

Article

Analyzing the Efficacy of Water Treatment Disinfectants as Vector Control: The Larvicidal Effects of Silver Nitrate, Copper Sulfate Pentahydrate, and Sodium Hypochlorite on Juvenile *Aedes aegypti*

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Abstract: For communities without access to uninterrupted, piped water, household water storage (HWS) practices can lead to adverse public health outcomes caused by water degradation and mosquito proliferation. With over 700,000 deaths caused by vector-borne diseases annually, the objective of this study was to determine whether water disinfectants, at concentrations deemed safe for human consumption and beneficial for water treatment, are effective in reducing the emergence of adult mosquitoes that transmit disease. Laboratory bioassays, designed to resemble the context of treating HWS containers, were conducted to assess the larvicidal effects of chemicals at concentrations below regulatory limits for drinking water: silver (20, 40, 80 µg/L Ag), copper (300, 600, 1200 µg/L Cu), and chlorine (500, 1000, 2000 µg/L free chlorine). The water disinfectants demonstrated the ability to significantly reduce the population of juvenile *Ae. aegypti*. Sodium hypochlorite was found to be the most effective in decreasing the survival rate of late first instar larvae, while silver nitrate exhibited the highest effectiveness in inhibiting the emergence of late third instar larvae. Ultimately, this study highlights the potential of an integrated approach to Water, Sanitation, and Health (WASH) solutions with vector control management.

Keywords: *Aedes aegypti*; household water storage; vector control management; water treatment



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1. Introduction

Eighty percent of the world's human population is at risk of contracting one or more vector-borne diseases (VBDs), which are caused by the spread of viruses, bacteria, and parasites from infected animals to a human [1,2]. These diseases account for more than 17% of the global burden of infectious diseases. With rising global temperatures driven by climate change, the risk of VBDs spreading to new regions continues to grow [3–5]. Many VBDs fall under the category of neglected tropical diseases (NTDs) due to their disproportionate impact on underserved or marginalized populations, particularly communities lacking access to basic healthcare, clean water, and sanitation [6,7]. In the absence of accessible vaccines and treatments for many VBDs, vector control management is essential to reducing

the risk of disease transmission by controlling vector populations or disrupting human–vector contact [8–11]. Effective vector control programs require a deep understanding of the infected animals responsible for spreading disease, which are often blood-sucking insects [2], with mosquitoes being the most prolific [12]. One of the two most abundant mosquito species is the *Aedes (Stegomyia) aegypti* (Linnaeus), responsible for transmitting several arboviral diseases such as Chikungunya, Dengue, Yellow fever, and Zika [2,13,14]. Table 1 highlights key public health data for each of these diseases.

Table 1. Public health information regarding VBDs transmitted by *Aedes aegypti*.

Vector-Borne Disease	Common Symptoms	Global Burden	Location of Prevalence	Vaccine for Prevention
Chikungunya (CHIKV)	Fever, severe joint pain, joint swelling, muscle pain [15]	Since 2023, 5+ million cases have been reported in last 15 yrs [16]	Detected in >100 countries as of 2021, circulating mainly in Africa, Asia, South America, and regions of the Pacific Ocean [17].	IXCHIQ (manufactured by Valenva) [16]
Dengue	High fever (40 °C/104 °F), severe headache, pain behind the eyes, muscle and joint pains, nausea, vomiting, swollen glands, rash [18]	In 2019, age-standardized incidence rate (ASIR) (A statistical measurement that compares the number of new cases of a disease in a population to a standard population. It's used to compare health metrics across populations with different age distributions.) estimated to be 7.40 per 1000 [19]. An estimated 100–400 million infections occur each year [18].	Endemic in >100 countries in WHO regions of Africa, the Americas, the Eastern Mediterranean, Southeast Asia and the Western Pacific. The Americas, Southeast Asia, and Western Pacific regions are most significantly affected [18].	Dengvaxia® (CYD-TDV), developed by Sanofi Pasteur, Qdenga® (TAK-003), developed by Takeda [18].
Yellow Fever	Fever, muscle pain, headache, loss of appetite, nausea or vomiting, jaundice, dark urine, abdominal pain [20]	In 2018, estimated 109,000 severe infections and 51,000 deaths in Africa and South America [21].	Thirty-four countries in Africa and thirteen countries in Central and South America are either endemic for, or have regions that are endemic for, yellow fever as of 2023 [20].	YF-VAX®, manufactured by Sanofi Pasteur [22]
Zika virus (ZIKV)	Fever, rash, headache, joint pain, conjunctivitis (red eye), muscle pains. Virus can be passed through sex and from a pregnant woman to her fetus [23]	In 2019, ASIR estimated to be 3.44 per 100,000 [24]	Eighty-nine countries and territories have documented evidence of current or previous transmission as of February 2022, circulating primarily in the Americas, South Asia, and the Pacific Islands [24,25].	No [24].

Understanding the life cycle of the *Ae. aegypti* (depicted in Figure 1), as well as its behaviors and habitats, is crucial for developing effective strategies to prevent and control the spread of these VBDs [9,26–28]. Typically living in tropical and subtropical regions, *Ae. aegypti* are found in close association with humans [29]. Jansen et al. cites urbanization,

socioeconomic factors, building design and constructions features, the quality of water supply and management, and the quality of other public health infrastructure services as significant influences shaping the geographic spread of *Ae. aegypti* [29]. The following behavioral traits have been observed of adult female *Ae. aegypti* mosquitoes [9,29–32]:

1. They prefer to bite hosts during the daytime, with peak activity occurring between dawn and dusk.
2. They feed on humans relative to other vertebrate species, obtaining bloodmeals which provide the necessary nutrients required for a female mosquito's egg production and reproduction.
3. They feed on several hosts within one reproductive cycle which increases the potential for the transmission of disease.
4. They prefer to lay eggs in manmade or artificial containers (e.g., household water storage containers, plant pots, tires, etc.), which is why they are commonly referred to as a container-breeding species.

***Aedes Aegypti* Life Cycle and Disease Transmission**

Water storage containers can serve as breeding grounds for *Aedes aegypti*. These mosquitoes go through a complete metamorphosis with an egg, larva, pupa, and adult stage. In the adult stage, *Ae. aegypti* can spread dengue fever, chikungunya, Zika fever, and yellow fever viruses, among other disease agents.

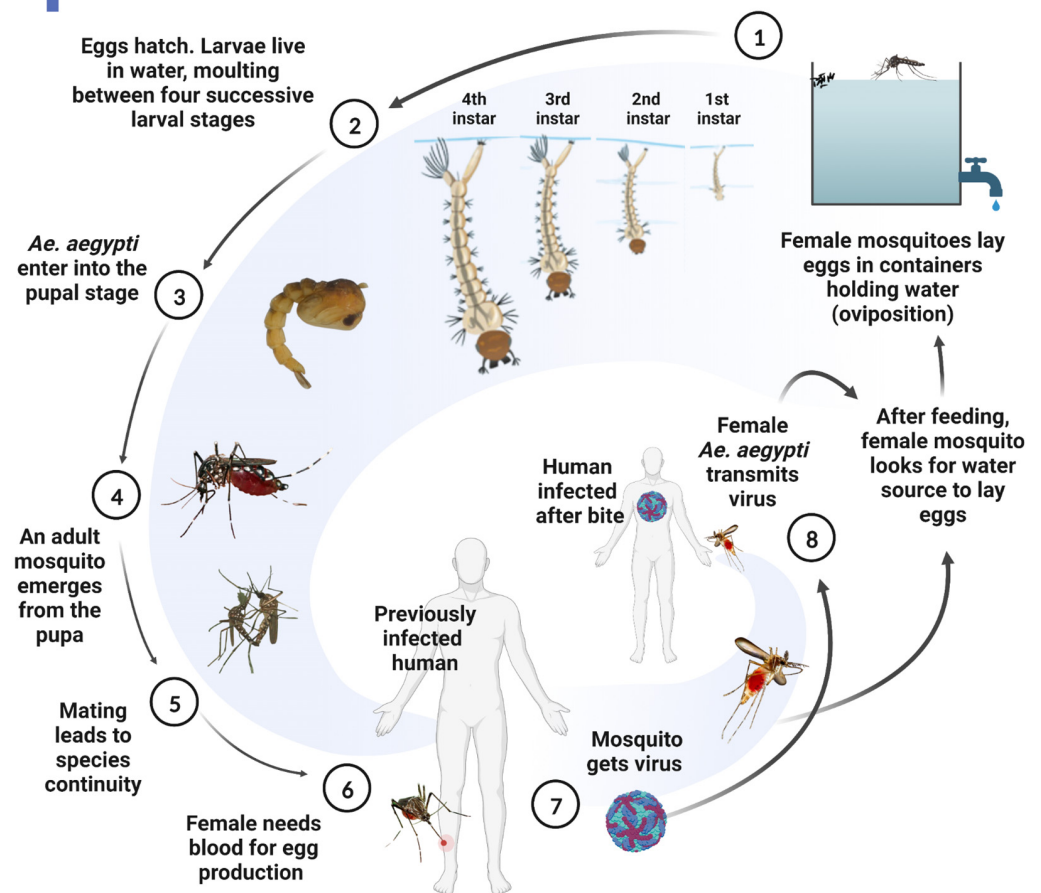


Figure 1. Life cycle of *Aedes aegypti* and its role in disease transmission. Figure created with Biorender.com.

