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EXPLORING THE LARVICIDAL POTENTIAL OF COMMON SALT IN COMBINATION WITH CITRONELLA LEAF EXTRACT AGAINST *AEDES AEGYPTI*

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Aedes aegypti primarily transmits dengue fever, a disease that poses a significant public health threat in tropical and subtropical regions. The present research evaluates the use of common salt in combination with an aqueous extract prepared from citronella leaves as a practical and eco-friendly approach. *Ae. aegypti* 3rd/4th instar larvae were exposed to five concentrations of the prepared citronella leaf aqueous extract (CLAE) @ 10, 11, 12, 13 and 14%. To explore the synergistic larvicidal effect of common salt (NaCl) in combination with CLAE, different formulations were created via Response Surface Methodology (RSM), utilising sub-lethal concentrations of CLAE and common salt (NaCl). Out of these, the most effective combination was considered for studying the larvicidal activity under simulated conditions in small and large containers. Additionally, a microscopic study of the treated

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larvae was performed to observe any morphological changes. Among the tested concentrations, 13% CLAE was identified as the most effective larvicidal concentration against *Ae. aegypti* larvae. Furthermore, the combinations of common salt + CLAE (out of the tested 9 combinations) at 0.5% + 11% and 0.75% + 9% exhibited significant larvicidal activity under laboratory conditions. Finally, the combination of 0.5% common salt + 11% CLAE showed effective synergistic larvicidal potential under simulated conditions. These treatments induced notable morphological changes in the larvae, including widening of the gut lumen, shrinkage and melanization of the alimentary canal, and damage to the anal gills. Using citronella leaf aqueous extract in combination with common salt may be a more effective approach as a bio-larvicide against *Ae. aegypti*.

INTRODUCTION

Mosquitoes are considered one of the most significant vectors responsible for transmitting various deadly diseases, including malaria, dengue, chikungunya, yellow fever, Japanese encephalitis, lymphatic filariasis, and Zika fever (TYAGI, 2025). In every corner of the globe, satisfactory climatic conditions, combined with socioeconomic and environmental factors, facilitate the survival of this vector and make eradication efforts more challenging (BECKER ET AL., 2020). Dengue, in particular, is the fastest-growing mosquito-borne disease transmitted by *Aedes aegypti*, with increasing incidence posing a significant threat to public health (BENELLI ET AL., 2005). Since the beginning of 2023, ongoing transmission, combined with an unexpected spike in dengue cases, has resulted in a historic increase of over 6.5 million cases and reporting more than 7,300 dengue-related deaths (WHO, 2025). According to the latest report from the National Vector-Borne Disease Control Programme, a total of 233,400 dengue cases were reported in India in 2024 (WHO, 2025).

Aedes aegypti, commonly known as the urban mosquito, is predominantly active during daylight hours. It typically prefers to lay eggs in artificial containers with clean, stagnant freshwater, such as earthen pots, desert coolers, gardens, and roadside ditches, often found in and around peri-domestic environments (KAUR ET AL., 2016). Over the years, *Ae. aegypti* has been the primary target of dengue vector control programs worldwide. Chemical insecticides remain the most widely employed method for mosquito control due to their rapid knockdown effect and cost-effectiveness. Frequently used insecticides include pyrethroids (resmethrin, sumithrin, pyrethrin, and permethrin), carbamates (carbaryl), organochlorines (aldrin), organophosphates (malathion, parathion) and larvicides such as temephos and methoprene (VAN DEN BERG ET AL., 2012). However, the extensive and repeated use of these insecticides has led to the development of insecticide resistance in mosquito populations across many regions of the world, including India (TYAGI, 2025, VONTAS ET AL., 2012). Resistance mechanisms may involve genetic mutations, metabolic detoxification, or behavioral changes, making chemical control increasingly ineffective. Furthermore, the over-reliance on chemical insecticides has raised significant concerns regarding their adverse effects on human health, non-target organisms, and the environment, contributing to ecological imbalances and bioaccumulation risks (MILAM ET AL., 2000). In response to these challenges, larval source management has emerged as an effective and environmentally sustainable strategy in integrated vector management (IVM) programs.

