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Preparation-dependent Dual Larvicidal and Oviposition-Attractant Effects of Microalgae on *Aedes aegypti*

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Abstract

Aedes aegypti, the primary vector of dengue, *Zika, chikungunya, and yellow fever viruses,* is among the most medically important mosquito species worldwide. Its close association with human habitats, coupled with the rapid emergence of insecticide resistance, renders conventional control approaches increasingly ineffective. Consequently, alternative, sustainable and eco-friendly control tools are urgently needed. In this study, the larvicidal potential of three microalgae species (*Chlorella* sp., *Tetradesmus obliquus*, and *Scenedesmus vacuolatus*) and their oviposition attractiveness to gravid *Ae. aegypti* females were investigated under laboratory conditions. Larval mortality rates showed difference among algae species, remaining low in *Chlorella* sp. (0–5.6%) but reaching high levels in *T. obliquus* and *S. vacuolatus* (up to 70.4% and 72.8%, respectively). Oviposition assays showed that lyophilised preparations of *T. obliquus* and *S. vacuolatus* exhibited significant oviposition attractiveness ($p < 0.05$), whereas fresh cultures did not elicit a significant response due to the variability in response within treatment. These results demonstrate that microalgae exert species-specific biological effects on *Ae. aegypti*, underscoring potential for integration of microalgae within IVM strategies.

Key words: *Aedes aegypti*; microalgae; oviposition activity index; vector management; *Chlorella* sp.; *Tetradesmus obliquus*

Introduction

Aedes aegypti is a major vector of medically important arboviruses including dengue, Zika, chikungunya, and yellow fever (Souza-Neto et al., 2019; WHO, 2024; ECDC, 2026a). These arboviruses pose significant health threats on a global scale, especially in tropics/subtropics (Lim et al., 2025; Kraemer et al., 2015), due to the rapid re-emergence and spatial expansion of *Aedes* mosquitoes including *Ae. aegypti* (Ryan et al., 2019; WHO, 2024; ECDC, 2026b) This is of particular importance in urban and semi-urban environments due to *Aedes* close association with humans and preference for breeding in anthropogenic materials containing stagnant water (Wilke et al., 2020).

Dengue is one of the fastest emerging diseases in the world, with over half of the global human population at risk from the disease; an estimated 5.6 billion people live in dengue-endemic

regions (Lim et al., 2025), and 14.6 million cases reported per annum (WHO, 2025). Although dengue vaccination is available in some settings, its use is currently targeted to specific populations, and there remains no specific antiviral treatment; consequently, vector control remains a central component of dengue prevention (Hadinegro et al., 2015). Other emerging *Aedes*-vectored diseases include the Chikungunya and Zika viruses; causing an estimated 693,000 and 500,000 annual cases respectively (Franklinos et al., 2019; Whitehorn et al., 2019). To date, estimated 2.8 billion people from 104 countries have experienced CHIKV transmission and every year 35 million CHIKV infection cases are reported globally (Santos et al., 2025).

Current preventative measures rely primarily on effective vector control, particularly the use of chemical insecticides, but including biocontrol agents such as *Bacillus thuringiensis israeliensis* (Bti) and insect growth regulators (McGregor and Connelly, 2021). Widespread use of chemical insecticides has, however, lead to serious concerns relating to human health, environmental damages and the prolific development of insecticide resistant mosquito populations (Ahmad et al., 2024; Hazarika et al., 2025; Tognarelli et al., 2025; Yavaşoğlu and Şimşek, 2021). As such, there has been a push for the development and deployment of environmentally benign technologies to reduce chemical applications; particularly with regards to biological control agents (BCAs) and natural products (Nehra et al., 2026).

Although *Bti* and insect growth regulators can provide effective and persistent control within treated containers, larvicidal interventions remain operationally challenging because *Ae. aegypti* females exploit numerous and widely dispersed artificial breeding sites and commonly distribute eggs among multiple containers through skip-oviposition behaviour (Nakazawa et al., 2020; McGregor and Connelly, 2021). An additional approach is to exploit the oviposition behaviour of gravid females through attract-and-kill strategies, in which attractive oviposition cues are used to direct females towards traps or aquatic habitats containing larvicidal agents (Faierstein et al., 2019; Mulatier et al., 2022). Such strategies may be particularly valuable for *Ae. aegypti*, as they exploit the mosquito's own oviposition behaviour to target the otherwise widely dispersed immature stages.

Microalgae are an important food source for mosquito larvae, consequently, gravid female mosquitoes preferentially oviposit in habitats rich in microalgae (Gil et al., 2021). However, not all microalgae are beneficial to mosquito larvae; some algae produce bioactive compounds

including fatty acids, polyphenols and lectins, that can impair larval development or cause mortality (Rey et al., 2009; Balakrishan et al. 2018; Cavalcanti et al. 2021; Hassan et al., 2021; Sigamani et al., 2020). Another mechanism underlying algal toxicity is the inability of mosquito larvae to digest sporopollenin, a highly resistant, triple-layered carotenoid polymer present in the cell walls of certain algal species (Ahmad et al., 2004; Hassan et al., 2021). Ingestion of such indigestible algae can lead to larval starvation and mortality due to impaired digestion in the midgut (Hassan et al., 2021; Tufan-Çetin and Çetin, 2023). Indigestible or toxic phytoplankton represent a promising biological control agent against mosquitoes because they naturally occur in larval habitats, can proliferate under favorable conditions, and persist without requiring repeated applications (Balakrishan et al. 2018).

Given that a water-borne organism with potential to function both as oviposition attractant and larvicide would be an ideal tool in larvicidal Integrated Vector Management (IVM), this study aimed to investigate the potential of three microalgae on both fronts using *Ae. Aegypti* as the model mosquito.