Ae. aegypti eggs can hatch within minutes after coming into contact with water. Eggs also have been observed to remain viable for over a year in dry conditions (i.e., desiccation-resistant) [33]. As a result, water storage practices play a crucial role in controlling their breeding.

Vector control management targets different life stages, including (1) larval and pupal control, involving the stages at which the living organism is typically aquatic, and (2) adult control, involving the stage which is responsible for transmitting diseases. Figure 2 depicts a range of vector control management strategies aimed at different stages of the *Ae. aegypti* life cycle. These strategies include *chemical* methods (spraying chemicals, introducing chemicals to water containers), *biological* methods (release of transgenic vectors with reduced capacity to transmit disease and/or reproduce), and *environmental* approaches (environmental modification, environmental manipulation) [34,35]. When these methods are employed in combination, it constitutes an *integrated vector management approach* [35,36]. The control of *Ae. aegypti* has primarily relied on the use of chemicals, particularly pyrethroids [37]. This has led to the onset of mass insect resistance [38–40] through the following four primary mechanisms: behavioral resistance, reduced penetration/cuticular resistance, metabolic detoxification, and target site resistance [41]. Thus, while chemicals serve as a useful tool, they need to be used in moderation within targeted approaches that apply lesser quantities of chemicals to delay or reduce the rate at which insects build resistance. Insecticides and larvicides, even at sublethal doses, have been shown to affect the different mechanisms associated with *Ae. aegypti*'s ability to reproduce such as their fecundity [42–44], egg hatching [44], immature development [44–46], adult longevity [47], sex proportion [48], adult size [48], and blood feeding [49,50]. One such targeted approach to applying chemicals, as a component in an integrated management plan, would be to treat water storage containers in or around the home.

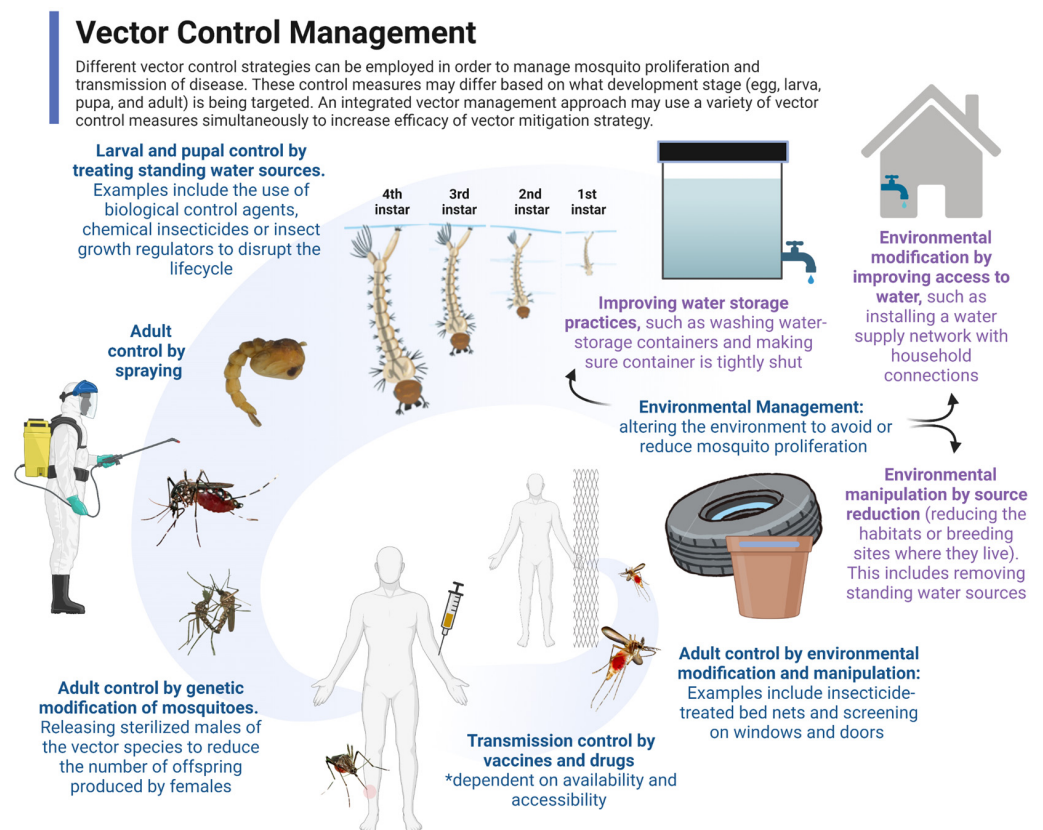


Figure 2. Vector control strategies that target different developmental phases of the *Aedes aegypti*. Vector control examples (biological, chemical, environmental management, and integrated practices) [35,51,52]. Figure created with Biorender.com.

In 2020, around one in four people lacked safely managed drinking water in their homes [53]. For regions experiencing water shortages and water stress, particularly within

communities without access to uninterrupted and reliable piped water connections [54,55], water storage within or around the home is a common coping strategy [56,57]; however, unsafe water storage practices, such as those illustrated in Figure 3, creates ideal breeding grounds for mosquitoes, leading to their proliferation [29,30,58–62] and consequently increasing the risk of contracting VBDs [60]. For this reason, it is important to implement proper water storage and hygiene practices, such as covering and/or treating containers, to reduce the risk of disease transmission [61]. Chemicals commonly used in HWS containers include temephos [63–68], diflubenzuron [69–74], pyriproxyfen [75–80], *Bacillus thuringiensis israelensis* (Bti) [81–83], Spinosad DT [69,84,85], and various plant oils [86,87]. The *Ae. aegypti*, among other species, have demonstrated resistance to many of these common insecticides currently employed in HWS containers, such as temephos, within the context of large vector-borne disease mitigation efforts [39,64,88–92]. Thus, in this study, we assess the efficacy of water disinfectants to mitigate larval growth, reduce adult mosquito emergence, and ensure water being stored is safe for human consumption. Since clean water access and VBD control are often interconnected, this approach can inform the design of interventions for water storage containers, such as improved point-of-use water treatment (POUWT) technologies that can address both issues simultaneously.



Figure 3. Water storage containers, such as buckets, drums, or jars, are commonly used to store and access water for daily use, particularly in settings with limited access to continuous water supply. Poor water storage practices can result in standing water, providing ideal conditions for mosquito-breeding sites. Addressing these issues typically falls under the umbrella of environmental management in integrated vector management. Photos captured by Sydney Turner in in Dzimali, South Africa, March of 2020, of water storage containers surrounding two different households. Standard sizes of water storage containers surrounding homes in these images typically range from 50 to 200 L, reflecting common household and community water storage practices in many regions.

This paper examines the larvicidal effects of water disinfectants commonly used in point-of-use water treatment (POUWT) technologies, on juvenile *Ae. aegypti* at concentrations within drinking water quality guidelines established by the U.S. Environmental Protection Agency (EPA) [93] and the World Health Organization (WHO) [94–96]. The study evaluates the effectiveness of sodium hypochlorite, silver nitrate, and copper sulfate pentahydrate for mosquito control while ensuring treated water remains safe for

human consumption. The findings provide insights for practical, integrated public health strategies that address both water quality and VBDs, enabling communities, governments, and organizations to implement effective, sustainable, and context-specific solutions. The study did find that elongated exposure to silver nitrate, copper sulfate pentahydrate, and sodium hypochlorite at these low concentrations negatively impacted juvenile *Ae. aegypti* development. While the goal was to achieve 100% inhibition of emergence, the study was constrained by the drinking water regulations, thus not permitting the use of chemicals at concentrations deemed unsafe for human consumption.

2. Materials and Methods

To analyze the efficacy of water disinfectants for mosquito larval source management of *Ae. aegypti*, the bioassay experiments in this study were designed to resemble the context of treating household water storage containers. This section describes the methods used to culture and rear *Ae. aegypti* in the laboratory (Section 2.1), select disinfectants for the bioassays and determine the test concentrations (Section 2.2), and test silver nitrate, copper sulfate pentahydrate, and sodium hypochlorite against *Ae. aegypti* larvae (Section 2.3).

