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Research Article

Influence of *Cassia occidentalis* leaf and stem extracts on the life parameters of *Aedes aegypti* (Linnaeus, 1762)

Aarti Sharma
Acharya Narendra Dev College, University of Delhi, Govindpuri, Kalkaji, New Delhi-110019, India; Galgotias University, School of Biological and Life Sciences (SBLS), Plot No. 2, Sector-17A, Yamuna Expressway, Greater Noida, Uttar Pradesh -203201, India
Monika Mishra
Acharya Narendra Dev College, University of Delhi, Govindpuri, Kalkaji, New Delhi-110019, India
Roopa Rani Samal
Acharya Narendra Dev College, University of Delhi, Govindpuri, Kalkaji, New Delhi-110019 India; Dyal Singh College, Lodhi Road, Pragati Vihar, New Delhi, Delhi -110003, India
Vinay Singh Dagar
Acharya Narendra Dev College, University of Delhi, Govindpuri, Kalkaji, New Delhi-110019 India; Deen Dayal Upadhyaya College, University of Delhi, Sector-3, Dwarka, New Delhi -110078, India
Manoj Kumar
Acharya Narendra Dev College, University of Delhi, Govindpuri, Kalkaji, New Delhi-110019, India
Anupama Shukla*
Acharya Narendra Dev College, University of Delhi, Govindpuri, Kalkaji, New Delhi-110019, India
Sarita Kumar*
Acharya Narendra Dev College, University of Delhi, Govindpuri, Kalkaji, New Delhi-110019, India

*Corresponding author: Email: anupamashukla@andc.du.ac.in

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Abstract
Mosquitoes are the most common disease vectors for several prevalent diseases, such as Dengue, Zika, Malaria, Encephalitis, Chikungunya, and yellow fever. Since the last few years, the world has recorded an unprecedented rise in *Aedes*-borne dengue incidences. Frequent use of chemicals has resulted in hazardous effects on the environment, non-targets and humans, necessitating the need to develop a safer and more efficient control strategy using plant-based products. The present study investigated the effects of *Cassia occidentalis* on various biological parameters of the *Aedes aegypti* larvae. The hexane extracts obtained from the leaf and stem of the plant were utilized to treat the larvae for 24 hours. The LC_{50} values for the leaf and stem extracts of *C. occidentalis* were determined as 0.103 mg/mL and 0.088 mg/mL, respectively. The corresponding lethal values of leaf and stem extracts obtained against pupae were 0.111 and 0.138 mg/mL. The extracts also imparted latent toxic effects and reduced the % adults developed from the survived larvae in the 60.00-61.67% range at the median lethal dose. The extract-treated larvae were restless and showed abnormal behaviour, like aggressive movements and self-biting of anal papillae. The inner membrane cuticle of anal papillae was shrunken and the gut region was damaged and disintegrated. The adults who emerged from the larvae, treated with 1.0 mg/mL of *C. occidentalis* hexane stem and leaf extracts, showed 1.8-fold and 2.29-fold decreased oviposition, respectively. Further study with identified bioactive constituents in the extracts can help to formulate green insecticides for *Ae. aegypti* management.

Keywords: *Aedes aegypti, Cassia occidentalis,* Larvicidal, Oviposition deterrent, Pupicidal

INTRODUCTION

Mosquitoes comprise about 3,500 species (El Hag *et al.*, 1999; Baik and Carlson, 2020). These are the vectors of several human diseases that can cause serious health problems. *Aedes aegypti* (*Ae. aegypti*) (Linnaeus, 1762) is a dominant mosquito which transmits Zika, Dengue, Yellow fever and Chikungunya

worldwide. Approximately 50% of the global population resides in dengue-predominant countries, while the Asia-Pacific countries bear around 60-75% of the global load of dengue. As per reports, in 2000, the total number of dengue cases recorded was 505,430, which rose to roughly 2.4 million in 2010 and 5.2 million in 2019. India has also encountered a substantial rise in dengue cases in 2019 about 55%. Nevertheless, the total number of cases worldwide apparently decreased during 2020 and 2021 (WHO, 2020). Such a high and rapid increase in dengue cases has raised human health concerns, particularly in tropical and subtropical regions. Several mosquito control interventions have been used since olden times. These measures include Indoor Residual Sprays (IRS) against adults, larvicides at the mosquito breeding sites, repellents to prevent mosquitoes' bites or knockdown agents in the form of mosquito coils and mats (Demirak and Canpolat, 2022; Hillary *et al.*, 2024). All these control strategies rely on the frequent use of harmful synthetic chemicals at indiscriminate dosages (Hillary *et al.*, 2024). This has developed insecticide resistance in mosquitoes, caused environmental pollution, biomagnified toxic residues in the food chain and imparted harmful effects on beneficial organisms and humans (Borase *et al.*, 2013). These complications have provided an edge to the herbal biorational measures, which have anti-mosquito potential and could be utilized against different development stages of mosquitoes without triggering any adverse effects on humans, outdoor settings and valuable biota (Ejeta *et al.*, 2021).