Material and Method

Mosquito rearing

Aedes aegypti (Bora Bora) were maintained in the insectarium at Evolutionary Ecology and Geographic Information Systems Laboratory in Biology Department of Aydın Adnan Menderes University. *Aedes aegypti* (Bora Bora) lineage has been maintained under insectarium conditions with 27±2°C temperature, 70±5% relative humidity and 12:12 hours light-dark photoperiod for 15 years, (Kauffman et al., 2017). Adults were maintained in 45 x 45 x 45 cm mosquito cages (Bugdorm, Taiwan) and fed once weekly on defibrinated sheep blood using an artificial membrane feeding system (Hemotek Ltd., UK) while a 10% sucrose provided through a wick for the rest of each week. Eggs from the cages were transferred to plastic containers containing distilled water, and the hatched larvae fed *ad libitum* on Tetramin flake fish food (TetraMin®, Germany) until either experimentation or adult emergence to continue the colony.

Microalgae collection and cultivation

So as to screen microalgae not commonly encountered by mosquitoes in larval breeding habitats, two water bodies were chosen that were unsuitable for mosquito habitation but rich

in microbial and algal diversity. The first, Kaklik Cave (Denizli, Turkiye, 37°5’23.7924"N 29°23'6.9684"E) was chosen to isolate algae from an extreme environment where thermal sulphur-containing waters dominate the habitat. From the samples, *Tetradesmus* (formerly *Scenedesmus) obliquus and Scenedesmus vacuolatus* were successfully isolated. The second site was chosen to isolate a control alga, but one gained from a haline lake so as to give an extremophilic comparator and strengthen the control through similar production methods. The chosen site was Salda Lake (Yelisova, Turkiye, 37°32’55.5”N 29°43'07.1’E), an area with a large mosquito population in the surrounding forests, but with soda lake for any mosquito larvae to be able to survive. *Chlorella* was the chosen target control genus due to its common occurrence in *Aedes* guts as a food source (Promprao et al., 2025; Fei et al., 2023; Ranasinghe and Amarasinghe, 2020), despite a few specific isolates produce mosquitocidal toxins (Cavalcanti et al. 2021).

All collected algae were successfully cultured on Blue-Green Medium (BG-11) (Day & Brand, 2005). Cultured microalgae samples were isolated by diluting with culture medium at regular intervals and using the pour plate method. Samples were stored in agar (1.5%) medium and horizontal agar by applying the streaking method (Sukatar, 2002). Cell isolates were incubated in 250 mL bottles at 22 ± 2˚ C and a light intensity of 30 µmol m^{-2} s^{-1} for 14 days.

Large-scale microalgae cultivation was carried out in 2 L aerated flasks at 22 ± 2°C, with a light intensity of 50 µmol/m^2 sec^{-1} and aeration rate of 1 L min^{-1}. After centrifugation at 3500 x g, a portion of the cells was counted using a Thoma chamber and diluted with distilled water to a final concentration of 10 x 10^5 cells mL^{-1} and placed in cylindrical plastic containers with a total volume of 5 mL. The biomass collected by filtration was washed with distilled water and freeze-dried (FreeZone-Labconco, USA), before storage at -20°C until analysis.

Identification of microalgae species

Taxonomic identification was initially carried out based on cell morphology. Morphological examinations were performed using 400X and 1000X brightfield microscopy (Olympus CH20, Japan), and green microalgae species were identified at the molecular level by partial 18S rRNA sequencing. Species identifications were made according to standard taxonomic keys (Fott and Komárek, 1974; Kessler et al., 1997) and verified via the AlgaeBase database (Guiry and Guiry, 2024). Total genomic DNA was manually extracted using the glass bead disruption

method applied by Jagielski et al. (2017) with minor modifications including mechanical cell lysis using 0.5 mm glass beads followed by standard phenol-chloroform extraction. PCR amplification of 18S rRNA gene regions from isolated microalgal DNAs was carried out using primers 18SF (5'-CCTGGTTGATCCTGCCAG-3') and 18SR (5'-TTGATCCTTCTGCAGGTTCA-3') (Liang et al., 2020). Amplification was performed using 1X Taq buffer, 2 mM MgCl2, 0.2 mM dNTP, 0.4 pmol per primer, 1.5U Taq polymerase (Thermo Scientific, USA) and 1 µL of total DNA was also added to the mixture. Amplification was performed under PCR conditions: 95 °C x 5 min, 35X {94 °C x 45 sec, 55 °C x 45 sec, 72 °C x 90 sec}, 72 °C x 10 min. PCR products were separated by 1.5% agarose gel electrophoresis. Similarity analysis of 18S rDNA sequences was performed using BLAST software from NCBI, and homologies were identified.

Feeding and larvicidal experiments

Larval feeding and mortality assays were each conducted using 25 third-instar (L_3) *Ae. Aegypti* larvae. Prior to experimentation, larvae were deprived of food for 12 h to standardise hunger and reduce residual intestinal contents. Assays were conducted in individual cylindrical plastic vials (1.5 cm radius × 3 cm height) containing a total working volume of 10 mL.

Microalgae were added, within the BG-11 medium, at an initial concentration of 10×10^5 cells mL^{-1}. For each microalgal species, five experimental treatments were established to distinguish the effects of the culture medium, food availability and short-term starvation from those associated with exposure to microalgae (table 1). In treatments receiving fish food, Tetramin (TetraMin®, Germany) flake food was supplied at 50 mg per container per day, equivalent to 2 mg per larva per day.