2.1. Culturing and Rearing

Ae. aegypti eggs were obtained commercially from Benzon Research, Inc. (Carlisle, PA, USA). The colony, derived from the USDA “Gainesville” strain, has been continuously colonized at Benzon Research since 1994. Eggs procured from Benzon were 2–3 weeks old. The mosquitoes were reared in the Water Quality Laboratory at the University of Virginia on a 12:12 h light–dark cycle. The Extech RHT20 Humidity and Temperature Datalogger (Teledyne FLIR LLC, Nashua, NH, USA) was used to monitor the environmental conditions. The *Ae. aegypti* eggs and larvae were cultured at 27.9 ± 0.2 (82.2 °F) in Sterlite plastic trays (35.6 × 27.9 × 8.3 cm) containing deionized (DI) water. The larvae were fed daily with ground larval food, a 3:1 mixture of liver powder–brewer’s yeast (MP Biomedicals™, Solon, Ohio, USA). Five grams of this mixture was added to 400 mL water. The DI water was deoxygenated by adding 1/8 oz of the food slurry to the rearing trays. Twenty-four hours later, eggs attached to strips of paper were submerged into the trays. Larvae of an intended instar were collected for each experiment. No food was added to rearing trays on the day of the hatch. The larvae were fed 0.25, 0.5, and 1 oz on the first three days post hatching, respectively. After day 3, larvae feed between 1 and 1.5 oz/day until pupation.

2.2. Water Treatment Disinfectants

Ae. aegypti larvae were assessed against varying concentrations of silver nitrate, copper sulfate pentahydrate, and sodium hypochlorite. These disinfectants were selected for this study due to their widespread use in water treatment processes, particularly in point-of-use water treatment (POUWT) technologies commonly employed in resource-limited settings [97]. Chlorine, particularly in the form of sodium or calcium hypochlorite, is one of the most widely used disinfectants in water treatment processes worldwide due to its strong oxidizing properties which allow it to eliminate a broad range of common water-borne pathogens such as *Escherichia coli*, *Legionella*, *Salmonella* Typhi, *Shigella*, adenoviruses, norovirus, and rotaviruses [97–99]. While cost effectiveness and availability make chlorine an attractive option for water treatment interventions, the taste and odor of chlorine has made it less culturally relevant in certain contexts [100,101], as well as its production of harmful disinfection byproducts at high concentrations [102]. Thus, silver and copper have gained traction as effective alternatives in water treatment applications [97,103–113]. Silver has become increasingly prominent as a disinfecting agent in water purification systems because of its high efficacy against killing Gram-negative bacteria at very low concentra-

tions, as well as its minimal impact on the taste or odor of treated water [94,114,115]. While copper has been observed in many studies to be less potent than silver at similar concentrations, it has been demonstrated as effective against both Gram-negative and Gram-positive bacteria, copper's higher drinking water quality guideline allows for greater flexibility in dosing and copper is approximately ten times less expensive than silver [97,113,116]. Established microbial efficacy ranges reported in previous laboratory and field studies for chlorine, silver, and copper provided the rationale for testing these water disinfectants at similar ranges for larvicidal efficacy.

The concentrations of disinfectants used in this study were selected based on established drinking water quality guidelines (DWQGs) set by public health agencies: the WHO [94–96] and the US EPA [93]. The DWQG values, reported in Table 2, served as the reference points for the creation of a range of concentrations to test each disinfectant that would maintain safety for human consumption when applied in the context of household water storage containers. For free chlorine, 2 mg/L was selected as the upper boundary condition as it aligns with the WHO guideline for POUWT contexts [117]. The WHO DWQG was chosen over the EPA's maximum contaminant level goal (MCLG: the level of a contaminant in drinking water below which there is no known or expected risk to health) of 4 mg/L [93] as it is more conservative, ensuring broader applicability across diverse global contexts, including taste and odor thresholds. The EPA's MCLG of 1.3 mg/L for copper [93] was selected as the upper boundary instead of the higher 2 mg/L guideline set by the WHO [95]. For silver, both the EPA and WHO DWQG align on a threshold of 100 µg/L, which was used as the upper boundary [93,96].

Table 2. Drinking water quality guidelines for disinfectants (silver, copper, and chlorine) and the low, medium, and high concentrations tested in larvicidal bioassays.

Disinfectant	Drinking Water Quality Guideline (DWQG)	Concentrations Tested (µg/L)		
		High (80–95% of DWQG)	Mid (40–50% of DWQG)	Low (20–25% of DWQG)
Silver (Ag): AgNO ₃	EPA and WHO: 100 µg/L	20	40	80
Copper (Cu): CuSO ₄ ·5H ₂ O	EPA: 1300 µg/L WHO: 2000 µg/L	300	600	1200
Chlorine (OCl ⁻ /HOCl): NaOCl	EPA: 4000 µg/L(EPA) WHO: 2000 µg/L free chlorine dose for clear water (<10 NTU) and 4000 µg/L for turbid water (≥10 NTU) for POUWT	500	1000	2000

To avoid potential health effects associated with concentrations exceeding recommended DWQG for consumption (e.g., high levels of chlorine may cause eye and nose irritation and stomach discomfort) and to address social acceptability factors (e.g., chlorine taste thresholds in different populations, cost implications), a range of concentrations were tested, none of which were directly at the uppermost boundary identified from reviewing DWQG set by public health organizations. The concentration ranges selected for this study represent high-, mid-, and low-range dosing for water treatment. A mid-range concentration was chosen at approximately 40–50% of the DWQG. The lower range was determined by halving the selected mid-range value, which resulted in about 20–25% of the DWQG value, while the higher range was set at double the mid-range value, making it 80–95% of the DWQG value of the specified disinfectant. For example, the DWQG set by the United States EPA and WHO for silver is 100 µg/L and the concentrations tested in the present study represent 20%, 40%, and 80% of that DWQG. This approach aligns with the design

and operational practices of POUWT technologies, which aim to dose disinfectants at levels below the maximum DWQG to ensure safety while simultaneously producing sufficient disinfectant to effectively target microbial disinfection of waterborne pathogens.

2.2.1. Silver Nitrate

The stock solution was made by dissolving 16.99 g of AgNO_3 powder (Artcraft Chemicals, CAS No. 7761-88-8, South Glens Falls, NY, USA) into 1000 mL of DI water. One milliliter of this 100 mM AgNO_3 stock solution (10.79 g of silver in 1000 mL) was added to 1077 mL of DI water to make a 10 mg/L silver solution. Serial dilutions of the stock solution (10 mg/L to 1 mg/L to 0.1 mg/L) were performed to make test concentrations of 80 $\mu\text{g/L}$ (high dose), 40 $\mu\text{g/L}$ (mid dose), and 20 $\mu\text{g/L}$ (low dose). Nominal silver concentrations were confirmed by inductively coupled plasma mass spectrometric analysis (ICP-MS) using the Agilent 7900 ICP-MS instrument (Agilent, Santa Clara, CA, USA). Samples for the ICP-MS were prepared by adding 2% trace metal grade nitric acid (HNO_3 , Fisher Chemical, Fair Lawn, NJ, USA).

2.2.2. Copper Sulfate Pentahydrate

The 100 mM stock solution was prepared by dissolving 25.22 g of copper sulfate pentahydrate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, Alfa Aesar, Thermo Fisher Scientific, CAS No. 7758-99-8, purity: 99%, Waltham, MA, USA) into 1000 mL of deionized (DI) water. The 100 mM was diluted to 10 mM by transferring 10 mL of the stock solution into a 100 mL volumetric flask and filling to the mark with DI water. A 1 mM solution was prepared by transferring 10 mL of the 10 mM solution into another 100 mL volumetric flask and diluted with DI water. For a 10 μM solution of copper sulfate pentahydrate (equivalent to 2497 $\mu\text{g/L}$), 1 mL of the 1 mM solution was transferred into a 1000 mL volumetric flask and diluted to the mark with DI water. Further serial dilutions were performed to make test concentrations of 1200 $\mu\text{g/L}$ (high dose), 600 $\mu\text{g/L}$ (mid dose), and 300 $\mu\text{g/L}$ (low dose). The copper level was confirmed using the ICP-MS. Samples for the ICP-MS were prepared by adding 2% HNO_3 .

2.2.3. Sodium Hypochlorite

Free chlorine is an indicator of the overall chlorine concentration in the water available for disinfection and lethality. Sodium hypochlorite (NaOCl) is a powerful oxidizing agent that can react with water to release free chlorine. Free chlorine is in a solution as hypochlorous acid (HOCl) or hypochlorite ion (OCl^-). When sodium hypochlorite is added to water, the hydrolysis reaction occurs. These processes are represented in the following equations:



The dissociation of HOCl in water has an equilibrium constant, K_a , much smaller than 1, indicating only a small fraction of HOCl dissociates into H^+ and OCl^- , making it a weak acid.

$$K_a = \frac{[\text{H}^+][\text{OCl}^-]}{[\text{HOCl}]} = 3.5 \times 10^{-8} \quad (3)$$

where $[\text{H}^+]$ is the concentration of hydrogen ions, $[\text{OCl}^-]$ is the concentration of hypochlorite ions, and $[\text{HOCl}]$ is the concentration of undissociated hypochlorous acid. Depending on factors such as pH, temperature, and pressure, the ratio of OCl^- and HOCl will change. For instance, higher temperatures might slightly increase the dissociation of HOCl in water. At lower pH, HOCl dominates over OCl^- and is more effective as a disinfectant. Depending on the conditions, the resulting mixture of free chlorine, OCl^- , and HOCl reacts with

and disinfects microorganisms present in the water, making it safe for consumption or other uses.