Ae. aegypti and *Ae. albopictus* larvae and pupae have traditionally been regarded as freshwater-restricted species (JINGUJI ET AL., 2021) and the concentration of inorganic salts in their habitats plays a critical role in influencing oviposition, egg hatchability and larval development, ultimately affecting the abundance and distribution of their aquatic life stages (EKECHUKWU AND FELICIA, 2011). These salinity-induced challenges can therefore reduce larval fitness and survival, potentially affecting mosquito population dynamics in saline or brackish water habitats (PATRICK ET AL., 2002). Under laboratory conditions, *Ae. aegypti* larvae were able to survive up to a maximum salt concentration of 14%, and the adults that emerged from the treated trials were found to have smaller body sizes compared to those that appeared in freshwater (CLARCK, FLIS AND REMOLD, 2004). Previously, we have also observed 100% mortality of *Ae. aegypti* 1st instar larvae when placed in water having a high concentration of common salt (9 ppt NaCl) (KAUR AND KOCHER, 2015).

In recent years, plant-derived products such as essential oils (EOs) and extracts have garnered considerable attention for their efficacy against mosquito larvae, making them promising candidates for eco-friendly mosquito control strategies (KOCHER ET AL., 2024). Unlike synthetic insecticides, which often target a single biochemical pathway, these products typically contain multiple active ingredients that act synergistically, reducing the likelihood of resistance development in mosquito populations (PIPLANI ET AL., 2019). Prior to the discovery of N,N-diethyl-meta-tolumide (DEET) in 1946, citronella was the most widely used plant-based insect repellent (KATZ ET AL., 2008). In India, 300-350 tonnes of citronella plant oil have been produced in the states of Assam, Karnataka, Uttar Pradesh, Madhya Pradesh, Maharashtra, Tamil Nadu and West Bengal during the past 6-8 years (KATIYAR ET AL., 2011). Volatile compounds, such as saponins, flavonoids, and polyphenols, present in the leaves and roots of citronella grass act as both repellents and toxicants against mosquitoes, resulting in their eco-friendly control (JANTAN AND ZAKI, 1999)

Researchers have explored various innovative approaches to enhance the larvicidal potential of plant-based products. Some reports are available regarding the synergistic effect of botanicals, indicating the higher larvicidal efficacy of phytochemical-mixed formulations than individual EOs, resulting in the development of less resistance (COTCHAKAEW AND SOONWERA, 2020). However, very little information is available in the literature regarding the larvicidal potential of EOs when integrated with non-lethal substances. 14 Among these substances, common salt (sodium chloride) can be employed in combination with EOs to improve the efficacy of formulating bio-larvicides due to its high solubility and stability in the aquatic habitats. Such integrated products could provide an effective, sustainable, and low-cost solution for controlling mosquito larvae in their breeding habitats. Keeping in mind the concept of IVM, the present study was designed to evaluate the larvicidal efficacy of common salt in combination with citronella leaf extract against *Ae. aegypti*.

MATERIALS AND METHODS

1. ***Collection of Ae. aegypti larvae*****:** Water samples were collected from a variety of small freshwater collections, including desert coolers, clay pots, plastic containers, rubber tyres, flower pots, and roadside ditches in peri-domestic areas in the urban region of Ludhiana district in Punjab state (India) from May to November 2022. These samples were allowed to settle for around 30 minutes. *Ae. aegypti* larvae were recognized and segregated from other mosquito larvae (if present) using standard keys (TYAGI, 2025; BECKER ET AL., 2020).

2. ***Preparation of citronella leaf aqueous extract (CLAE)*:** Fresh leaves were picked from citronella plants growing near Punjab Agricultural University, Ludhiana, Punjab (India) from May to July 2022. The contaminated leaves were discarded, and fresh leaves were thoroughly washed to eliminate the dust. The cleaned citronella leaves were shade dried by spreading them out on filter paper for three to five days at room temperature. To prevent bacterial or fungal infections, the filter paper was replaced daily. Dried leaves were gently crushed by hand before being ground into a fine powder using an electric grinder. The powder was autoclaved for 30 minutes to eliminate any possibility of contamination. For the preparation of the aqueous extract, 20 g of citronella leaf powder was thoroughly mixed in 100 mL of distilled water in a conical flask. The flask was wrapped with aluminium foil and kept overnight. The next morning, the mixture was filtered using a muslin cloth, and the filtrate was collected in a clean polycarbonate vial, which was tightly closed and wrapped in aluminium foil. To prevent any contamination, the prepared extract was stored in a deep freezer at -18°C for further use.