Table 1. Experimental groups and their corresponding rearing conditions, culture media, and feeding regimes applied to larvae

Group	Experimental treatment
G1	Larvae maintained in distilled water and provided with fish food
G2	Larvae maintained in distilled water + BG-11 medium and provided with fish food
G3	Larvae maintained in distilled water without supplemental food

Group	Experimental treatment
G4	Larvae maintained in BG-11 medium containing microalgae at 10 x 10^5 cells mL^{-1} without supplemental fish food
G5	Larvae maintained in BG-11 medium containing microalgae at 10 x 10^5 cells mL^{-1} and provided with fish food

Larval survival was recorded at 24 hrs and 48 hrs following establishment of the assays. In the microalgae-containing treatments (G4 and G5), algal abundance was also monitored throughout the experiment. To do this, a 10 µL water sample was collected from each container and cells were counted by light microscopy using a Thoma counting chamber, following the approach described by Houessou et al. (2023). Five replicate assays were conducted for each treatment, with a total of 125 *Ae. aegypti* exposed to each treatment.

An additional experiment was conducted to determine algal reproduction in the absence of mosquito larvae. In this, the same experimental protocol was followed as above, with the omission of mosquito larvae. Algal concentration was counted, as above, at the 24 and 48 hr timepoints.

Oviposition experiments

Oviposition experiments were conducted to determine the attractiveness of each of the algae to gravid female *Ae. aegypti*. These were conducted in mosquito cages measuring 45 x 45 x 45 cm under a photoperiod of 12:12 (L:D), 27±2°C temperature and 70±5% relative humidity. Ten gravid female *Ae. aegypti* were transferred to the experimental cages using an aspirator 48 hours after blood feeding. Two variations of the experiment were performed: **(1)** Oviposition preference test of each live algal species at 10 x 10^5 cells mL^{-1}, and **(2)** Oviposition preference test of each algal species in lyophilised form at 0.05 g L^{-1}.

For the first assay, two plastic containers (5 x 5 x 4 cm), one containing distilled water inoculated with sterile BG-11 medium, and the other containing distilled water inoculated with the BG-11 medium containing a final concentration of 10 x 10^5 cells mL^{-1} were deployed into the cages. Microalgae suspensions were prepared by taking 1 mL exponential growth

phase culture directly and diluting it with 49 mL distilled water. Similarly, the control group consisted of 1 mL cell-free culture medium into 49 mL distilled water.

For the second assay, similar to the first assay, two plastic containers (5 x 5 x 4 cm), one containing distilled water as the control and the other containing lyophilised algal biomass at a final concentration of 0.05 g L^{-1} were placed in the cages. Suspensions were prepared by dissolving 0.0025 g lyophilised microalgal form in 50 mL distilled water.

Both containers were lined with Whatman No. 2 filter paper extending above the water line to collect eggs, and each container was placed within a corner of the mosquito cage and rotated randomly, daily. Six replicates were performed for each treatment in each of the experiments. After 4 days, the filter papers in the plastic containers were collected and the accumulated eggs were counted using a Leica S8 Apo stereomicroscope.

Data analysis

Algae concentrations were calculated based on the counts on Thoma chamber at 48 hours and alterations in algal concentrations (remaining algae concentration) were evaluated by comparing the algal concentrations on the first day and the algal concentration at the 48^{th} hour. To determine the differences in the average microalgae concentrations between the groups (1-5) in the feeding experiments, ANOVA analysis was first performed, followed by Tukey's HSD test. Larval mortality was calculated as percentages (%) in groups 1,2,3,4 and 5 of each algae species. Larval mortality differences among each group were calculated via ANOVA analysis followed by Tukey's HSD test. In oviposition experiments, average percentages of total egg numbers were calculated. The oviposition attraction index (OAI) was calculated according to the Kramer and Mulla (1979) as OAI = (Nt-Ns)/(Nt+Ns) where Nt is total egg number in treatment group and Ns is total egg number in control group. The differences in the number of eggs laid between the experimental and corresponding control groups were analysed using a paired samples t-test. Statistical calculations were performed using IBM SPSS version 28 software. Graphs were created using R Studio (R core team, 2023).

Results

Microalgae species identification

Results showed that the three microalgae species isolated from sampling area were *Chlorella sp*., *Tetradesmus obliquus*, and *Scenedesmus vacuolatus.*

Chlorella sp. was observed as green, unicellular, and spherical (coccoid) or hemispherical. The chloroplast was parietal and cup-shaped with a single pyrenoid under light microscope. Morphometric observations showed a cell diameter of 2–11 µm (Figure 1A). Amplified PCR products created approximately 1900 bp amplicons with 18S rRNA primer pairs. Similarity analysis of 18S rDNA sequences, showed 100% similarity with *Chlorella sp.* (Figure 1B, Table 2)

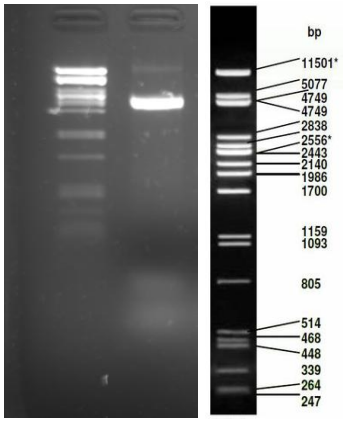
A)  B) 

Figure 1: *Chlorella sp.* A) Light-field microscope image (400X), B) Gel image of PCR-amplified gene region, Marker: λ Pst

Tetradesmus obliquus was observed as four or eight-celled coenobia with oval-shaped cells averaging 9-11.5 µm in length and 4.5–5.5 µm in width (Fig 2A). Each cell of *T. obliquus* had a single chloroplast and a single pyrenoid within it. Amplified PCR products created approximately 1900 bp amplicons with 18S rRNA primer pairs. Similarity analysis of 18S rDNA sequences, showed 99.9% similarity with *Tetradesmus obliquus.* (Figure 2B, Table 2)