The stock solution of sodium hypochlorite with 10–15% available chlorine was procured from Sigma-Aldrich (CAS No. 7681-52-9, St. Louis, MO, USA). Serial dilutions were performed to create a new stock solution at a concentration of 25 mg/L. Further serial dilutions to make free chlorine test concentrations of 0.5 mg/L (low dose), 1 mg/L (mid dose), and 2 mg/L (high dose) were performed directly before the start of an experiment. Concentrations of free chlorine were measured directly, utilizing the USEPA DPD Method 8021 for low-range free chlorine, before and after the addition of larvae, as well as at 4 and 8 h, using a HACH DR6000 spectrophotometer.

2.3. Survival Bioassays: Evaluation of Dose Response to Water Treatment Disinfectants

The main objective of the survival bioassays was to determine the optimum application of common water disinfectants silver, copper, and chlorine against juvenile *Ae. aegypti* to create recommendations for water treatment within household water storage containers for the purpose of vector control. Each experiment was conducted with three replicates of each test concentration and control. Each set of experiments was performed in triplicate on different days, using fresh solutions and batches of larvae each time to counter confounders in the bioassay. The resulting data, which included counts of larvae, pupae, and adult mosquitoes in each container, were subject to statistical analysis.

2.3.1. Experimental Setup

Experimental methods for the laboratory study were influenced primarily by WHO's *Guidelines for laboratory and field testing of mosquito larvicides* [118]. Per test concentration, 25 larvae were placed in aliquots of 200 mL prepared solution within a 250 mL beaker (3 replicates) for bioassays. Control beakers (three replicates) were filled with DI water. The larvae were transferred to test beakers by means of disposable transfer pipettes. The test beakers were held at the same environmental conditions described in Section 2.1: 27.9 ± 0.2 °C, photoperiod of 12L:12D.

Larval activity (surviving larvae, the emerging pupae, and adult mosquitoes) was recorded in both treated and control beakers. Observations were made every 24 h until completion of adult emergence in all treatment groups or until the experiment reached day 16 post exposure. Test and control beakers were covered with netting to prevent successfully emerged adults from escaping. The number of successfully emerged adults were confirmed by observations of the empty pupal cases left in the solution in the instance that an adult mosquito may have escaped. The water disinfectant's inhibitory effect on mosquito emergence was indicated by the observation of adult mosquitoes that failed to fully separate from their pupal cases and the presence of moribund or dead larvae and pupae. Larvae were considered moribund or dead when they did not move when their aquatic environment was disturbed and, furthermore, could not be induced to move even after being probed. Larvae matching this description were counted as dead larvae for calculating survival. To calculate mortality, any larvae that successfully completed their metamorphosis into adult mosquitoes were considered alive for the duration of the experiment.

This study examined two distinct larval stages: late third instar larvae and late first instar larvae. By testing these stages, the study aimed to illustrate two potential scenarios within the context of household water storage:

1. A mosquito deposits eggs in a water storage container, resulting in newly hatched larvae being exposed to a freshly applied disinfectant. This scenario evaluates whether

a POUWT technology introduced into the water storage container can effectively reduce the survival and emergence of newly hatched larvae.

2. Larvae are already present in the source water supplying the household water storage (HWS) system, allowing them to develop further before encountering the water storage container and disinfectant.

The older instar experiments took place over 16 days (when the control larvae reached full emergence and larvae in the treatment groups either emerged, died, or were moribund). If adult emergence in the control was less than 80%, the test was discarded and repeated. For the younger instar experiments, larvicidal data were collected for 3 days.

The older larvae were fed after observations on day 4, and the younger instar larvae were fed after observations on day 2. Two drops of the slurry were added per test beaker at each feeding, which occurred every other day until the experiment was terminated. While delaying the feeding, as described above, is suboptimal for typical lab-grown *Ae. aegypti* growth rates as observed in the literature, this particular food regiment was chosen for this particular study for two main reasons: (1) to simulate the scenario when the water within the household water storage container has been disinfected/safe to drink, and (2) to decrease the confounding variable of the water treatment disinfectant interacting with the food slurry (e.g., volatility of the chlorine, interactions between silver or copper ions and brewer's yeast or liver powder that could affect toxicity).

2.3.2. Data Analysis

Larvicidal activity was calculated using the WHO's bioassay protocol [118] and methods established in Ngonzi et al. [119]. Data from all replicates in an experiment were pooled for analysis. Where the emergence was between 80% and 95%, mortality was calculated using Abbott's (1925) formula [120].

$$\text{Survival (\%)} = 100 - ((C - T)/C * 100) \quad (4)$$

where C = percentage survival in the untreated control and T = percentage survival in the treated sample. Larvae that developed into successfully emerging adults was expressed in terms of emergence:

$$\text{Emergence (\%)} = 100 - ((C - T)/C * 100) \quad (5)$$

where C = percentage emergence in the untreated control and T = percentage emergence in the treated sample. Inhibition of emergence (IE) was calculated on the basis of the number of larvae exposed. IE% is calculated using the following formula:

$$\text{Inhibition of Emergence (IE\%)} = 100 - (T * 100)/C \quad (6)$$

where T = percentage emergence in treated batches and C = percentage emergence in the control. Abbot's correction was also applied when appropriate according to the WHO guidelines for laboratory testing of mosquito larvicides [118].

All statistical analyses were performed using R (version 4.2.2) and RStudio (2022.07.2 Build 576). To assess the dose response of silver, copper, and chlorine on the number of larvae, pupae, and emerged adult mosquitoes, the sample mean, standard deviation (SD), and standard error of mean (SEM) were calculated on the observed data.

Probit analysis, a method first published in *Science* by Chester Ittner Bliss in 1934 [121] and then popularized by the work of Finney [122], is commonly used in toxicology in order to analyze the relationship between a dose and a response [123]. To investigate the impact of the treatments on the probability that a larva survives and/or has emerged, a mixed-effect

probit regression model was fit to the data in R. The response variable was the classification of percent survival or percent emergence, while the independent variables were time (days), dosage, and their interaction. A natural spline with two degrees of freedom was applied to time to account for non-linearity in the rate of change per day. Each experiment was treated as a random effect. The function *glmer* (which stands for “generalized linear mixed effects regression”), from the *lme4* package, was employed to produce predictive models for each of the treatments. The function *emmeans* (which stands for “estimated marginal means”) was used to perform pairwise comparisons of the estimated means.

For the inhibition of emergence predictive model, day 16 post exposure values for the control were obtained from the emergence model. Bootstrapping was used to generate a confidence interval using the upper and lower limits of the 95% confidence interval for emergence. The *bootMer* (which stands for “bootstrap for mixed effects models”) function simulated 100 samples based on the bounds of the model.

The probit regression curves presented in the analysis represent the probability of the binary response variable taking on the value of 1 (“alive” or “has emerged”) as a function of the predictor variables with a 95% confidence interval. Thus, the probit regression model predicts the probability of survival or emergence for the juvenile *Ae. aegypti* over the course of the experiment. Within the analysis in this paper, extrapolation of the inhibition of emergence model beyond the experimental range of this study was performed to estimate the conditions required to achieve a predicted 100% inhibition of emergence by day 16. This estimation was used to assess whether the required concentration would remain within drinking water quality guidelines. Further experimentation would be necessary to validate these predictions and confirm whether 100% inhibition can be achieved under these conditions.

3. Results and Discussion

Within this study, testing larvae at different life stages allowed the researchers to understand if susceptibility of the larvae to the water disinfectants’ effects was dependent on the age of the larvae as reflected in previous studies [124–126]. For older instar experiments, survival and emergence data are presented over a 16-day post exposure period. For the younger larvae, results reflect survival over the course of 72 h of exposure to the treatments.

3.1. Late Third Instar Experiments

This section reports the survival and emergence results for the experiments conducted on late third instar *Ae. aegypti* utilizing silver nitrate, copper sulfate pentahydrate, and sodium hypochlorite treatments. Observed data (summarized in Table 3) serve as input for probit regression models. Observed data results are expressed in terms of Percentage Mean \pm SEM, and the model data results (summarized in Table 4) are expressed in terms of Predicted Probability Mean [Upper 95% Confidence Interval, Lower 95% Confidence Interval].