Plant-derived products have rich pools of chemical constituents that engage in diverse biological activities. These are considered safer than chemical products because of easy and quick degradability and comparatively lesser hazardous effects. The insecticidal, repellent and growth regulatory activity of various plant derivatives and phytochemical constituents have been reported against mosquitoes (Sukumar *et al.*, 1991; Shaalan *et al.*, 2005; Sharma *et al.*, 2016). Since plant extracts contain a pool of constituents, identifying the bioactive constituents in these botanicals becomes significant. As the plant species, its geographical location, the part and solvent used for extraction, and the methodology adopted can affect its efficacy significantly, the foremost requirement is the preliminary screening of different plants' crude extracts and assessing their relative impact (Anibogwu *et al.*, 2021). However, most such studies have been conducted with widely known economically and medically important flora. Since the commercial use of this vegetation can threaten these species and disturb the ecosystem, using weeds for mosquito management could be advantageous (Demirak and Canpolat, 2022; Arunthirumeni *et al.*, 2023).

Cassia occidentalis (*C. occidentalis*) is a plant that grows vertically and exhibits light branching. It belongs to the Fabaceae family and can be found as a shrub or tree. It is also known as coffee weed or senna and grows well in semi-arid, tropical, and subtropical regions. It is particularly common in India, where it is frequently observed multiplying in roadside patches soon after rainy spells, exhibiting opportunistic growth patterns (Vashishtha *et al.*, 2009). *C. occidentalis* exerts many pharmacological effects, including antimicrobial, anthelmintic, insecticidal, antioxidant, antianxiety, antidepressant, anti-mutagenic antidiabetic, wound healing, hepatoprotective, renoprotective, sun protective, smooth muscles relaxation, immune-modulating, anti-inflammatory, analgesic, antipyretic and other effects (Al-Snafi, 2015; Choudhary *et al.*, 2021).

Various studies have investigated the potential of *C. occidentalis* as a mosquito control agent and reported its efficacy against different mosquito species, including *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus* (Tona *et al.*, 1999; Tona *et al.*, 2001; Dhandapani *et al.*, 2011; Bukar and Zainab, 2019). However, these studies have investigated only the toxic potential of extracts. The current study was, thus, held to investigate not only the impact of the *C. occidentalis* leaf and stem extracts on the survival of *Ae. aegypti* larvae and their impact on larval growth, behavioral alterations, and morphology. The study was further extended to elucidate the oviposition capacity of adult *Ae. aegypti* that emerged from these extract-treated larvae.

MATERIALS AND METHODS

Maintenance of *Aedes aegypti* culture

The *Ae. aegypti* population was maintained in an insect-rearing laboratory of Acharya Narendra Dev College, University of Delhi, India. The rearing conditions were set at 28±1 °C temperature, 70 ± 10% moisture and l14h/10h; Light/Dark conditions (Sharma *et al.*, 2015). The larvae were reared in de-chlorinated water and were fed on finely crushed dog biscuits and live yeast (3:1 w/w). The pupae formed were collected in a bowl and kept in the cloth cages for adult emergence. The split deseeded raisins were kept in the cages as adult food and a water-soaked cotton pad was placed on the top of the cage to provide water and moisture. Female mosquitoes were fed on the blood meals for egg maturation. A water-filled plastic bowl, lined with strips of Whatman paper, was placed inside the cage to collect ova.

Collection of *Cassia occidentalis*

Fresh and healthy *C. occidentalis* plants were collected from the premises of Acharya Narendra Dev College, New Delhi, India and its vicinity. The leaves and stems were carefully harvested and placed in clean polybags. Upon arrival in the laboratory, the plant parts were

washed thoroughly and dried under shade at ambient room temperature (27 °C) for approximately 10-15 days.

Preparation of *Cassia occidentalis* extract

The stems and leaves *C. occidentalis* were separated and finely powdered using a grinder. Each part was individually extracted in 1 L hexane for 3 days, @ 8 hours/day, using a Soxhlet apparatus. During extraction, the temperature was set below the boiling point of hexane. The extracts were collected and concentrated in a rotary evaporator at 60 °C under low pressure. The concentrated extracts were packed in an air-tight bottle and kept at 4 ºC and used when required.

Impact of *Cassia occidentalis* extracts on larval and pupal survival

The toxic potential of both the stem and leaf extracts of *C. occidentalis* was estimated on the early fourth instars and pupae of *Ae. aegypti* as per WHO protocol (WHO, 2016). A series of extract concentrations (0.04 – 0.2 mg/mL) were prepared for the treatment. In the larvicidal bioassay, sets of 20 early fourth instars were treated for 24 h with a homogeneous solution of 99 mL of distilled water and 1 mL of *C. occidentalis* extract. The experiment was concurrently repeated thrice for each concentration. The extract was replaced with 1 mL of ethanol in the control sets. The dead larvae were scored after 24 h. The assays with >20% larval mortality in control or >20% pupae formation in any treatment were repeated. The results of the tests showing 5%-20% larval mortality in the control sets was corrected as per Abbott's formula (Abbott, 1925).

The toxic effects of both the leaf extract (CLE) and stem extract (CSE) of *C. occidentalis* were also evaluated on the pupae of *Ae. aegypti* based on the same protocol described for larvicidal bioassay.

Statistical analysis

The data of all the assays were subjected to regression analysis using PASW Program (SPSS-Version: 19.0). Different lethal levels of extracts were computed along with 95% confidence limits, regression coefficient (RC), standard error (SE), degree of freedom (df) and chi-square (χ^2). The values were assessed for their significance at $p < 0.05$ probability level.