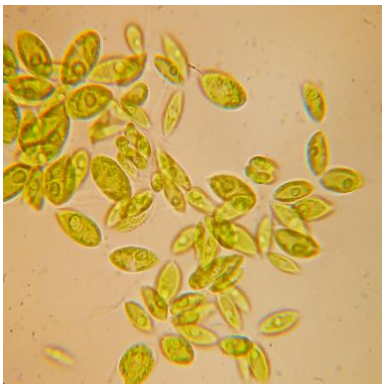
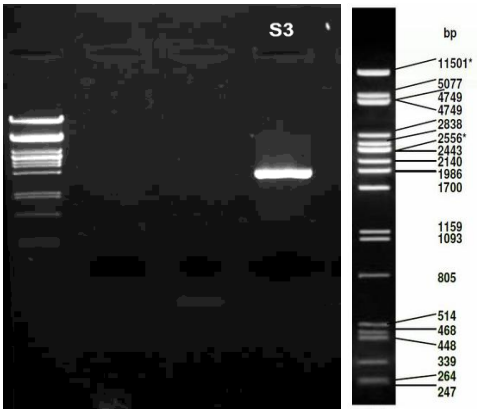
A)  B) 

Figure 2: *T. obliquus* A) Light-field microscope image (1000X) B) Gel image of PCR-amplified gene region, Marker: λ Pst

Scenedesmus vacuolatus was observed with ellipsoidal cells are 5-12 µm long and 3-11 µm wide. A single chloroplast and a single pyrenoid were observed in each *S. vacuolatus* cell (Figure 3C). Amplified PCR products created approximately 1900 bp amplicons with 18S rRNA primer pairs. Similarity analysis of 18S rDNA sequences, showed 99.88% similarity with *Scenedesmus vacuolatus* (Figure 3A, Table 2)

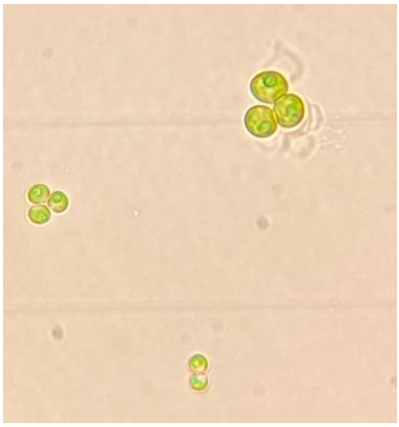
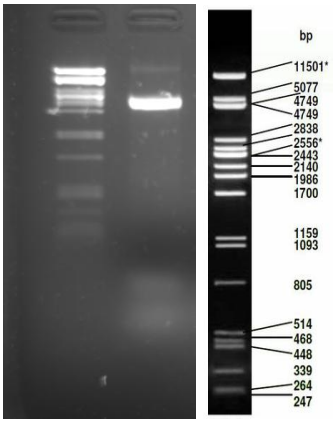
A)  B) 

Figure 3: *S. vacuolatus* A) Light-field microscope image (1000X), B) Gel image of PCR-amplified gene region, Marker: λ Pst

Table 2: Diagnosis of 18S rDNA sequence with nucleotid BLAST software *in-silico*

Strain	Microalgae Name	Identity Percentage	Reference

1	*Chlorella sp.* UTEX EE97 18S ribosomal RNA gene, partial sequence	%100	KU291900.1
2	*Tetradesmus obliquus* strain CE2 small subunit ribosomal RNA gene partial sequence	99.9%	MH307949.1
3	*Scenedesmus vacuolatus* strain NG small subunit ribosomal RNA gene partial sequence	99.88%	MH459062.1

Response of *Aedes aegypti* larvae exposed to algal culture

Larval mortality differed among treatment groups for all three microalgal species (Table 3; Figure 4). For *Chlorella* sp., mortality remained low across all treatments, with mean mortality rates of 0, 1.6, 2.4, 5.6 and 3.2% in G1–G5, respectively. Nevertheless, an overall difference among treatment groups was detected (one-way ANOVA: $F_{(4,20)}=3.190$, $p < 0.001$). Mortality was highest in G4 (microalgae without supplemental fish food), although post-hoc Tukey HSD analysis identified a significant difference only between G4 and G1 ($p < 0.001$).

A substantially stronger treatment effect was observed for *T. obliquus*. Mean larval mortality was 2.4, 3.2, 3.2, 70.4 and 19.2% in G1–G5, respectively, and differed significantly among treatments ($F_{(4,20)}=44.909$, $p<0.001$). Mortality was highest in G4, reaching 70.4%, and Tukey HSD comparisons showed that G4 differed significantly from all other treatment groups ($p < 0.001$). The addition of fish food in G5 was associated with substantially lower mortality (19.2%) than observed in G4.

A similar pattern was observed for *S. vacuolatus*. Mean larval mortality was 2.4, 2.4, 1.6, 72.8 and 18.4% in G1–G5, respectively. Mortality differed significantly among treatments ($F_{(4,20)} = 73.558$, $p < 0.001$), with the highest mortality again occurring in G4. Tukey HSD analysis showed that mortality in G4 was significantly greater than in all other treatment groups ($p < 0.001$). As observed for *T. obliquus*, mortality was markedly reduced when fish food was provided alongside the microalgae in G5.