3.1.1. Silver Nitrate Exposure to Older Instar

Introducing silver nitrate into the aquatic environment of juvenile *Ae. aegypti* negatively impacted their growth and development at all concentrations tested (20, 40, and 80 $\mu\text{g/L}$ Ag) when compared to the growth and development of the control larvae by the end of the experimental period ($p_{\text{control}} < 0.001$). Within the environmental conditions tested, silver nitrate treatment shows great potential to inhibit emergence. The data reflected a dose–response relationship: 80 $\mu\text{g/L}$ achieved the highest inhibition of emergence of $88.66 \pm 5.70\%$ on day 16 of exposure, followed by 40 $\mu\text{g/L}$ at $81.56 \pm 3.39\%$ and 20 $\mu\text{g/L}$ at $72.40 \pm 4.13\%$. Emergence and survival values are comparable, suggesting that

most of the larvae that survived the treatment emerged into adult mosquitoes during the experimental period.

Table 3. Observed data for silver nitrate (AgNO₃) treatments of 20, 40, and 80 µg/L; copper sulfate pentahydrate (CuSO₄·5H₂O) treatments of 300, 600, and 1200 µg/L; and for sodium hypochlorite (NaOCl) treatments of 0.5, 1, and 2 mg/L free chlorine (OCl⁻/HOCl). Standard deviation (St. dev) and standard error of mean (SEM) is presented with the mean percentage survival, emergence percentage, and inhibition of emergence (IE) percentage of older instar *Ae. aegypti* larvae on day 16 post exposure. Data are corrected with Abbot’s formula (1925).

Observed Data												
Silver Treatment	20 µg/L Ag			40 µg/L Ag			80 µg/L Ag			Control		
Variable	Mean	St. Dev	SEM	Mean	St. Dev	SEM	Mean	St. Dev	SEM	Mean	St. Dev	SEM
Survival (%)	26.85	6.25	3.61	17.09	4.58	2.64	10.75	8.64	4.99	91.11	0.77	0.44
Emergence (%)	25.01	6.44	3.72	16.69	5.24	3.02	10.29	8.94	5.16	90.67	1.33	0.77
IE (%)	72.40	7.16	4.13	81.56	5.86	3.39	88.66	9.87	5.70			
Copper Treatment	300 µg/L Cu			600 µg/L Cu			1200 µg/L Cu			Control		
Variable	Mean	St. Dev	SEM	Mean	St. Dev	SEM	Mean	St. Dev	SEM	Mean	St. Dev	SEM
Survival (%)	42.55	7.13	4.12	25.80	10.91	6.30	11.92	7.50	4.33	89.78	0.77	0.44
Emergence (%)	40.30	5.38	3.11	24.88	9.94	5.74	11.44	7.51	4.34	89.33	0.00	0.00
IE (%)	54.89	6.02	3.48	72.15	11.12	6.42	87.19	8.41	4.86			
Chlorine Treatment	500 µg/L Free Cl			1000 µg/L Free Cl			2000 µg/L Free Cl			Control		
Variable	Mean	St. Dev	SEM	Mean	St. Dev	SEM	Mean	St. Dev	SEM	Mean	St. Dev	SEM
Survival (%)	58.40	21.88	12.63	24.75	12.85	7.42	17.39	14.70	8.49	90.67	2.67	1.54
Emergence (%)	56.53	20.75	11.98	24.98	12.99	7.50	17.59	14.95	8.63	89.78	2.78	1.60
IE (%)	37.44	21.35	12.32	72.46	13.77	7.95	80.65	16.04	9.26			

Table 4. Predicted mean probability of survival and predicted probability of emergence of older instar *Aedes aegypti* on day 16 after contact with silver nitrate (AgNO₃) treatments of 20, 40, and 80 µg/L; copper sulfate pentahydrate (CuSO₄·5H₂O) treatments of 300, 600, and 1200 µg/L; and for sodium hypochlorite (NaOCl) treatments of 500, 1000, and 2000 µg/L free chlorine (OCl⁻/HOCl). Modeled inhibition of emergence (IE) percentage produced with the probit regression model for emergence. Data are corrected with Abbot’s formula (1925).

Probit Regression Model								
Silver Treatment	20 µg/L Ag		40 µg/L Ag		80 µg/L Ag		Control	
Survival (%)	23.57 [18.10, 28.57]		15.91 [11.49, 20.01]		11.21 [8.25, 14.29]		91.39 [87.75, 94.15]	
Emergence (%)	21.04 [15.48, 29.37]		14.10 [8.79, 20.55]		8.20 [4.81, 11.96]		92.84 [88.22, 95.93]	
IE (%)	78.96 [71.87, 85.19]		85.90 [78.13, 90.51]		91.80 [86.72, 94.84]			
Copper Treatment	300 µg/L Cu		600 µg/L Cu		1200 µg/L Cu		Control	
Survival (%)	42.56 [34.02, 53.57]		27.50 [19.17, 37.88]		12.52 [7.05, 20.42]		90.52 [84.18, 94.76]	
Emergence (%)	42.65 [36.46, 49.15]		24.43 [18.71, 30.17]		11.77 [7.96, 15.68]		93.36 [90.36, 95.58]	
IE (%)	57.35 [49.48, 64.47]		75.57 [70.91, 82.82]		88.23 [84.47, 91.90]			
Chlorine Treatment	500 µg/L Free Cl		1000 µg/L Free Cl		2000 µg/L Free Cl		Control	
Survival (%)	57.53 [8.14, 17.24]		19.77 [14.32, 26.56]		12.19 [7.64, 18.30]		91.63 [86.98, 94.90]	
Emergence (%)	58.34 [45.76, 69.53]		21.57 [14.00, 34.51]		13.73 [7.55, 21.99]		92.85 [86.58, 96.58]	
IE (%)	41.66 [31.12, 54.55]		78.43 [67.94, 86.38]		86.27 [79.95, 92.99]			

Figure 4 depicts the experimental data for survival with curves fitted by probit regression overlaid, illustrating the relationship between the model and observed experimental data. The probit regression curves for probability of survival across the different treatments are plotted together on a single graph in Figure 5. The model predicted that 11.21% [8.25, 14.29] of the juvenile *Ae. aegypti* treated with 80 µg/L survived by day 16, followed by 15.91% [11.49, 20.01] at 40 µg/L, and 23.57% [18.10, 28.57] at 20 µg/L.

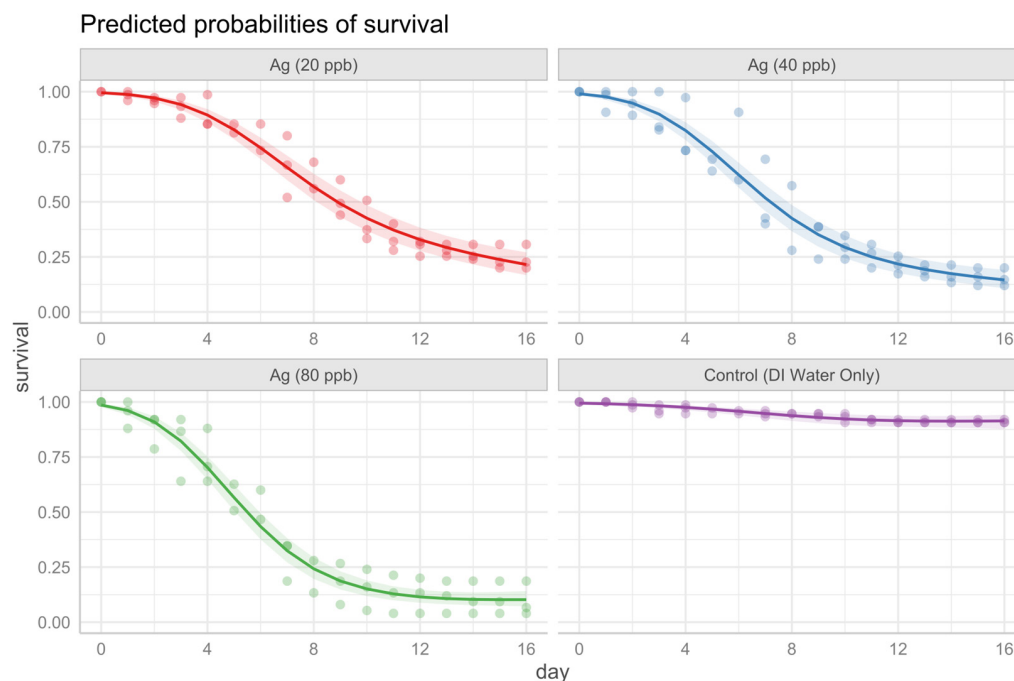


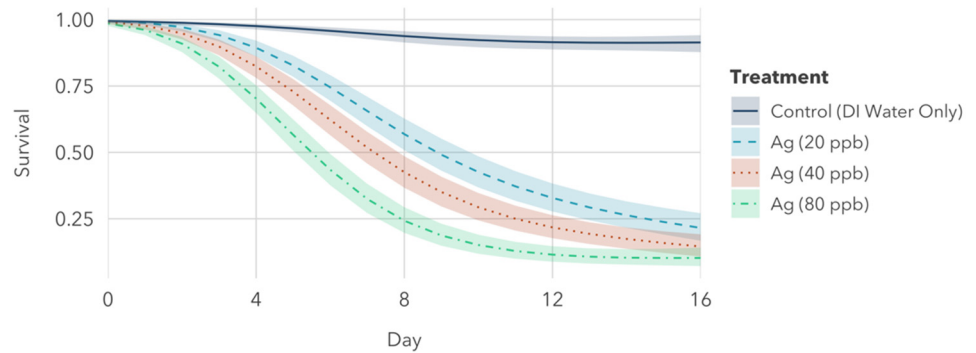
Figure 4. The predicted probabilities of survival of older instar *Ae. aegypti* during the experimental period for larvae treated with silver nitrate (20, 40, and 80 µg/L) and controls. The shaded areas represent the 95% confidence interval.