3. ***Larvicidal bioassay of citronella leaf aqueous extract (CLAE) against Ae. aegypti:*** The standard protocol suggested by WHO (1981) was followed with minor variations for carrying out mosquito larvicidal assays in laboratory conditions. Preliminary testing for the larvicidal potential of prepared CLAE was carried out against $3^{rd}/4^{th}$ instars of *Ae. aegypti* by random selection of its higher and lower concentrations. On the basis of this preliminary screening, five different concentrations of CLAE @ 10, 11, 12, 13 and 14% were prepared in 250ml of de-chlorinated water and 20 *Ae. aegypti* larvae ($3^{rd}/4^{th}$ instars) were exposed to these concentrations. A control set containing 250 ml of dechlorinated water with twenty *Ae. aegypti* larvae ($3^{rd}/4^{th}$ instars) were also run simultaneously. Treated and control experimental sets were performed in triplicate in plastic beakers with a 250 mL capacity. These beakers were properly covered with a net and tightly closed with a rubber band. Dog biscuits and a crushed yeast mixture at a 3:1 ratio (2 mg/100 g) were also supplemented as feed for the larvae in the experimental sets. The mortality of larvae kept in various concentrations of prepared CLAE and in the control set was determined by counting dead larvae after 3, 6, 9, 12, 24, 36, and 48 hours. Larvae that showed no response after being disturbed with a brush were considered dead. The lowest concentration of CLAE, which resulted in the highest mortality within a short duration (hours), was used as the effective

concentration for subsequent experiments. For calculating LC_{50} and LC_{90} values after 24 hours of post-exposure, a log concentration-mortality regression was determined using the log probit technique (FINNEY, 1971), employing the computer program POLO (ROBERTSON ET AL., 1980).

4. **Larvicidal potential of common salt in combination with citronella leaf aqueous extract (CLAE) against *Ae. aegypti*:** In our previous study, 100% killing of *Ae. aegypti* larvae (1st instar) have already been reported after exposure to determine the effective concentration of common salt at 9 ppt (0.9% NaCl) (KAUR AND KOCHER, 2015). The ranges of standard salt concentration (below its effective larvicidal concentration i.e. 0.9% NaCl) and CLAE (below its effective larvicidal concentration determined during dose-response larvicidal bioassay) were selected for making different combinations by using Response Surface Methodology (RSM) software with a Central Composite Design (CCD) approach so as to evaluate their combination(s) having best synergistic larvicidal potential against *Ae. aegypti*. The experimental design was generated using "Design-Expert-9.0.3" software (Stat-Ease, Inc., Minneapolis, USA). A 13-run factorial central composite experimental design was employed, involving two factors and five replicates at the center point, resulting in 13 experimental combinations (Table 1). Out of these, nine unique combinations were selected for further larvicidal bioassay (as there was repetition of one combination, i.e. Combination V, five times, as depicted in Table 1). Nine combinations of common salt+CLAE (through RSM design) were prepared in per cent concentrations @ 0.15+11, 0.25+9, 0.25+11, 0.5+8.2, 0.5+11, 0.5+13.8, 0.75+9, 0.75+13, and 0.85+11 in plastic beakers containing dechlorinated water, with a total volume of 250 mL. Twenty *Ae. aegypti* larvae (3rd/4th instars) were introduced into each treatment beaker. Control sets were maintained alongside the treatment trials, consisting of 250 ml of dechlorinated water with 20 larvae in each beaker. All treatment and control experiments were conducted in triplicate using 250 ml plastic beakers, which were securely covered with mesh netting fastened with rubber bands. Larval mortality was recorded at 3, 6, 9, 12, 24, 36 and 48 hours post-exposure in both treatment and control sets. The combination(s) of common salt and CLAE that demonstrated the highest larval mortality in the shortest exposure duration among the tested treatments were considered adequate and taken for further experimental studies.