Effects of *Cassia occidentalis* extracts on the adult emergence

A total of 20 larvae, in three replicates, were treated for 24 h with CLE and CSE at different lethal concentrations (LC_{30}, LC_{50} and LC_{90}) calculated after larvicidal bioassay. The surviving larvae were reared till they developed into adults. The total adult emergence was recorded and the latent impact of extracts on the larval growth and development was evaluated.

Influence of *Cassia occidentalis* extracts on the larval behavior

The early fourth instars of *Ae. aegypti* were treated with LC_{50} value of each *C. occidentalis* extract for 24 h. Both normal and abnormal movements were photographed with Canon Power Shot SX50HS to analyze the behavioral alterations in the larvae. Three replicates were conducted with each extract.

Effects of *Cassia occidentalis* extracts on the larval morphology

Three batches of 20 early fourth instars of *Ae. aegypti* were treated with 199 mL water and 1 mL of leaf or stem *C. occidentalis* extract (CLE and CSE) at LC_{30}, LC_{50} and LC_{90} levels for 24 h. The dead larvae were isolated. Each larva was mounted on a microscopic slide with Hoyer's medium and observed under the compound microscope (Nikon Eclipse Model: E100). The morphology of externally visible body parts, eyes, appendages, siphon and anal gills was recorded. Each alteration was photographed with Canon Power Shot SX50HS. Parallel studies were performed with the larvae of the control set.

Influence of *Cassia occidentalis* extracts on the oviposition of females

Fifty 3-day old *Ae. aegypti* adults; 25 blood-fed females and 25 males, were released in a mosquito cage. Two oviposition cups were randomly placed in the cage, one filled with 99 mL water and 1 mL *C. occidentalis* extract (experimental), and the other with 100 mL water (control). The position effect on egg laying of female mosquitoes was prevented by arbitrarily changing the place of each cup after 12 h. The total number of eggs laid in each cup was counted after 24 h with the help of a dissecting microscope (40 X). Alike experiments were carried out with different concentrations of each *C. occidentalis* extract ranging from 0.05 to 1.0 mg/mL. Each assay was run in triplicates.

The oviposition rate of individual female *Ae. aegypti* in each extract concentration was calculated by dividing the total eggs laid by the total number of females. The oviposition deterrent potential of both CLE and CSE was evaluated by computing the % effective oviposition deterrence (ED) values using the formula given in Equation 1. Along with, Oviposition Activity Index (OAI) was also estimated for each concentration of CLE and CSE using the formula denoted by Equation 2.

$$ED\% = \frac{(NC-NT)}{NC} \times 100 \quad \text{Equation 1}$$

$$OAI = \frac{(NT-NC)}{(NT+NC)} \quad \text{Equation 2}$$

ED is the Effective deterrence, NC is the total number of eggs in the control cup, and NT is the total number of eggs in the extract-treated cup.

RESULTS

Larval and pupal survival

The larvicidal bioassay with *C. occidentalis* leaf and stem hexane extracts (CLE; CSE) conducted on *Ae. aegypti* early fourth instars resulted in 100% mortality at concentrations ≥ 0.2 mg/mL (Fig. 1). No larval mortality in controls or pupal formation was observed in any assays. The bioassay showed higher CLE toxicity against larvae of *Ae. aegypti* in comparison to the CSE. The CLE (0.0883 mg/mL) exhibited a 0.85-fold higher LC_{50} value than CSE (0.1037 mg/mL). The bioassay with *C. occidentalis* extracts against *Ae. aegypti* pupae also revealed similar results. The respective LC_{50} values obtained with CLE and CSE treatment were 0.1111 mg/mL and 0.1388 mg/mL, which showed 0.80-fold higher pupal toxicity imparted by CLE than CSE (Table 1).

Adult emergence

The treatment of early fourth instars of *Ae. aegypti* with the leaf and stem extracts of *C. occidentalis* diminished the adult emergence significantly compared with the control set ($p < 0.05$), indicating the latent toxic effects of the extracts. Larval subjection to the LC_{30} dosage of extracts resulted in 45-56% adult emergence, while treatment with LC_{50} dosage reduced the adult emergence to 38-40% and that with LC_{90} dosage caused just 3-7% emergence. No adult emergence was recorded at the dose of LC_{99} extract (Table 2) .

Larval behavior

The control larvae of *Ae. aegypti* showed calm, zig-zag or wriggling dynamic movement (Fig. 2A), whereas the CLE and CSE-treated larvae showed restless behavior. Significant larvae agitation was observed after 2 min treatment with the extracts (Table 3). After 10 min of treatment, the