As seen in Figure 5, there is a clear indication that the final proportion of individuals that have emerged into adult mosquitoes by day 16 in the treatment groups is significantly lower than that of the control ($p_{\text{Ctrl-20Ag}} \leq 0.001$, $p_{\text{Ctrl-40Ag}} \leq 0.001$, $p_{\text{Ctrl-80Ag}} \leq 0.001$). The visual interpretation of the model shows separation between all treatment groups from the control by day 7, with the first four days of the experiment characterized by minimal emergence in the controls. While the predicted probability of emergence for the controls by day 16 was 92.84% [88.22, 95.91], the emergence predicted for larvae in water containing 20, 40, and 80 µg/L Ag was 21.04% [15.48, 29.37], 14.10% [8.79, 20.55], and 8.20% [4.81, 11.96], respectively. In the model, the 20 µg/L and 40 µg/L Ag treatments were found to be significantly different [$p_{40\text{Ag}-20\text{Ag}} = 0.032$] for the predicted probability that a larva has emerged by day 16.

While the ultimate objective of vector control is to achieve a 100% inhibition of emergence (IE% = 100%), excessive use of disinfectants beyond what is necessary to achieve this goal may lead to diminishing returns in terms of cost-effectiveness and human health; thus, it is important to balance the concentration of the chemical used with the desired outcome. Depicted in Figure 6, extrapolation of the IE% model outside of this study's experimental range suggests that 100% inhibition of emergence on day 16 would occur at a concentration of 117.32 µg/L Ag, which is slightly greater than the drinking water quality guideline.

A) Predicted probabilities of survival

Silver concentrations



B) Predicted probabilities of emergence

Silver concentrations

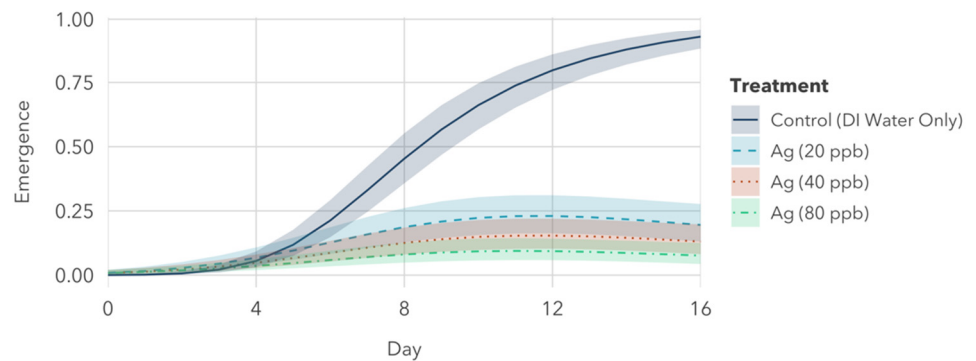


Figure 5. Predicted probabilities of (A) survival of *Ae. aegypti* larvae exposed to 20, 40, and 80 µg/L of silver nitrate and (B) emergence of *Ae. aegypti* larvae into adult mosquitoes following exposure to these varying concentrations of silver nitrate. Shaded areas indicate the 95% confidence interval.

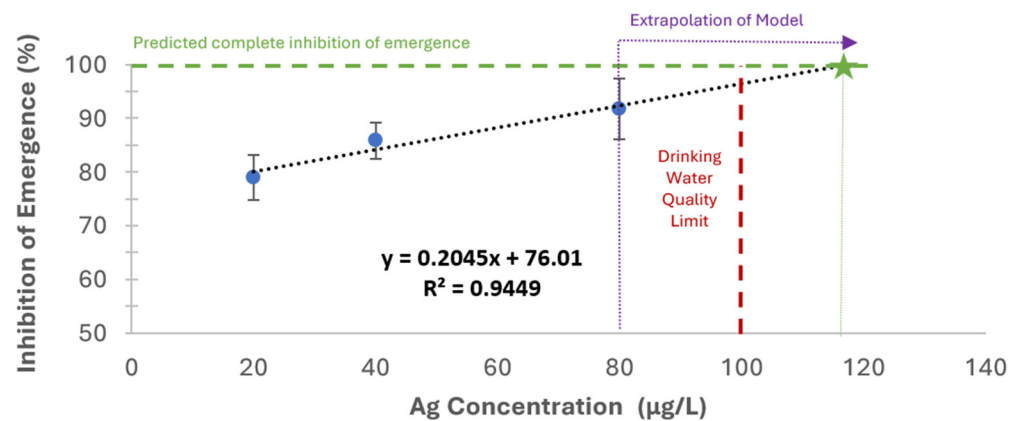


Figure 6. The modeled inhibition of emergence of *Ae. aegypti* larvae on day 16 of exposure to silver nitrate, with concentrations of 20, 40, and 80 µg/L Ag. Model error bars represent a 95% confidence interval.

To validate this prediction, more experiments would need to be conducted within a wider range of silver concentrations, including those concentrations not safe for human consumption, to validate whether linear regression is the most appropriate model for this relationship between concentration of the larvicide and percent inhibition of emergence.

Prolonged exposure of the larvae to higher concentrations of the water disinfectant may lead to compound effects (e.g., threshold toxicity concentration reached for the larvae or cumulative developmental effects over the duration of the experiment) that may cause the relationship to be more non-linear in fit.

3.1.2. Copper Sulfate Pentahydrate Exposure to Older Instar

All copper concentrations tested (300, 600, and 1200 µg/L Cu) resulted in decreased survival and emergence of juvenile *Ae. aegypti* compared to the control group by the end of the experimental period ($p_{\text{control}} < 0.001$), as reported in Table 3. The control group exhibited a survival and emergence of roughly 90%, while those that came into contact with 300 µg/L Cu had a survival and emergence of only $42.55 \pm 4.12\%$ and $40.30 \pm 5.38\%$, respectively. Increasing the dose to 1200 µg/L resulted in a more drastic reduction in both the emergence and survival of the *Ae. aegypti*, with on average $11.44 \pm 4.34\%$ having emerged by day 16 and $11.92 \pm 4.33\%$ probability of survival. A dose response is reflected within the inhibition of emergence data, with 300, 600, and 1200 µg/L Cu, resulting in IE% on day 16 of $54.89 \pm 3.48\%$, $72.15 \pm 6.42\%$, and $87.19 \pm 4.86\%$, respectively.

The probit model for the predicted probability of survival of juvenile *Ae. aegypti* in the presence of different concentrations of copper sulfate pentahydrate and the predicted probability that a larva has emerged is illustrated in Figure 7. When compared to the control group on day 16, the predicted survival and emergence of *Ae. aegypti* exposed to copper treatments was significantly lower at all concentrations tested ($p_{\text{ctrl-300Cu}} \leq 0.001$, $p_{\text{ctrl-600Cu}} \leq 0.001$, $p_{\text{ctrl-1200Cu}} \leq 0.001$). As was observed with silver nitrate, a dose response is evident in emergence with copper sulfate ($p_{600\text{Cu}-300\text{Cu}} \leq 0.001$, $p_{1200\text{Cu}-600\text{Cu}} \leq 0.001$, $p_{1200\text{Cu}-300\text{Cu}} \leq 0.001$).