For the conduct of simulated trials, water was collected from small freshwater habitats, representing typical breeding sites of *Ae. aegypti*, and water was filtered prior to use. Fifty 3rd/4th instar larvae of *Ae. aegypti* were exposed to the effective combination(s) of common salt+CLAE (determined from the laboratory trials) in small containers (plastic cups with a capacity of 250 ml) and large containers (plastic buckets with a capacity of 10 L).

Table 1. Optimization of different combinations of common salt with citronella leaf aqueous extract (CLAE) against *Aedes aegypti* larvae (3rd/4th instars) obtained by RSM design

Run	**Concentration as obtained by RSM design**		**Combinations taken for testing in per cent (Common salt + CLAE)**	
	Common salt (%)	**CLAE (%)**		
1	0.1464	11	0.15+11	Combination-I
2	0.25	9	0.25+9	Combination-II
3	0.25	13	0.25+13	Combination-III
4	0.5	8.172	0.5+8.2	Combination-IV
5	0.5	11	0.5+11	Combination-V
6	0.5	13.828	0.5+13.8	Combination-VI
7	0.5	11	0.5+11	Repeat
8	0.75	9	0.75+9	Combination-VII
9	0.5	11	0.5+11	Repeat
10	0.5	11	0.5+11	Repeat
11	0.75	13	0.75+13	Combination-VIII
12	0.5	11	0.5+11	Repeat
13	0.8536	11	0.85+11	Combination-IX

The total volume of water containing the respective combinations of common salt and CLAE was adjusted to 250 mL in plastic cups and 1000 mL in plastic buckets. Corresponding control sets were also maintained, consisting of dechlorinated water (250 ml in cups and 1,000 ml in buckets) with 50 larvae. Treatment and control experimental sets were conducted in triplicate. Larval mortality was recorded after 24 and 48 hours of exposure for both the treatment and control sets.

5. ***Effect of treatment on the morphological characters of Ae. aegypti larva***: *Ae. aegypti* larvae (3rd/4th instars) exposed to the effective concentration of CLAE alone, as well as to the most effective combination of common salt+CLAE,

were carefully observed for any morphological changes. For this purpose, moribund about to die) and dead *Ae. aegypti* larvae were separated from the treated sets and examined under a light microscope (Olympus CX21i). The observed larvae were photographed using a SONY colour video camera (SSC-G818) to document any morphological changes or abnormalities induced by the treatments, and these were compared with those of larvae from the control set.

6. ***Statistical analysis*****:** The data were statistically analysed by comparing larval mortality between treatment and control sets using Analysis of Variance (ANOVA), followed by Tukey's test at a 5% level of significance.

RESULTS

CLAE prepared in the laboratory was brown with a floral, pleasant smell and was soluble in water. Approximately 130-140 mL of CLAE was obtained from 50 g of citronella leaf powder after extraction with 250 mL of water overnight.

1. ***Larvicidal potential of citronella leaf aqueous extract (CLAE) against Ae. aegypti:*** Exposure of *Ae. aegypti* larvae exposed to 10 and 11% CLAE resulted in 16.66±5.77 % and 23.33±5.77% mortality within 3 hours, which further increased with time, and 100% mortality was observed within 48 hours. Increasing the concentration to 12% CLAE resulted in the killing of all larvae within 36 hours, while concentrations of 13% and 14% led to 100% mortality of larvae within 24 hours (Table 2). Thus, the percentage of larval mortality was found to increase with both an increase in the concentration of CLAE and an increase in the exposure period. A comparative analysis of larval mortality among the tested concentrations revealed that CLAE at 13% was found to be an effective larvicidal concentration. No larval mortality was observed in the control sets performed along with the treatment sets. LC_{50} and LC_{90} toxicity values computed for *Ae. aegypti* larvae based on the record of mortality up to 24 hours of exposure to CLAE were calculated to be 9.67 and 11.54 mg/L, respectively (Table 3).