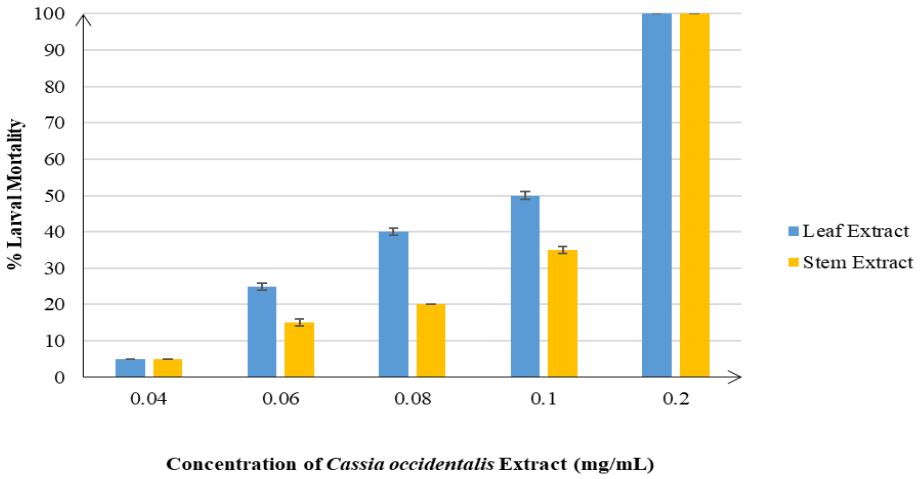

Fig. 1. *Relative % mortality of Aedes aegypti early fourth instar when treated with leaf and stem hexane extracts of Cassia occidentalis for 24 h*

Table 1. Lethal concentrations of *Cassia occidentalis* leaf and stem extracts obtained after treatment of early fourth instar larvae and pupae of *Aedes aegypti* for 24 h

Developmental Stage	Lethal Concentration of *C. occidentalis* Extracts					χ^2 (df)	Regression Coefficient (R.C.)	Standard Error (S.E.)
	LC_{30} (mg/mL)	LC_{50} (mg/mL)	LC_{70} (mg/mL)	LC_{90} (mg/mL)	LC_{99} (mg/mL)			
Leaf Extract								
Early fourth instar larvae	0.0696 (0.0583-0.0798)	0.0883 (0.0770-0.0104)	0.1122 (0.0963-0.1445)	0.1580 (0.1275-0.2406)	0.2551 (0.1837-0.4972)	2.128 (4)	5.053	0.919
Pupae	0.0678 (0.0506-0.0853)	0.1111 (0.0883-0.1458)	0.1819 (0.1395-0.2756)	0.3706 (0.2506-0.7439)	0.9896 (0.5411-3.0415)	4.771 (5)	2.450	0.391
Stem Extract								
Early fourth instar larvae	0.0824 (0.070-0.095)	0.1037 (0.0902-0.1254)	0.1305 (0.1109-0.1722)	0.1820 (0.1450-0.2803)	0.2878 (0.2064-0.5592)	5.214 (4)	5.249	0.933
Pupae	0.0888 (0.0676-0.1108)	0.1388 (0.1111-0.1844)	0.2169 (0.1666-0.3363)	0.4134 (0.2806-0.8527)	1.0065 (0.5576-3.1808)	6.338 (4)	2.704	0.461

Values are mean of three replicates, LC – Lethal Concentration that kills 30% (LC_{30}), 50% (LC_{50}), 70% (LC_{70}), 90% (LC_{90}) and 99% (LC_{99}) of the treated larvae; χ^2 = Chi-square, df = degree of freedom, no heterogeneity factor used while computing confidence limits

12-17% larvae appeared restless and aggressive. They tried to bite their own anal papillae resulting in ring-shaped body (Fig. 2B). The convulsion and paralysis followed by substantial larval mortality were recorded after just 2 h of treatment; CLE caused 47% mortality and CSE resulted in 41% mortality. All larvae were recorded dead after 3 h of *C. occidentalis* extract treatment.

Morphological aberrations

The CLE and CSE-treated larvae of *Ae. aegypti* did not exhibit any noticeable malformations in the head region. However, the anal papillae exhibited considerable damage showing shrinkage in their inner cuticle (Figs. 3A; 3B). In addition, the alimentary canal was found to be distorted, with a broken gut and damaged membranes.in comparison to control (Fig. 4A; 4B).

Oviposition deterrent studies

Both the extracts of *C. occidentalis* imparted significant oviposition deterrence in *Ae. aegypti* females (Table 4, 5). The effects observed were dose-dependent, and the oviposition deterrence augmented with the increase in

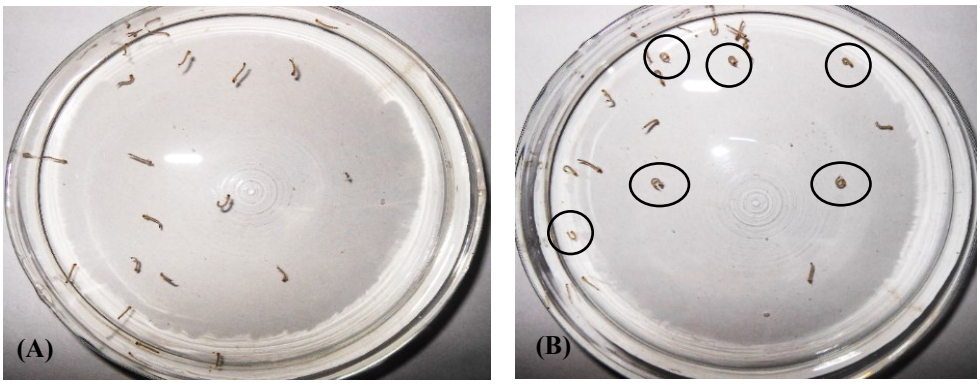

Fig. 2. *Movement in early fourth instars of Aedes aegypti (A) Control larvae; (B) Extract- treated larvae showing anal gill biting (Encircled)*

Table 2. Percent adults emerged from the *Aedes aegypti* early fourth instar treated with the lethal concentrations of leaf and stem extracts of *Cassia occidentalis* for 24 h.