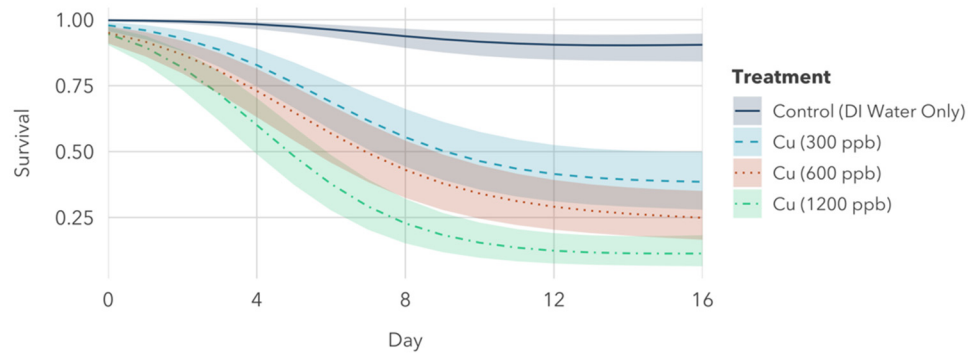
The probit regression model was applied to create a model for inhibition of emergence, depicted in Figure 8. At the conclusion of the experimental period, this model predicts that 1200 µg/L concentration results in an 88.23% [84.47, 91.90] inhibition of emergence, which is roughly 1.5× more effective than the 300 µg/L treatment (IE%: 57.35% [49.48, 64.47]); however, the concentration is four times greater. From the linear regression model which establishes a relationship between concentration of copper and percentage inhibition of emergence, and through extrapolation, it is predicted that 100% inhibition of emergence could potentially occur at roughly 1500 µg/L Cu. This concentration is over the drinking water quality standard set by the EPA, but does not surpass the guideline set by the WHO. The linear regression line appears to underestimate the inhibition of emergence potential of the 600 µg/L Cu treatment, which may indicate that there is a non-linear relationship between the concentration of the water disinfectant and percent inhibition of emergence at these low concentrations after a prolonged exposure to the treatment.

3.1.3. Sodium Hypochlorite Exposure to Older Instar

The results indicate that all concentrations of free chlorine tested (500, 1000, and 2000 µg/L) adversely affected the development and survival of the *Ae. aegypti* when compared to the control group ($p_{\text{Ctrl-500Cl}} \leq 0.001$, $p_{\text{Ctrl-1000Cl}} \leq 0.001$, $p_{\text{Ctrl-2000Cl}} \leq 0.001$), as reported in Table 3. The control group survival and emergence were both near 90% by day 16, while the treatment groups of 500, 1000, and 2000 µg/L free chlorine caused survival to drop down to $58.40 \pm 12.63\%$, $24.75 \pm 7.42\%$, and $17.39 \pm 8.49\%$, respectively. Inhibition of emergence for free chlorine concentrations on day 16 for 500, 1000, and 2000 µg/L were $37.44 \pm 12.32\%$, $72.46 \pm 7.95\%$, and 80.65 ± 9.26 , respectively.

A) Predicted probabilities of survival

Copper concentrations



B) Predicted probabilities of emergence

Copper concentrations

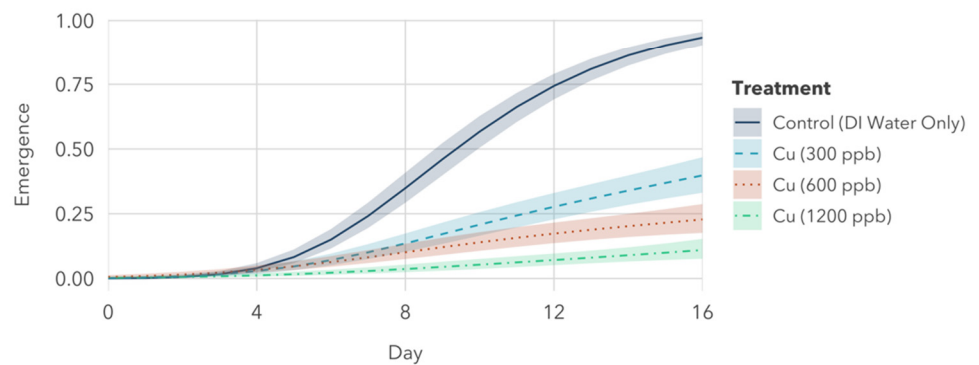


Figure 7. Predicted probabilities (A) of survival and (B) that *Ae. aegypti* larvae have emerged into adult mosquitoes after being exposed to copper sulfate pentahydrate at concentrations of 300, 600, and 1200 µg/L. Shaded areas indicate the 95% confidence interval.

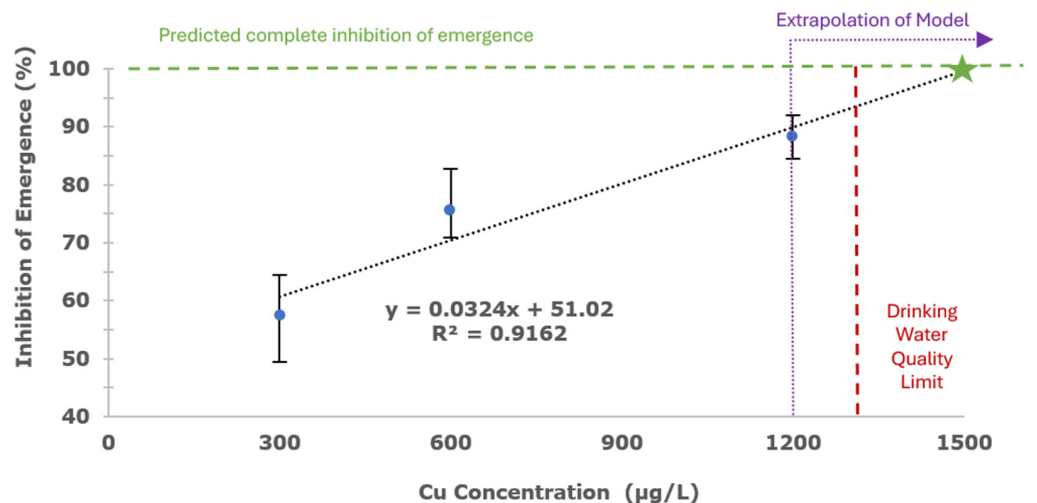


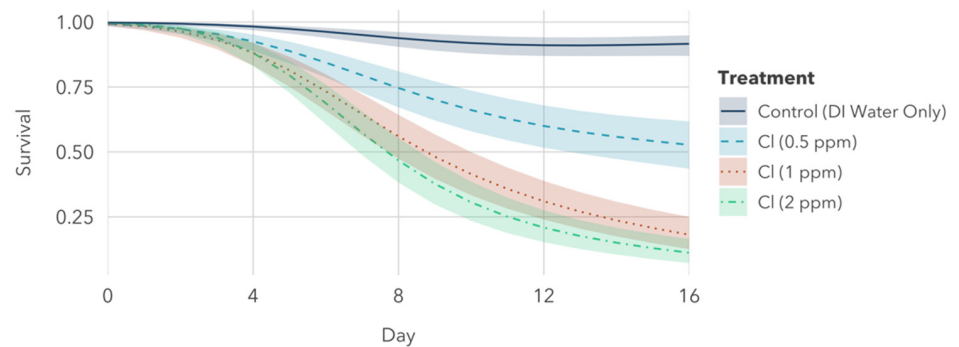
Figure 8. The modeled inhibition of emergence of *Ae. aegypti* larvae on day 16 of exposure to copper sulfate pentahydrate, with concentrations of 300, 600, and 1200 µg/L Cu. Observed error bars represent a 95% confidence interval.

The probit model that predicts the survival of juvenile *Ae. aegypti* after they have encountered a single dose of free chlorine of concentration 500, 1000, and 2000 µg/L

is illustrated in Figure 9. Similar to the response observed with the other two water disinfectants, a dose response was evident with free chlorine, with predicted survival probabilities of 57.53% [8.14, 17.24], 19.77% [14.32, 26.56], and 12.19% [7.64, 18.30] at the end of the observational period for 500, 1000, and 2000 $\mu\text{g}/\text{L}$, respectively. Similarly, the chlorine treatment decreased the larvae's ability to reach adulthood ($p_{\text{Ctrl-500Cl}} \leq 0.001$, $p_{\text{Ctrl-1000Cl}} \leq 0.001$, $p_{\text{Ctrl-2000Cl}} \leq 0.001$). As the concentration of free chlorine increased, the predicted probabilities that a larva has emerged decreased: 58.34% [45.76, 69.53] for 500 $\mu\text{g}/\text{L}$, 21.57% [14.00, 34.51] for 1000 $\mu\text{g}/\text{L}$, and 13.73 [7.55, 21.99] for 2000 $\mu\text{g}/\text{L}$. These results suggest that chlorine has a dose-dependent effect on the development of *Ae. aegypti* ($p_{500\text{Cl-1000Cl}} \leq 0.001$, $p_{2000\text{Cl-1000Cl}} = 0.008$, $p_{2000\text{Cl-500Cl}} \leq 0.001$). Depicted in Figure 9, the model also portrays that while a single dose of 500 $\mu\text{g}/\text{L}$ free chlorine reduced emergence, the treatments of 1000 and 2000 $\mu\text{g}/\text{L}$ were roughly twice as effective.