Table 2: Effect of different concentrations of citronella leaf aqueous extract (CLAE) on mortality of *Aedes aegypti* larvae (3rd/4th instars)

Concen-tration (%)	Per cent larval mortality up to (Mean±S.D) (n=20)							Range of mortality (Within hours)
	3hr	6hr	9hr	12hr	24hr	36hr	48hr	
10	16.66±5.77^{ab} (2-4)	33.33±15.27^{b} (4-10)	46.66±15.27^{b} (6-12)	56.66±5.77^{b} (10-12)	66.66±15.27^{b} (10-16)	76.66±5.77^{b} (14-16)	100.00±0.00^{b} (20)	3-48
11	23.33±5.77^{bc} (4-6)	36.66±5.77^{b} (6-8)	46.66±5.77^{b} (8-10)	56.66±5.77^{b} (10-12)	73.33±5.77^{b} (14-16)	86.66±5.77^{c} (16-18)	100.00±0.00^{b} (20)	3-48
12	30.00±10.00^{bcd} (2-8)	66.66±5.77^{c} (12-14)	73.33±15.27^{bc} (12-18)	80.00±10.00^{c} (14-18)	96.66±5.77^{c} (18-20)	100.00±0.00^{c} (20)	-	3-36
13	36.66±5.77^{cd} (6-8)	66.66±5.77^{c} (12-14)	73.33±11.54^{bc} (12-16)	83.33±5.77^{c} (16-18)	100.00±0.00^{c} (20)	-	-	3-24
14	43.33±5.77^{d} (8-10)	70.00±10.00^{c} (12-16)	86.66±5.77^{c} (16-18)	96.66±5.77^{c} (18-20)	100.00±0.00^{c} (20)	-	-	3-24
0 (Control)	0.00±0.00^{a} (0)	0.00±0.00^{a} (0)	0.00±0.00^{a} (0)	0.00±0.00^{a} (0)	0.00±0.00^{a} (0)	0.00±0.00^{a} (0)	0.00±0.00^{a} (0)	-

- n represents the number of larvae taken
- Figures in parentheses indicate the range in number of dead larvae from the start of the experiment till that period
- Figures followed by different superscripts indicate a significant difference ($p < 0.05$) with respect to the control and treatment sets using Tukey's test.

Table 3. Toxicity values of citronella leaf aqueous extract (CLAE) against *Aedes aegypti* larvae (3rd/4th instar) after 24 hours of exposure

Toxicity Value (mg/L)	**Fiducial limits**		χ^2
	Lower limit (mg/L)	**Upper limit (mg/L)**	
LC_{50}=9.67	8.00	10.27	6.99
LC_{90}=11.54	10.87	12.85	

2. ***Synergistic larvicidal potential of common salt in combination with citronella leaf aqueous extract (CLAE) against Ae. aegypti:*** Out of the tested nine combinations of common salt+CLAE obtained with RSM design, combination - I, II and IV showed 100, 83.33 and 76.66% larval mortality, respectively, within 48 hrs. Two combinations, i.e. III and IX, showed 100% larval killing within 24 hrs of exposure. However, combinations V and VII showed 100% mortality within 36 hours of treatment, while combinations VIII and showed

killing of all larvae within 9 and 12 hours of exposure. Out of these nine combinations, two combinations, i.e. combination-V having CLAE + common salt @ 0.5+11% and combination-VII having CLAE+common salt @ 0.75+9% were found to be the best larvicidal effective combinations, which resulted in 100% larval mortality within 36 hrs of treatment, as these had minimum concentration of CLAE and common salt (Table 4).

Two effective combinations of CLAE + common salt, i.e. combination-V and VII @ 0.5+11% and 0.75+9% were taken into consideration for their validation under simulated conditions. Their testing in plastic cups and plastic buckets resulted in per cent larval mortality of 70.66±8.08 and 45.33±7.02 in plastic cups, and 40.00±7.21 and 31.33±6.42 in plastic buckets, respectively, within 24 hours of treatment. After 48 hours of exposure, the percentage of larval killing was found to be 82.66±7.57% and 76.00±9.16% in plastic cups, and 63.33±6.11% and 52.00±7.21% in plastic buckets, respectively. No larval mortality was observed in the control sets. Combination-V, which contains CLAE and common salt at 0.5% and 11%, was found to be a better combination compared to Combination-VII, as it showed statistically higher larvicidal potential within 24 hours of treatment (Table 5).