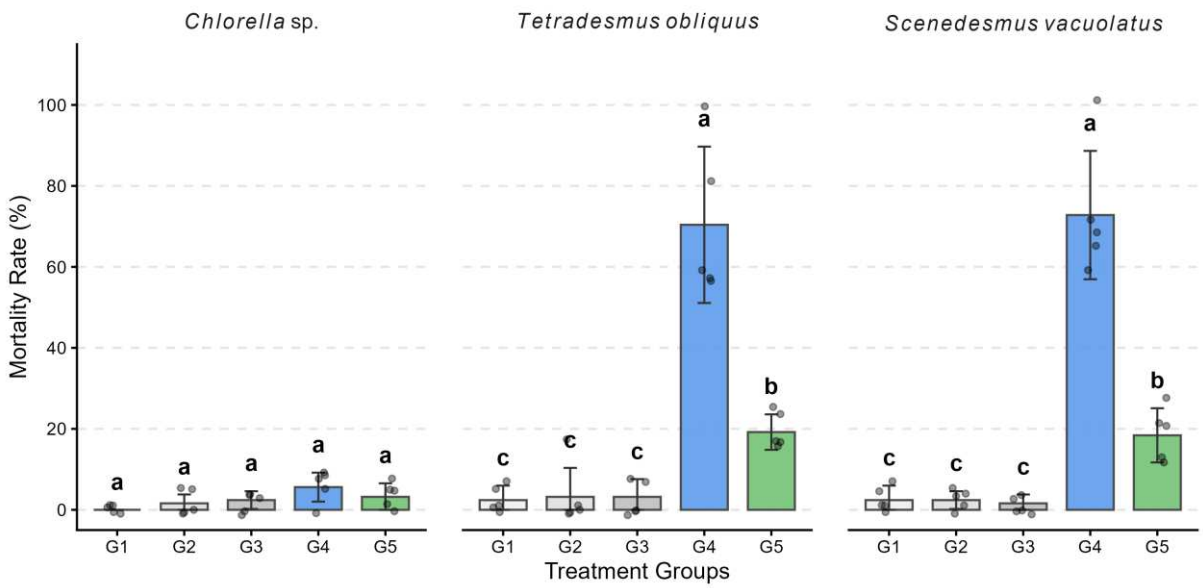


Figure 4. Mortality of 3rd instar *Aedes aegypti* larvae exposed to each of the treatments (G1: Larvae + distilled water + fish food; G2: Larvae + BG-11 medium + fish food; G3: larvae + distilled water; G4: Larvae + BG-11 medium + microalgae; G5: Larvae + BG-11 medium + microalgae + fish food) over 48 hrs period. Data shown represent mean mortalities at 48 hrs, including individual replicate data points, ± SD. Letter demarcation above bars indicate significant differences between groups ($p < 0.05$).

Table 3. Larvicidal activity of three microalgae species at 48th hr (*Chlorella* sp., *Tetradesmus obliquus*, and *Scenedesmus vacuolatus*) against *Aedes aegypti* larvae across different experimental groups G1, G2, G3, G4, G5 (G1: Larvae + distilled water + fish food; G2: Larvae + BG-11 medium + fish food; G3: larvae + distilled water; G4: Larvae + BG-11 medium + microalgae; G5: Larvae + BG-11 medium + microalgae + fish food). Data are presented as mean ± standard deviation (SD).

Treatment	*Chlorella sp.*	*Tetradesmus obliquus*	*Scenedesmus vacuolatus*
G1	0.00 ± 0.00	2.40 ± 3.58	2.40 ± 3.58
G2	1.60 ± 2.19	3.20 ± 7.16	2.40 ± 2.19
G3	2.4 ± 2.19	3.2 ± 4.38	1.6 ± 2.19
G4	5.6 ± 3.57	70.40 ± 19.31	72.80 ± 15.85
G5	3.20 ± 3.35	19.20 ± 4.38	18.40 ± 6.69

Mortality within the G4 treatment differed significantly among the three microalgal species ($F_{(2,12)} = 34.236$, $p < 0.001$; Figure 5). Mean mortality was 5.6% for *Chlorella* sp., compared with 70.4% for *T. obliquus* and 72.8% for *S. vacuolatus*. Tukey HSD analysis showed that mortality following exposure to *Chlorella* sp. was significantly lower than that observed with either *T. obliquus* or *S. vacuolatus* ($p < 0.001$), whereas mortality did not differ significantly between *T. obliquus* and *S. vacuolatus*.

The same overall pattern was observed in G5, where fish food was provided alongside the microalgae. Mortality differed significantly among algal species ($F_{(2,12)} = 16.213$, $p < 0.001$), with mean mortality of 3.2% for *Chlorella* sp., 19.2% for *T. obliquus* and 18.4% for *S. vacuolatus*. Tukey HSD analysis again showed significantly lower mortality in the *Chlorella* sp. treatment than in either *T. obliquus* or *S. vacuolatus* ($p < 0.001$), while no significant difference was detected between the latter two species.