Lethal Concentration (mg/mL)	Leaf Extract of *Cassia occidentalis*		Stem Extract of *Cassia occidentalis*	
	Total number of adults emerged (% emergence)	% Adult Emergence Inhibition	Total number of adults emerged (% emergence)	% Adult Emergence Inhibition
Control	19.666 ± 0.333 ^{a} (98.33)	1.67	19.333 ± 0.666 ^{v} (96.66)	3.34
LC_{30}	9.000 ± 0.577 ^{b} (45.00)	55.00	11.333 ± 0.333 ^{w} (56.666)	43.34
LC_{50}	7.666 ± 0.666 ^{b} (38.33)	61.67	8.000 ± 1.000 ^{x} (40.00)	60.00
LC_{70}	3.666 ± 0.333 ^{c} (18.33)	81.67	4.666 ± 0.333 ^{y} (23.33)	76.67
LC_{90}	0.666 ± 0.333 ^{d} (3.33)	96.67	1.33 ± 0.333 ^{z} (6.66)	93.34
LC_{99}	Nil	100.00	Nil	100.00

LC – Lethal Concentration that kills 30% (LC_{30}), 50% (LC_{50}), 70% (LC_{70}), 90% (LC_{90}) and 99% (LC_{99}) of the treated larvae; Mean ± SEM (Standard Error of Mean) calculated for 3 replicates, each comprised of 20 larvae. Values in each column followed by different letters are significantly different ($p < 0.05$), one-way ANOVA followed by Tukey's all pair wise multiple comparison test.

Table 3. Impact of the leaf and stem hexane extracts of *Cassia occidentalis* on the behavior of *Aedes aegypti* early fourth instars, when treated with LC_{-50} dosage of extracts

Duration of Treatment	Leaf Hexane Extract of *Cassia occidentalis*	Stem Hexane Extract of *Cassia occidentalis*
	LC_{50} (0.088 mg/mL)	**LC_{50} (0.103 mg/mL)**
00 min	Natural and dynamic movement in all larvae	
02 min	Excitation and irregular movement	
10 min	Agitation, convoluting movements because of self-biting of anal papillae	
	17.33 ± 0.33 ^{a}	12.33 ± 0.33 ^{a}
15 min	30.33 ± 0.33 ^{a}	23.66 ± 0.33 ^{a}
30 min	Extensive restlessness, paroxysms and struggling behavior	
	45.00 ± 0.57 ^{a}	29.66 ± 0.33 ^{a}
1 h	Shocks, tremors and finally paralysis	
	58.66 ± 0.33 ^{a}	67.66 ± 0.33 ^{a}
	27.33 ± 0.33 ^{b}	21.33 ± 0.33 ^{b}
	15.33 ± 0.33 ^{c}	12.33 ± 0.33 ^{c}
2 h	49.66 ± 0.33 ^{a}	48.66 ± 0.33 ^{a}
	04.00 ± 0.57 ^{b}	11.00 ± 0.57 ^{b}
	47.00 ± 0.33 ^{c}	41.00 ± 0.33 ^{c}
3 h	50.00 ± 0.00 ^{c}	50.00 ± 0.00 ^{c}
24 h	50.00 ± 0.00 ^{c}	50.00 ± 0.00 ^{c}

a = % larvae sank± SEM; b = % moribund larvae ± SEM; c = % dead larvae ± SEM

extract concentration, whether from leaves or stems of *C. occidentalis*. However, the leaf extract resulted in higher deterrence than the stem extract, causing 2.29-fold and 1.8-fold respective decrease in oviposition at 1 mg/mL. The oviposition rate of female adults declined from 10.21 in control (255.33 eggs) to 4.45 in 1.0 mg/mL CLE-containing water (111.33 eggs), while in water containing 1.0 mg/mL CSE, it decreased to 6.17 (154.33 eggs) from 11.17 in control (111.33 eggs) (Table 4).

The % Effective Deterrence of 1.0 mg/mL CLE and CSE ranged from 44.748-56.396%; the hexane leaf extract induced oviposition deterrence more effectively than the hexane stem extract (Table 5). The oviposition activity index (OAI) of the hexane leaf and stem extracts of *C. occidentalis* decreased with increased extract concentration. The highest decline of (-) 0.392 and (-) 0.288 in OAI was observed with 1.0 mg/mL leaf and stem extract of *C. occidentalis*, respectively (Fig. 5).

DISCUSSION

Ae. aegypti is a crucial vector of human ailments. It spreads various arboviruses, such as dengue, chikungunya, yellow fever and zika, inflicting high mortality and morbidity in human beings. Despite continuous efforts of scientists, successful medication and effective vaccines against these viruses are still unavailable. Thus, management and control of *Ae. aegypti* is the only effective intervention against these diseases.