Table 4. Synergistic larvicidal effect of RSM-designed combinations of common salt with citronella leaf aqueous extract (CLAE) against *Aedes aegypti* (3rd/4th instars)

Common salt + CLAE (%)	**Per cent larval mortality up to (Mean±S.D) (n=20)**							**Range of mortality (Within hours)**
	3hr	**6hr**	**9hr**	**12hr**	**24hr**	**36hr**	**48hr**	
Combination-I (0.15+11)	23.33±5.77^{b} (4-6)	36.66±5.77^{b} (6-8)	46.66±5.77^{b} (8-10)	56.66± 5.77^{c} (10-12)	73.33±5.77^{c} (14-16)	86.66±5.77^{c} (16-18)	100.00±0.00^{c} (20)	3-48
Combination-II (0.25+9)	0.00±0.00^{a} (0)	0.00±0.00^{a} (0)	0.00±0.00^{a} (0)	20.00±10.00^{b} (2-6)	40.00±10.00^{b} (6-10)	70.00±10.00^{b} (12-16)	83.33±15.27^{bc} (14-20)	12-48
Combination-III (0.25+13)	23.33±5.77^{b} (4-6)	53.33±11.54^{bc} (8-12)	70.00±10.00^{c} (12-16)	86.66±5.77^{d} (16-18)	100.00±0.00^{d} (20)	-	-	3-24
Combination-IV (0.5+8.2)	0.00±0.00^{a} (0)	0.00±0.00^{a} (0)	0.00±0.00^{a} (0)	20.00±10.00^{b} (2-6)	33.33±5.77^{b} (6-8)	70.00±10.00^{b} (12-16)	76.66±11.54^{b} (14-18)	12-48
Combination-V (0.5+11)	**26.66±5.77^{bc} (4-6)**	**40.00± 10.00^{b} (6-10)**	**50.00±10.00^{b} (8-12)**	**60.00±10.00^{c} (10-14)**	**73.33±5.77^{c} (14-16)**	**100.00±0.00^{c} (20)**	-	**3-36**
Combination-VI (0.5+13.8)	46.66±5.77^{d} (8-10)	73.33±5.77^{cd} (14-16)	100.00±0.00^{e} (20)	-	-	-	-	3-9
Combination-VII (0.75+9)	**0.00±0.00^{a} (0)**	**0.00±0.00^{a} (0)**	**0.00±0.00^{a} (0)**	**26.66±5.77^{b} (4-6)**	**46.66±5.77^{b} (8-10)**	**100.00±0.00^{c} (20)**	-	**12-36**
Combination-VIII (0.75+13)	43.33±5.77^{d} (8-10)	70.00±10.00^{cd} (12-16)	86.66±5.77^{de} (16-18)	100.00±0.00^{d} (20)	-	-	-	3-12
Combination-IX (0.85+11)	36.66±5.77^{cd} (6-8)	66.66±5.77^{cd} (12-14)	76.66±5.77^{cd} (14-16)	83.33±5.77^{d} (16-18)	100.00±0.00^{d} (20)	-	-	3-24
0 (Control)	0.00±0.00^{a} (0)	0.00±0.00^{a} (0)	0.00±0.00^{a} (0)	0.00±0.00^{a} (0)	0.00±0.00^{a} (0)	0.00±0.00^{a} (0)	0.00±0.00^{a} (0)	-

- n represents the number of larvae taken
- Figures in parentheses indicate the range in number of dead larvae from the start of the experiment till that period.
- Figures followed by different superscripts indicate a significant difference (<0.05) with respect to the control and treatment sets using Tukey's test.

