. M., Pontual, E. V., Ximenes, R. M., de Oliveira, A. R. C. & Souza, C. D. S., et al. (2015). *Schinus terebinthifolius* leaf extract causes midgut damage, interfering with survival and development of *Aedes aegypti* larvae. *PLoS One.*, 10: e 0126612.
  53. Qasim, M., Islam, W., Ashraf, H. J., Ali, I. & Wang, L. (2020). Saponins in insect pest control. In *Co-Evolution of Secondary Metabolites*, (pp. 1-28). Springer, Cham.
  54. Radwan, I. T., Baz, M. M., Khater, H., Alkhaibari, A. M. & Selim, A. M. (2022). Mg-LDH nanoclays intercalated fennel and green tea active ingredient: Field and laboratory evaluation of insecticidal activities against *Culex pipiens* and their non-target organisms. *Molecules*, 27(8), 2424.
  55. Reiss, J. (1975). Insecticidal and larvicidal activities of the mycotoxins aflatoxin B1, rubratoxin B, patulin and diacetoxyscirpenol towards *Drosophila melanogaster*. *Chemico-Biological Interactions*, 10(5), 339-342.
  56. Riddick, E. W. (2024). Evaluating the effects of flavonoids on insects: Implications for managing pests without harming beneficials. *Insects*, 15(12), 956.
  57. Ruttanaphan, T., Songoen, W., Pluempanupat, W. & Bullangpoti, V. (2023). Potential insecticidal extracts from *Artocarpus lacucha* against *Spodoptera litura* (Lepidoptera: Noctuidae) larvae. *Journal of Economic Entomology*, 116(4), 1205-1210.
  58. Sabit, F. A., Abdullah, M. A. & Elhadeeti, S. A. K. (2025). Study of the contact effect of stearic acid on *Trogoderma granarium* Everts, 1898 (Coleoptera: Dermestidae) in the laboratory. *Asian Journal of Agriculture*, 9(1).
  59. Salinas-Sánchez, D. O., Flores-Franco, G., Avilés-Montes, D., Valladares-Cisneros, M. G., Arias-Ataide, D. M., Mendoza-Catalán, M. Á. & Sotelo-Leyva, C. (2021). Bioactivity of a linoleic acid-rich fraction of *Ricinus communis* L.(Euphorbiaceae) leaves against the yellow sugarcane aphid, *Sipha flava* (Hemiptera: Aphididae). *Journal of Food Protection*, 84(9), 1524-1527.
  60. Saxena, R.C., Harshan, V., Saxena, A., Sukumaran, P., Sharma, M.C. & Lakshamana, K.M. (1993) Larvicidal and chemosterilant activity of *Annona squamosa* alkaloids against *An. stephensi*. *J. Am. Mosq. Control. Assoc.*, 9 (1), 84 – 87.
  61. Saxena, S. C. & Yadav, R. S. (1983). A new plant extract

- to suppress the population of yellow fever and dengue vector *Aedes aegypti* L. (Diptera: Culicidae). *Current Science*, 713-715.
62. Sengul Demirak, M. S. & Canpolat, E. (2022). Plant-based bioinsecticides for mosquito control: Impact on insecticide resistance and disease transmission. *Insects*, 13(2), 162.
  63. Shaalan, E.A., Canyon, D.V., Younges, M.W.F., Abdel-Wahab, H. & Mansour, A. (2005) A review of botanical phytochemicals with mosquitocidal potential. *Environ. Int.* 31, 1149–1166.
  64. Shah, V. K. & Gupta, K. K. (2025). Assessment of larvicidal, growth-suppressing, and development-altering bioefficacy of *Ageratum houstonianum* against *Aedes aegypti* (L.). *Journal of Vector Borne Diseases*, 62(1), 67-77.
  65. Sharawi, S. E. (2023). Larvicidal effect of some traditional Saudi Arabian herbs against *Aedes aegypti* larvae, a vector of dengue fever. *BioRxiv.*, 2023-09.