Various plant-based products have been investigated as potential mosquito control tools that are safer than the frequently used synthetic chemical-based measures. The present study also attempts to estimate the bio-efficacy of a weed, *C. occidentalis,* against different developmental stages of *Ae. aegypti*. The hexane extract of the leaf and stem of *C. occidentalis* was prepared and tested against the larvae, pupae and adults of *Ae. aegypti.* A few studies have been carried out in the past to explore the potential of this weed; however their focus has primarily been on its toxic effects. The present study, nevertheless, not only investigates the weed's toxic potential but also assesses the impact of the extracts on larval growth, behavior and morphology, as well as the reproductive fitness of emerging females. As far as we know, this study is the first of its kind, with *C. occidentalis* against *Ae. aegypti.*

The present study demonstrated that 0.2 mg/mL leaf and stem hexane extract of *C. occidentalis* could induce 100% mortality in *Ae. aegypti* IV instar, resulting in the respective LC_{50} dosages of 0.088 mg/mL and 0.103 mg/mL, respectively (Table 1). Remia and Logaswamy (2010) reported comparable results when treating the 4th instar larvae of *Ae. aegypti* with the *C. occidentalis* acetone leaf extract for 24 h and obtained LC_{50} value of 0.156 mg/mL. Although higher than those

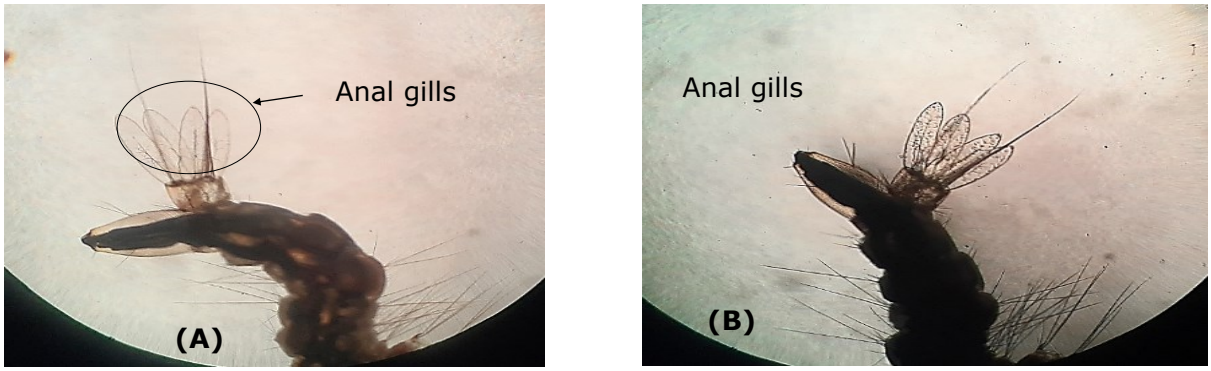

Fig. 3. *Light micrographs of the Aedes aegypti early fourth instars showing anal gills; (A) Control larva with normal anal gills, (B) Cassia occidentalis--treated larva with shrunk inner cuticle in the anal gills*

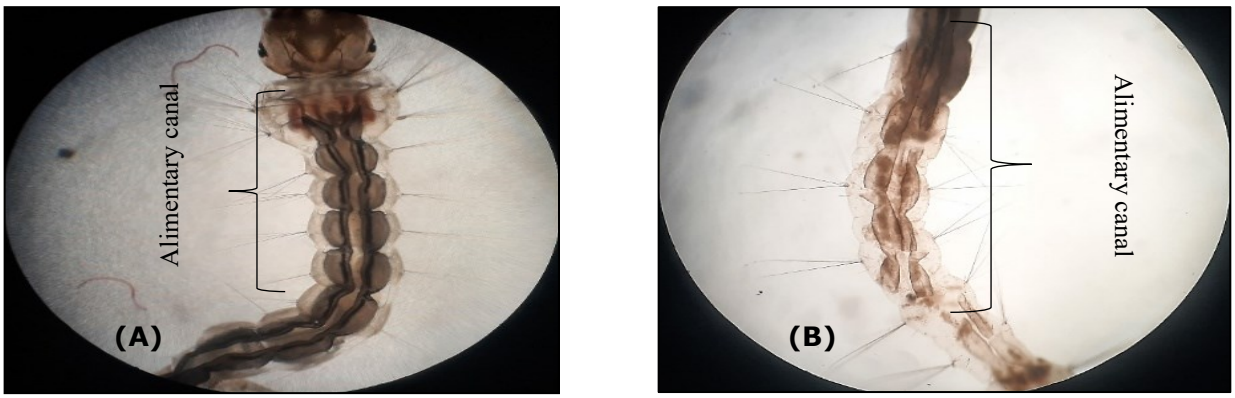

Fig. 4. *Light micrographs of the gut of early fourth instars of Aedes aegypti; (A) Control larva, (B) Cassia occidentalis--treated larva showing damaged and distorted alimentary canal*

Table 4. Oviposition deterrence induced by the leaf and stem extracts of *Cassia occidentalis* in female adults of *Aedes aegypti*