Table 5. Larvicidal potential of RSM-designed effective combinations of common salt with citronella leaf aqueous extract (CLAE) against *Aedes aegypti* (3rd/4th instars) under simulated conditions

Common salt + CLAE (%)	**Per cent larval mortality up to (Mean±SD) (n=50)**			
	24hr		**48hr**	
	Plastic Cups	**Plastic Buckets**	**Plastic Cups**	**Plastic Buckets**
Combination-V **(0.5+11)**	70.66 ± 8.08^{c} (31-39)	40.00 ± 7.21^{b} (27-31)	82.66 ± 7.57^{b} (37-44)	63.33 ± 6.11^{b} (29-35)
Combination-VII **(0.75+9)**	45.33 ± 7.02^{b} (19-23)	31.33 ± 6.42^{b} (12-18)	76.00 ± 9.16^{b} (33-42)	52.00 ± 7.21^{b} (22-29)
0 (Control)	0.00 ± 0.00^{a} (0)	0.00 ± 0.00^{a} (0)	0.00 ± 0.00^{a} (0)	0.00 ± 0.00^{a} (0)

- n represents the number of larvae taken
- Figures in parentheses indicate the range in number of dead larvae from the start of the experiment till that period
- Figures followed with different superscripts indicate significant difference ($p<0.05$) with respect to control and treatment sets by using Tukey's test

3. ***Changes in the morphological characters of Ae. aegypti larvae after treatment*:** When *Ae. aegypti* larvae were exposed to effective larvicidal concentration of CLAE i.e. 13%, it resulted in widening of gut lumen (Fig. 1a) and distortion of anal gills (Fig. 1b), while in case of treatment having effective combination of CLAE+common salt @ 0.5+11% showed shrinkage and melanization of larval alimentary canal (Fig. 1c) along with damaged anal gills (Fig. 1d). While control (untreated) larvae were found to be intact and normal (Fig. 1e and f).

DISCUSSION

Plant-based products (either extracts or EOs) are a promising alternative to control mosquitoes in comparison to the synthetic chemical insecticides, as these are readily available, effective and environment-friendly (KOCHER ET AL., 2024). Citronella extract contains several naturally occurring chemical compounds with proven larvicidal properties, including 6-methyl-5-hepten-2-one, β-myrcene, cis-ocimene, linalool, trans-verbenol, neral, geraniol, geranial, 2-undecanone, saponins, steroids, and tannins. Among these, the primary active constituents are geranial and neral. Larvae ingest the bioactive components of citronella extract through their gut, respiratory system, or via spiracles located on the body surface, thereby interfering with normal physiological functions and leading to neurological disorders (COSTA ET AL., 2013). Saponins, another group of bioactive compounds found in citronella, contribute towards damaging cell membranes and disrupting metabolic processes, thus resulting in the mortality of *Ae.* aegypti larvae (ADITAMA ET AL., 2021), as also reported during the present study (Table 2). Moreover, extracts and essential oils derived from various plant species have demonstrated inhibitory effects on the development of different mosquito species, including morphological deformities (RIAT AND KOCHER, 2017), prolongation of larval and pupal durations (KAUR AND KOCHER, 2022) and larval mortality due to toxicity during developmental stages (KAUR AND KOCHER, 2023).

It has been observed that the concentration of sodium chloride in the surrounding medium significantly influences fitness parameters in insects, such as growth and development, by disturbing several physiological processes, including osmoregulation, digestion, and neurophysiological functions. *Ae. aegypti*, being a freshwater mosquito, exhibits limited tolerance to salinity during its larval stages. Any increase in salinity imposes considerable stress on the larvae, particularly in the early instars, thereby reducing their survival rate (SONG AND BROWN, 2002). Previous work carried out in our laboratory has also demonstrated 100% mortality of *Ae. aegypti* 1st instar larvae being exposed to 9 ppt of NaCl (KAUR AND KOCHER, 2015). Some mosquito species have evolved specialized osmoregulatory adaptations, such as an additional rectal segment, which facilitates active ion transport from the haemolymph, allowing them to excrete concentrated urine and maintain haemolymph osmolarity across a range of salinities. However, *Aedes* species that lack this additional rectal segment are unable to eliminate excess ions effectively, leading to ion imbalance and subsequent larval mortality (PATRICK AND BRADLEY, 2000).