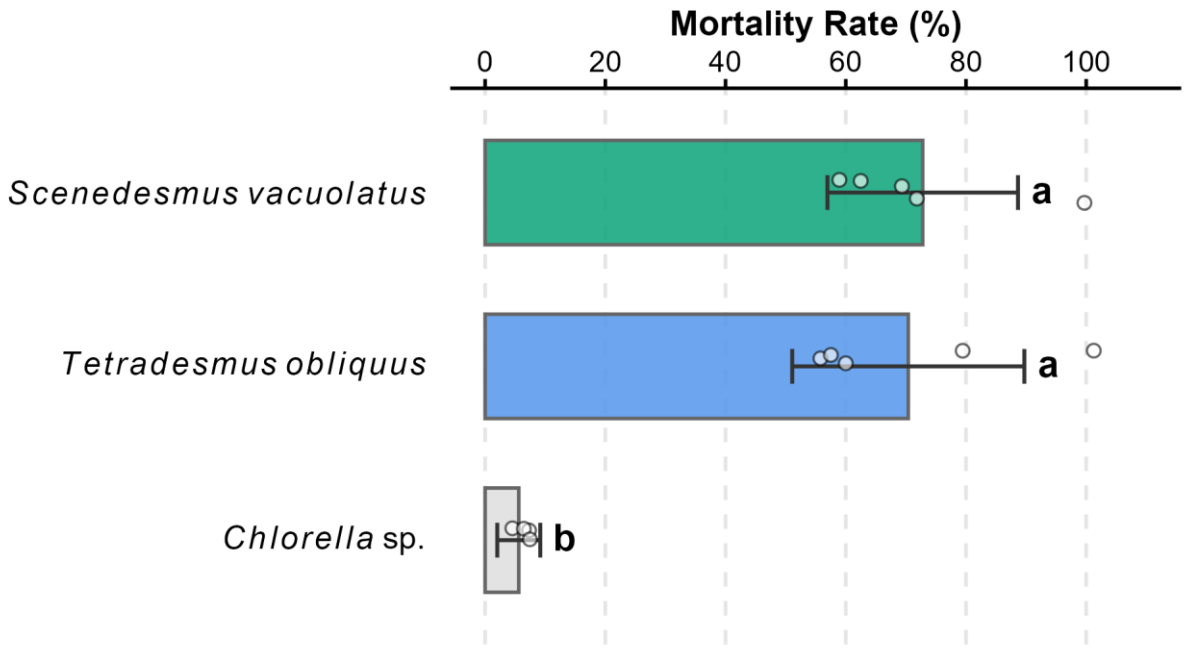


Figure 5. Mortality rates of 3rd instar *Aedes aegypti* larvae exposed to $10x10^5$ algal cells mL^{-1} over a period of 48 hours. Values represent mean mortality (%), with individual data points shown ± SD. Different lowercase letters indicate statistically significant differences between treatments.

Microalgal concentration alteration

Microalgal abundance changed markedly over the 48 hrs assay period, with the magnitude of change differing among treatments and algal species (Figure 6). For *Chlorella* sp., the initial concentration of 10 × 10^5 cells mL^{-1} decreased to 3.4 × 10^5 cells mL^{-1} in G4 after 48 hrs, whereas it increased slightly to 13 × 10^5 cells mL^{-1} in G5. In the algae-only control (ACG), cell abundance increased to 20 × 10^5 cells mL^{-1}. Overall differences in cell concentration were significant ($F_{(3,23)}$ = 3.714, p = 0.028), with Tukey HSD analysis identifying a significant difference between G4 and the ACG ($p < 0.001$).

For *T. obliquus*, cell concentration decreased from 10 × 10^5 mL^{-1} to 2.13 × 10^5 cells mL^{-1} in G4 and to 7.7 × 10^5 cells mL^{-1} in G5 after 48 hrs. By contrast, substantial algal proliferation occurred in the ACG, where the concentration reached 5.08 × 10^6 cells mL^{-1}. Differences among treatments were significant ($F_{(3,23)}$ = 134.972, $p < 0.001$), with Tukey HSD analysis showing a significant difference between G4 and the ACG ($p < 0.001$).

A similar pattern was observed for *S. vacuolatus*. The initial concentration of 10× 10^5 cells mL^{-1} decreased to 3.95 × 10^5 cells mL^{-1} in G4, while increasing to 1.358 × 10^6 cells mL^{-1} in G5 and 2.13 × 10^6 cells mL^{-1} in the ACG. Significant differences in cell concentration were detected among treatments ($F_{(3,23)}$ = 5.401, p = 0.007), with the post-hoc analysis identifying a significant difference between G4 and the ACG ($p < 0.004$ in all cases).

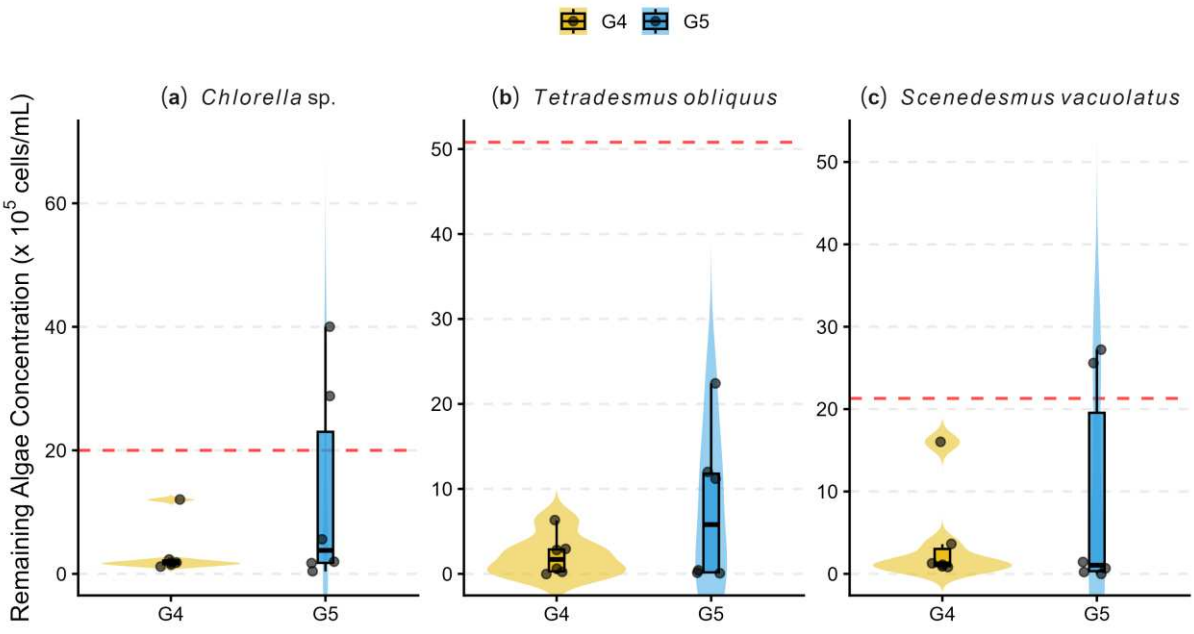


Figure 6. Remaining algae concentrations after feeding experiments (red-dots indicates algae control group) using fed and unfed larvae exposed to 10 x 10^5 cells mL^{-1} (G4: Larvae + BG-11

medium + microalgae; G5: Larvae + BG-11 medium + microalgae + fish food). Values are presented as mean ± SD.