Extract of *Cassia occidentalis*	Concentration of *Cassia occidentalis* Hexane Extracts (mg/mL)							
	Control	**0.05**	**0.1**	**0.2**	**0.4**	**0.6**	**0.8**	**1.0**
Leaf Extract (CLE)	255.33 ^{a} ± 3.17* (10.21)	254.00 ^{a} ± 2.08 (10.16)	234.66 ^{a} ± 2.33 (9.38)	208.00 ^{b} ± 1.52 (8.32)	176.00 ^{c} ± 1.52 (7.04)	144.33 ^{cd} ± 2.60 (5.7)	128.00 ^{d} ± 1.154 (5.12)	111.33 ^{d} ± 1.85 (4.45)
Stem Extract (CSE)	279.33 ^{a} ± 2.33 (11.17)	280.00 ^{a} ± 3.21 (11.2)	254.00 ^{b} ± 2.64 (10.16)	247.33 ^{b} ± 2.33 (9.89)	225.00 ^{b} ± 3.46 (9.00)	187.33 ^{c} ± 4.09 (7.49)	174.33 ^{cd} ± 3.28 (6.97)	154.33 ^{c} ± 4.05 (6.17)

* The figures represent mean number of eggs laid in three replicates ± S.E.M. (Standard Error of Mean) ($p < 0.05$). Each replicate consisted of 25 female adults, Figures in parentheses represent the oviposition rate of female adults. Means in each row followed by different letters are significantly different ($p < 0.05$), one-way ANOVA followed by Tukey's all pair wise multiple comparison test

Table 5. Oviposition deterrence induced by the leaf and stem extracts of *Cassia occidentalis* in female adults of *Aedes aegypti*

Extract of *Cassia occidentalis*	Concentration of *Cassia occidentalis* Hexane Extracts (mg/mL)						
	0.05	**0.1**	**0.2**	**0.4**	**0.6**	**0.8**	**1.0**
	Percent Effective Deterrence						
Leaf Extract (CLE)	0.520	8.092	18.536	31.069	43.471	49.868	56.396
Stem Extract (CSE)	ND*	9.068	11.454	19.450	32.934	37.588	44.748

*ND- Non-Deterrent

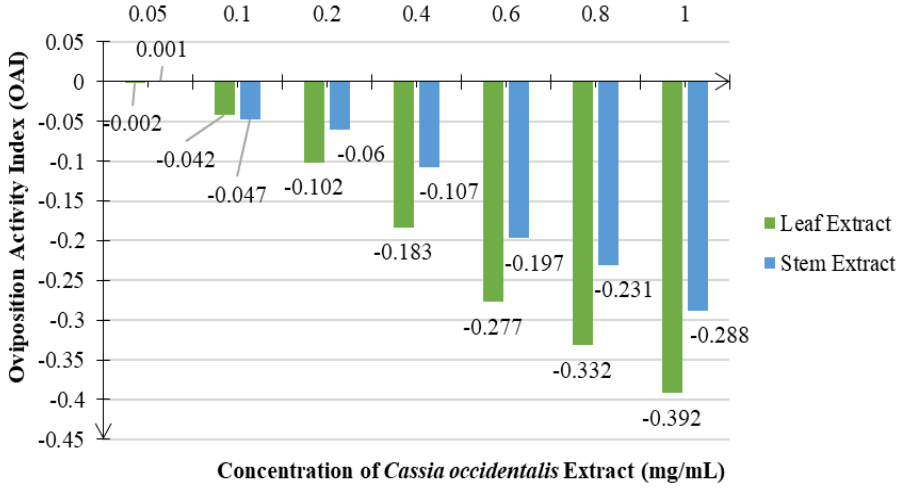

Fig. 5. *Oviposition activity index (OAI) of the Cassia occidentalis leaf and stem extracts against female adults of Aedes aegypti*

obtained in the present study, similar values were revealed when *Cx. quinquefasciatus* larvae were treated with acetone and ethanol extract of *C. occidentalis* for 24 h, resulting in respective LC_{50} values of 0.240 and 0.397 mg/mL (Vairavan *et al.*, 2018). On the contrary, the 24 h assay of *C. occidentalis* petroleum ether leaf extract against the I, II, III and IV instars of *An. stephensi* revealed respective LC_{50} values of 3.34, 4.48, 5.90 and 8.17 mg/mL, much higher than those obtained in the present work (Panneerselvam *et al.*, 2013). The differences in lethality against diverse mosquito species may be ascribed to the species diversity, occurrence of variable phytoconstituents in extracts due to geographical diversity, and use of different solvents for extraction.

The present investigation also demonstrated the detrimental effects of *C. occidentalis* extracts on the pupae of *Ae. aegypti*. The LC_{50} dosage was calculated to be 1.26 -fold higher for pupae (LC_{50} = 0.111 mg/mL) compared to the early fourth instars larvae (LC_{50} = 0.088 mg/mL) (Table 1). According to our knowledge, the pupicidal effects of *C. occidentalis* have not yet been studied against any insect. However, pupicidal toxicity of *Nicotiana plumbaginifolia* leaves was tested against *Culex vishnui* mosquito, revealing an equivalent LC_{50} value of 0.111 mg/mL after 24 h of exposure (Singh and Chandra, 2022). Nevertheless, the leaf extract of *Alchornea cordifolia* (Schum. & thonn.) was found much less effective against *Anopheles gambiae* pupae with LC_{50} and LC_{90} values reported as 189.97 mg/L and 857.00 mg/L (Koomson *et al.*, 2022).