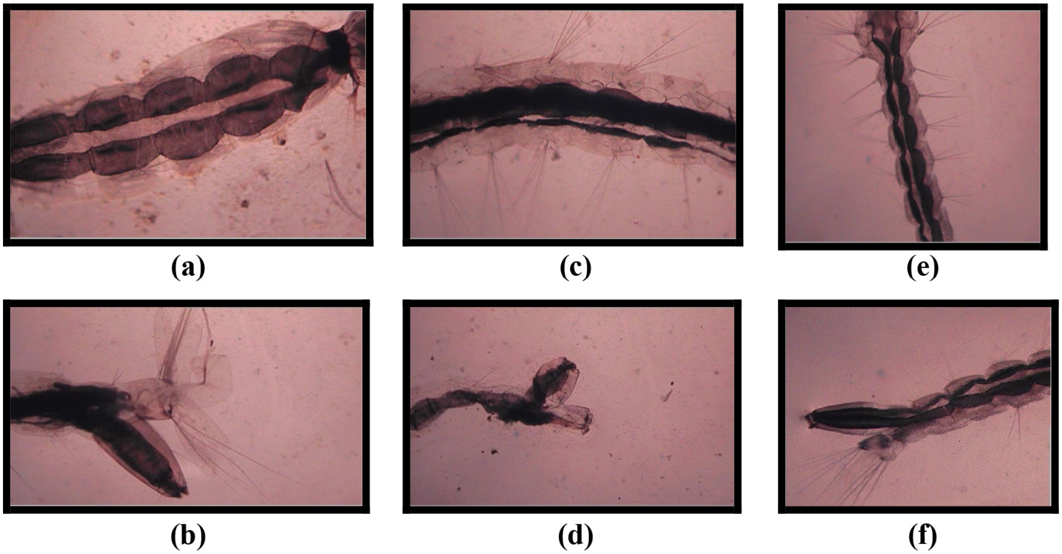

Fig. 1. Morphological changes observed in *Aedes aegypti* larvae after exposure to citronella leaf aqueous extract (CLAE) alone and in combination with common salt (10X × 4X), (a) Widening of gut lumen and loss of body hairs; (b) Distorted anal gills; (c) Shrinkage and melanization of gut; (d) Damaged anal gills; (e) and (f) Control larvae.

Synthetic chemical insecticides are generally targeted towards a single biochemical pathway, whereas plant-derived products, when used in combination with other non-lethal chemicals, such as common salt, result in effective larval mortality due to synergistic action (KAUR AND KOCHER, 2023). Similar observations have been recorded during the current study after exposing *Ae. aegypti* larvae to different combinations of CLAE and common salt (as depicted in Table 4). It was observed that when larvae were exposed to CLAE alone, the effective larvicidal concentration was recorded to be 13% (Table 2); however, mixing the extract with common salt @ 0.5% resulted in a reduction of CLAE concentration to 11% (Table 5), indicating the synergistic larvicidal effect. Microscopic study of the larvae exposed to citronella extract and its combination with NaCl solution depicted morphological deformities during the present study like widening of larval gut lumen, loss of body hairs and damage to anal gills (Fig. 1), this may be due to the flavonoids present in citronella extract, which have the potential to dissolving the integument, degradation of the chitin layer and abnormal stretching of the larval body (GAUTAM ET AL., 2013). Other degenerative changes like necrosis, and darkening of the body (as also observed during the current study) may occur because of a number of reasons and are generally influenced by some mechanisms, including melanization caused by phenoloxidase cascade activation (SHAO ET AL., 2012). However, the treatment having plant extract and common salt in combination primarily targets the gut, especially the midgut epithelium, which is the main absorption region of the mosquito larva (KAUR AND KOCHER, 2023). As a result, any modification or disruption in this gut region leads to poor or no absorption, which in turn causes larval death.

CONCLUSION

Interestingly, reducing the concentration of common salt by integrating it with citronella leaf aqueous extract (CLAE) was found to be an effective strategy for killing *Ae. aegypti* larvae. The determined best combination, having CLAE+NaCl @ 0.5+11% not only minimized the required dose of each individual agent, but also enhanced larvicidal efficacy through their combined action. Therefore, the idea of integrating common salt with CLAE explored in the present study targets the improvement of *Ae. aegypti* larval management. This integrated approach resulted in observable morphological deformities in larvae, including modifications to the alimentary canal, loss of hair, and darkening and damage to the anal gills upon exposure to the effective combination of CLAE and NaCl, suggesting a pronounced synergistic effect.

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