Oviposition preference

Oviposition responses varied among algal species and between fresh and lyophilised preparations (Figure 7; Table 4). Fresh *Chlorella* sp. produced a positive numerical oviposition response, with a mean of 378.2 ± 182.7 eggs laid per cage in the algal treatment compared with 178.5 ± 107.6 eggs in the corresponding control. This resulted in an oviposition activity index (OAI) of 0.36; however, the difference in egg number was not statistically significant (t = 1.817, df = 5, p = 0.129). Lyophilised *Chlorella* sp. similarly produced a positive but non-significant response, with 357.7 ± 208.6 eggs laid in treatment containers compared with 273.8 ± 121.5 eggs in controls (OAI = 0.13; t = 0.664, df = 5, p = 0.536).

Fresh *Tetradesmus obliquus* produced the strongest numerical oviposition response among the fresh algal preparations, with 386.2 ± 178.6 eggs laid in treatment containers compared with 174.7 ± 112.0 eggs in controls, corresponding to an OAI of 0.38. Despite this marked numerical preference, the difference did not reach statistical significance (t = 2.227, df = 5, p = 0.076). In contrast, lyophilised *T. obliquus* produced a significant oviposition preference, with 429.5 ± 105.8 eggs laid in treatment containers compared with 189.2 ± 67.2 eggs in controls (OAI = 0.39; t = 3.726, df = 5, p = 0.014).

Fresh *Scenedesmus vacuolatus* did not significantly influence oviposition, with 229.2 ± 163.5 eggs laid in treatment containers compared with 213.2 ± 172.9 eggs in controls (OAI = 0.04; t = 0.186, df = 5, p = 0.860). In contrast, lyophilised *S. vacuolatus* produced a significant oviposition preference, with 456.7 ± 139.4 eggs laid in treatment containers compared with 246.8 ± 93.3 eggs in controls, corresponding to an OAI of 0.30 (t = 3.112, df = 5, p = 0.026). OAI values for the fresh and lyophilised preparations of each algal species are presented in Table 4.

Table 4. Oviposition attractance indexes of fresh and lyophilised form of each algae species

Algae	*Chlorella sp.*	*Scenedesmus vacuolatus*	*Tetradesmus obliquus*
(a) Fresh Algae	+0.35	+0.03	+0.37
(b) Lyophilized Algae	+0.13	+0.30	+0.38

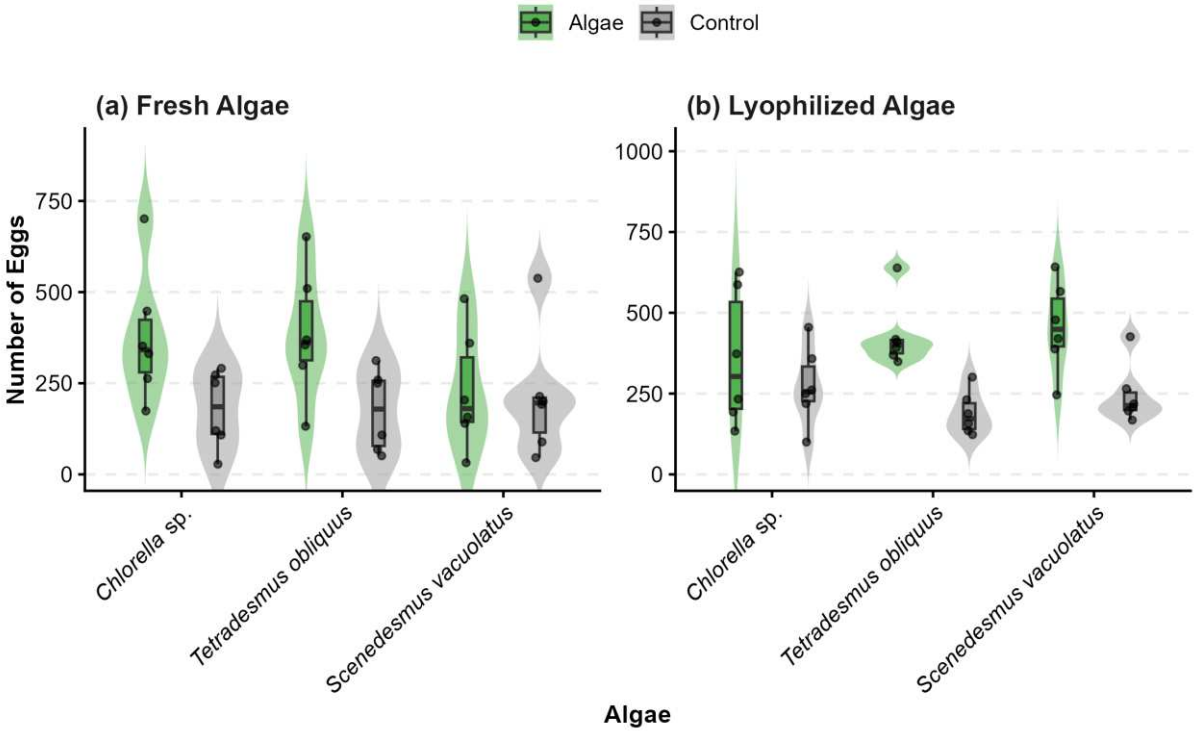


Figure 7. Comparison of mean number of eggs attracted by each algae species. Values are presented as mean ± SD.