The *C. occidentalis* extracts also significantly inhibited adult emergence in *Ae. aegypti* by 60-61% when larvae

were treated at LC_{50} levels and 93-96% when larvae were treated at LC_{90} dosages of *C. occidentalis* stem and leaf extracts (Table 2). These results are in congruence with those of Ilahi *et al.* (2019), who examined the dose-dependent adult emergence inhibition in C*x. quinquefasciatus* induced by larval treatment with *Cymbopogon nardus* extract. They recorded 31% adult emergence (66.2% emergence inhibition) after treatment with 1.0 mg/mL extract, while 82.3% emergence (12.2% emergence inhibition) was noticed on treating the larvae with 0.125 mg/mL extract.

The investigation also showed the antagonistic impact of the *C. occidentalis* extracts on the behaviour of *Ae. aegypti* larvae resulting in an irregular movement with excitation, restlessness, violent vertical and horizontal wriggling and self-biting of anal papillae (Fig. 2). Such studies have not been conducted previously with *C. occidentalis* extracts, though similar studies exist in literature with ethanol extract of *Apium graveolens* seeds (Kabir *et al.*, 2011; Spinozzi *et al.*, 2021), ethanolic seed extract of *Seseli diffusum* (Kabir *et al.*, 2013; Bibi *et al.*, 2020; Ragavendra *et al.*, 2024), leaves, stems, and roots of *Argemone mexicana* (*A. mexicana*) extracted in petroleum ether and hexane (Warikoo and Kumar, 2013; Kumar and Arya, 2022) and leaf and stem hexane extract of *Achyranthes aspera* (*A. aspera*) (Sharma *et al.*, 2015; Afsheen *et al.*, 2022) against *Ae. aegypti.* It is possible that the extracts might act as cytolysin and disrupt the neuromuscular synchronization in chemical synapses. It resulted in aggression, agitation, tremors, convulsions, paralysis, and death which are identical to those brought on by synthetic neurotoxins (Warikoo and Kumar, 2013; Sharma *et al.*, 2015; Bibi *et al.*, 2020; Afsheen *et al.*, 2022).

The *C. occidentalis* extracts also damaged the anal papillae leading to the shrinking of the inner cuticle, indicating the possible effect on their functions (Fig. 3). Comparable morphological changes in the anal papillae of *Ae. aegypti* fourth instars were reported by Warikoo and Kumar (2013) on exposure to *A. mexicana* extracts. Our observations are also in agreement with the outcomes reported by Kumar *et al.* (2010) and Sharma *et al.* (2015) who also testified the noticeable shrinkage in the internal structure of anal papillae in *Ae. aegypti* larvae when treated with *Piper longum, Piper nigrum* and *A. aspera* extracts. It has been proposed that structural distortion of anal papillae could possibly led to their anomalous function, especially transport mechanism and osmotic balance, which may be the intrinsic cause of the larval mortality (Chaithong *et al.*, 2006; Dey *et al.*, 2020; Ganesan *et al.*, 2023).

The *C. occidentalis* extract also caused 44.748% - 56.396% oviposition deterrence in *Ae. aegypti* (Table 5). The hexane leaf extract was a comparatively more effective oviposition deterrent than the hexane stem extract. This difference in effectiveness may be attributed to the presence of phytochemicals in the leaves, which exhibit a higher deterrent potential compared to those found in the stems. The proposition, though, needs further investigations. Warikoo and Kumar (2014a; b) evidenced the noteworthy oviposition deterrence and reproductive disadvantage in *Ae. aegypti* caused by the stem of *A. mexicana* extracted in five different solvents. They reported the greatest efficacy of the 1000 ppm petroleum ether stem extract of *A. mexicana,* causing 85-99% oviposition deterrence while the acetone stem extract resulted in the lowest oviposition deterrence. Likewise, the ethanol leaf extract of *Cassia obtusifolia* could deter 75.5% oviposition in *An. stephensi* at 100 mg/L, while 92.5% deterrence was noted with 400 mg/L extract (Rajkumar and Jebanesan, 2009). These results evidently showed the significant efficacy of *C. occidentalis* extract against different developmental stages of *Ae. aegypti.*

The strong deterrence efficacy of some indigenous plants extracted in various solvents; methanol, ethyl acetate, and acetone, @500 ppm has been demonstrated against oviposition by *Anopheles subpictus* females (Elango *et al.*, 2009). Recently, Baz *et al.* (2024) investigated the oviposition deterrence of *Acacia nilotica* extracts against *Culex pipiens* and observed suppressed hatching of the mosquito eggs. Another study examined the effects of leaf extracts from *Combretum micranthum*, *Xienmia americana* and *Aloysia citrodora* on female *An. gambiae* mosquitoes' oviposition and egg viability were examined and documented that the ethyl-acetate extracts of *A. citrodora* significantly reduced oviposition by 42.85% compared to controls (Muhammad *et al.*, 2024).

Conclusion

The present study demonstrated that leaf and stem hexane extract of *C. occidentalis* induced lethality, behavioral and structural modifications, and reproductive disadvantage in *Ae. aegypti* with the leaf extract exhibiting greater efficacy than the stem extract. The results indicate that using *C. occidentalis* can be advantageous for the management of *Ae. aegypti* larvae in the fields. Considering the detrimental effects of synthetic chemicals on the environment and its living organisms, the current investigation can serve as an alternative mosquito-control strategy. It is proposed that the identification of bioactive components in the extract and field testing with the identified component will contribute in the formulation of a green insecticide, which could be an ecofriendly and cost-effective approach to control *Ae. aegypti*.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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