  66. Sharma, A., Kumar, S. & Tripathi, P. (2015). Impact of *Achyranthes aspera* leaf and stem extracts on the survival, morphology and behaviour of an Indian strain of dengue vector, *Aedes aegypti* L. (Diptera: Culicidae). *Journal of Mosquito Research*, 5(7), 1-9.
  67. Shazad, M., Kayesth, S., Kumar, S., Kapoor, N. & Gupta, K. K. (2024). Impact of *Catharanthus roseus* leaf ethanol extract on survival, growth and development of *Dysdercus koenigii* (Heteroptera: Pyrrhocoridae). *Indian Journal of Natural Products and Resources (IJNPR) [Formerly Natural Product Radiance (NPR)]*, 15(2), 302-312.
  68. Sheng, Z., Jian, R., Xie, F., Chen, B., Zhang, K., Li, D., ... & Hong, W. D. (2020). Screening of larvicidal activity of 53 essential oils and their synergistic effect for the improvement of deltamethrin efficacy against *Aedes albopictus*. *Industrial Crops and Products*, 145, 112131.
  69. Sofi, M. A., Nanda, A., Sofi, M. A., Maduraiveeran, R., Nazir, S., Siddiqui, N., ... & Rehman, M. U. (2022). Larvicidal activity of *Artemisia absinthium* extracts with special reference to inhibition of detoxifying enzymes in larvae of *Aedes aegypti* L. *Journal of King Saud University-Science*, 34(7), 102248.
  70. Sowa, I., Moidoch, J., Dresler, S., Kubrak, T., Soluch, A., Szczepanek, D., ... & Wójciak, M. (2023). Phytochemical profiling, antioxidant activity, and protective effect against H<sub>2</sub>O<sub>2</sub>-induced oxidative stress of *Carlina vulgaris* extract. *Molecules*, 28(14), 5422.
  71. Tang, K. J., Zhao, Y., Tao, X., Li, J., Chen, Y., Holland, D. C., ... & Xiang, L. (2023). Catecholamine derivatives: natural occurrence, structural diversity, and biological activity. *Journal of Natural Products*, 86(11), 2592-2619.
  72. Vilvest, J., John Milton, M. C., Yagoo, A. & Irine, J. (2025). *Coffea canephora* leaf extracts as mosquito larvicidal, pupicidal and ovicidal activity against *Aedes aegypti* and *Culex quinquefasciatus*. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences*, 95(1), 133-139.
  73. Viswakethu, V., Ramasamy, V., Balakrishnan, P., Narayanasamy, B. & Karthic, R. (2025). Efficacy of botanical pesticides in insecticidal activity against the banana fruit scarring beetle *Basilepta subcostata* an *In vitro* analysis. *Journal of Natural Pesticide Research*, 11, 100101.
  74. W. S. Abbott. (1925) A method of computing the effectiveness of an insecticide, *Journal of Economic Entomology*, 18, 2, 265–267, <https://doi.org/10.1093/jee/18.2.265a>
  75. War, A. R., Paulraj. M. G., Ahmad, T., Buhroo, A. A., Hussain, B. & Ignacimuthu, S., et al. (2012). Mechanisms of plant defense against insect herbivores. *Plant Signal Behav.*, 7:1306–20.
  76. World Health Organization. (2024). Dengue - Global situation (Disease Outbreak News, 30 May 2024). [www.who.int/emergencies/disease-outbreak-news/item/2024-DON518](http://www.who.int/emergencies/disease-outbreak-news/item/2024-DON518)).
  77. Xu, Y. Q., Ji, W. B., Yu, P., Chen, J. X., Wang, F. & Yin, J. F. (2017). Effect of extraction methods on the chemical components and taste quality of green tea extract. *Food Chem.*, 248, 146– 154, DOI: 10.1016/j.foodchem.,12.060
  78. Zabar, A., Cvetkovic, V., Rajkovic, J., Jovic, J., Vasiljevic, P. & Mitrovic, T. (2013) Larvicidal activity and *in vitro* effects of green tea (*Camellia sinensis* L.) water infusion. *Biol. Nyssana* , 4, 75–79