Discussion

This study demonstrates the effects of microalgae on both larval survival and oviposition behaviour of *Aedes aegypti*. Most notably, *Tetradesmus obliquus* displayed a dual phenotype, causing a high degree of larval mortality within 48 hrs, while also significantly increasing oviposition when prepared as lyophilised bait. Likewise, *Scenedesmus vacuolatus* produced comparable larval mortality and the lyophilised cells also attracted gravid females. *Chlorella*, by contrast functioning as a control, caused little mortality or oviposition attractancy. These contrasting responses identify *T. obliquus* as the strongest candidate for further development as a dual-function oviposition-active larvicide using different preparations.

The development of single biological control agents with dual-active oviposition larvicidal properties would represent a distinctly advantageous combination for *Aedes* control. Larvicidal interventions depend on gravid females depositing eggs within treated habitats, while *Ae. aegypti* commonly distributes eggs among multiple breeding containers. Attract-

and-kill strategies seek to overcome this limitation by coupling an attractive oviposition substrate with a lethal agent. Faierstein et al. (2019), for example, demonstrated increased oviposition into traps combining larval-derived cues with *Bacillus thuringiensis israelensis*. The present results extend this concept by demonstrating that a single biological material can potentially provide both components: an oviposition cue and a subsequent lethal effect on larvae.

The mechanism underlying mortality remains unresolved, although the treatment structure provides useful insight. Mortality following 48 h without supplemental food was low, indicating that simple starvation over the experimental period cannot explain the approximately 70% mortality associated with *T. obliquus* and *S. vacuolatus*. However, addition of fish food reduced mortality to 19.2 and 18.4%, respectively, demonstrating a strong interaction with nutritional conditions. Green microalgae can impair mosquito larvae through poor digestibility and displacement of nutritionally suitable food (Marten, 2007). More recently, it was shown that a *Chlorella sorokiniana* strain could cause concentration-dependent inhibition of *Ae. aegypti* larval growth, pupation and adult emergence (Soe et al. 2026). In this case, high algal proportions produced substantial mortality, further supporting the capacity of microalgal diets to disrupt mosquito development (Soe et al. 2026). Extracts of *S. obliquus* have also previously demonstrated larvicidal activity, indicating that bioactive metabolites cannot be excluded (Hassan et al., 2021). The present phenotype may therefore reflect nutritional interference, bioactivity associated with the algae, or a combination of both, rather than starvation alone.

The marked decline in algal abundance in containers containing larvae is consistent with active consumption or removal of algal cells, although ingestion was not directly quantified. This interpretation has ecological support: microalgae constitute an important component of mosquito larval diets (Souza-Neto et al., 2019), and recent metabarcoding of *Ae. aegypti* breeding sites demonstrated *Tetradesmus* in both larval guts and associated water samples, with the genus detected across all sites examined (Promprao et al., 2025). Thus, interaction between *Ae. aegypti* larvae and *Tetradesmus* generally is not restricted to an artificial laboratory association; albeit the specific samples here were obtained from sites where no interactions would take place. Importantly, therefore, this also indicates that the strong

mortality observed here should be considered an isolate-specific phenotype rather than a general property of the genus.

The oviposition results further support the potential application of *T. obliquus*. Both fresh and lyophilised preparations produced strong positive oviposition responses, with similar OAI values, although statistical significance was reached only for the lyophilised material. This suggests that the behavioural cue is retained, and perhaps concentrated, during processing; an encouraging feature for future storage and formulation. The operational relevance of such stability is supported by Fairestein et al. (2025), who demonstrated that lyophilised *Ae. aegypti* larval extracts retained oviposition activity when combined with *Bti* in field-deployed lethal oviposition traps. Nevertheless, the present two-choice assay demonstrates oviposition preference rather than unequivocal long-range attraction, because such assays cannot distinguish attraction before landing from short-range stimulation of egg laying (Mulatier et al., 2022). Moreover, larvicidal activity was demonstrated only for live cultures; determining whether lyophilised *T. obliquus* retains both oviposition activity and larvicidal efficacy will therefore be important before a single formulated product can be proposed.

Collectively, these findings provide proof of concept for the use of microalgae as dual-function components of an Ae. aegypti attract-and-kill strategy. T. obliquus is particularly promising because oviposition preference and high larval mortality occurred within the same biological system, rather than requiring separate lure and killing agents. Further work should now define the dose-response relationship, determine the basis of larval mortality and oviposition activity, establish whether both effects persist after formulation, and evaluate performance in organically enriched water and semi-field conditions. If these properties can be retained under operational conditions, T. obliquus could provide the basis for a biologically derived lethal oviposition system that exploits mosquito behaviour to direct progeny towards an otherwise attractive but unsuitable developmental habitat.

Conclusions

In summary, *T. obliquus* showed the strongest dual activity, combining significant oviposition preference with high larval mortality against *Ae. aegypti*. Mortality was strongly influenced by nutritional conditions, while oviposition activity was retained after lyophilisation. Together,

these findings support further development of *T. obliquus* as a microalgae-based lure-and-kill tool, with future work required to resolve mechanism, formulation stability and efficacy under semi-field and field conditions.

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Authors' contributions:

SIY: Conceptualization; Investigation; Methodology; Writing-original draft. MJW: Investigation; Writing-original draft; Writing-review & editing. NA: Formal analysis; Investigation; Methodology; Visualisation. ED: Formal analysis; Investigation; Methodology; Software. DYB: Investigation, Methodology, Writing original-draft. MY: Investigation; Methodology. FMS: Investigation; Methodology. TMB: Conceptualization; Supervision; Writing-original draft; Writing-review & editing.

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