A) Predicted probabilities of survival

Chlorine concentrations



B) Predicted probabilities of emergence

Chlorine concentrations

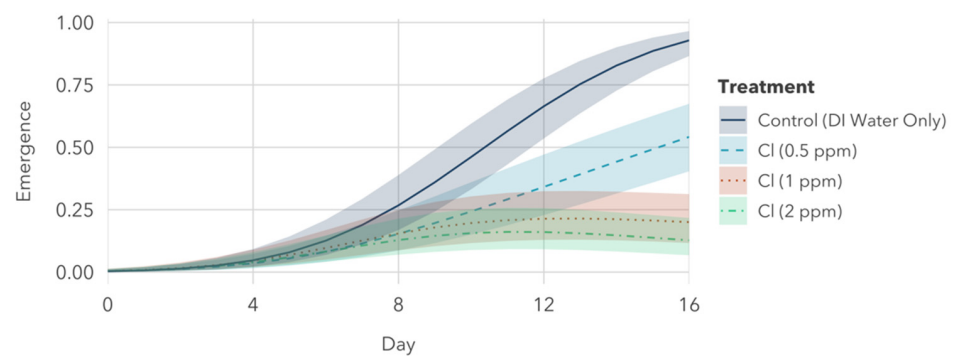


Figure 9. Predicted probabilities of (A) survival of *Ae. aegypti* larvae in contact with chlorine and (B) emergence of *Ae. aegypti* larvae in contact with chlorine at concentrations of 500, 1000, and 2000 $\mu\text{g}/\text{L}$ of free chlorine.

When considering the other treatments tested in this study (silver and copper), there is greater variability in the chlorine data for larval emergence, as indicated by the larger standard deviation in the observed data and the wider confidence interval in the model. Three possible explanations for this variation include the following:

1. The volatility of chlorine, which makes it less stable compared to silver and copper during long exposure periods, especially in elevated temperatures that can increase chlorine evaporation;

2. Differences in the rate of free chlorine consumption within each beaker, potentially caused by larvae, external contamination sources, or variations in pH and temperature;
3. The ratio of hypochlorous acid (HOCl) to hypochlorite ion (OCl⁻), as HOCl is the more effective disinfectant of the two forms and may also exhibit stronger larvicidal effects (ratio is influenced by the pH of the solution; higher pH values correspond to a greater concentration of OCl⁻).

These factors lead to different contact times and forms of chlorine exposure for the larvae, depending on the specific environmental conditions in each beaker.

The relationship between free chlorine concentration and the inhibition of *Ae. aegypti* emergence is shown in Figure 10. Data extrapolation suggests that a free chlorine concentration of approximately 2.3 mg/L would completely inhibit larval emergence under the tested environmental conditions. The linear model for inhibition of emergence underestimates the potential for the 1 mg/L free chlorine treatment, indicating that prolonged larval exposure may not follow a strictly linear relationship.

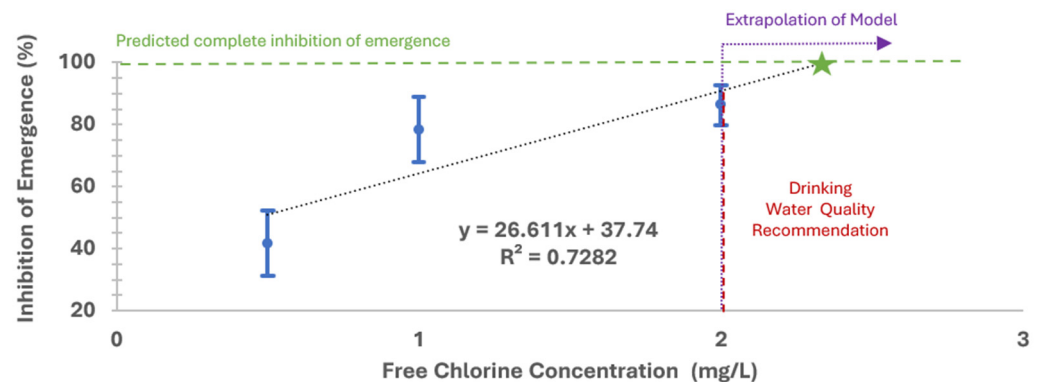


Figure 10. The modeled inhibition of emergence of *Ae. aegypti* larvae on day 16 of exposure to copper sulfate, with concentrations of 0.5, 1, and 2 mg/L free chlorine. Observed error bars represent a 95% confidence interval.

3.2. Younger Instar Experiments

This section reports the results for the experiments conducted on late first instar *Ae. aegypti* utilizing silver nitrate, copper sulfate, and sodium hypochlorite treatments. Each of the following sections will start with observed data (summarized in Table 5), which will serve as input for probit regression models (represented in Table 6). Significant differences will then be examined through analysis of the models. Observed data results are expressed in terms of Percentage Mean \pm SEM, and the model data results are expressed in terms of Predicted Probability Mean (UCI_{95%}, LCI_{95%}).

3.2.1. Silver Nitrate Exposure to Younger Instar

Silver nitrate was effective against first instar *Ae. aegypti*, contributing to the observed $57.73 \pm 7.10\%$, $36.44 \pm 11.23\%$, and $7.95 \pm 6.41\%$ survival of the larvae after 72 h of exposure to 20, 40, and 80 $\mu\text{g/L}$ treatments, respectively. Table 5 presents the observed survival of larvae after 24, 48, and 72 h exposure to the silver nitrate treatments. The probit regression model was built using the observed data as the input. Table 6 and Figure 11 present the predicted probability of survival models. By 72 h, each of the treatments performed significantly differently from each other ($p_{40\text{Ag}-20\text{Ag}} \leq 0.001$, $p_{80\text{Ag}-40\text{Ag}} \leq 0.001$, $p_{80\text{Ag}-20\text{Ag}} \leq 0.001$), with the highest concentration of silver nitrate (80 $\mu\text{g/L}$) leading to the greatest mortality.

Table 5. Observed data for silver nitrate (AgNO₃) treatments of 20, 40, and 80 µg/L, copper sulfate pentahydrate (CuSO₄·5H₂O) treatments of 300, 600, and 1200 µg/L, and sodium hypochlorite (NaOCl) treatments of 500, 1000, and 2000 µg/L. Standard deviation (St. dev) and standard error of mean (SEM) is presented with the mean percentage survival of younger instar *Ae. aegypti* larvae after 24, 48, and 72 h of exposure. Data are corrected with Abbot’s formula (1925).

Silver Treatment	20 µg/L Ag			40 µg/L Ag			80 µg/L Ag			Control		
Time (h)	Mean	St. Dev	SEM	Mean	St. Dev	SEM	Mean	St. Dev	SEM	Mean	St. Dev	SEM
24	92.78	4.47	2.58	89.70	1.92	1.11	86.06	7.46	4.31	99.11	1.54	0.89
48	81.13	11.95	6.90	65.55	2.36	1.36	54.11	15.69	9.06	94.22	2.04	1.18
72	57.73	12.30	7.10	36.44	19.45	11.23	7.95	11.10	6.41	82.22	2.04	1.18
Copper Treatment	300 µg/L Cu			600 µg/L Cu			1200 µg/L Cu			Control		
Time (h)	Mean	St. Dev	SEM	Mean	St. Dev	SEM	Mean	St. Dev	SEM	Mean	St. Dev	SEM
24	82.59	14.96	8.64	79.47	14.77	8.53	68.31	24.64	14.23	99.11	1.54	0.89
48	57.24	25.06	14.47	49.21	25.64	14.80	32.41	33.08	19.10	94.22	2.04	1.18
72	21.85	23.74	13.71	17.71	12.43	7.18	12.81	15.23	8.79	82.22	2.04	1.18
Chlorine Treatment	500 µg/L Free Cl			1000 µg/L Free Cl			2000 µg/L Free Cl			Control		
Time (h)	Mean	St. Dev	SEM	Mean	St. Dev	SEM	Mean	St. Dev	SEM	Mean	St. Dev	SEM
24	76.67	6.55	3.78	72.56	12.53	7.23	36.91	11.13	6.43	93.78	5.39	3.11
48	25.67	3.79	2.19	14.72	17.20	9.93	6.13	5.36	3.10	81.33	4.00	2.31
72	15.40	1.20	0.69	8.60	11.93	6.89	0.00	0.00	0.00	75.11	3.36	1.94

Table 6. Predicted mean probability of survival (%) of younger instar *Aedes aegypti* after 24, 48, and 72 h of exposure to silver nitrate treatments of 20 µg/L, 40 µg/L, and 80 µg/L; copper sulfate pentahydrate treatments of 300 µg/L, 600 µg/L, and 1200 µg/L; and sodium hypochlorite (NaOCl) treatments of 500, 1000, and 2000 µg/L. Data corrected by Abbot’s formula (1925).

Silver Treatment	20 µg/L Ag		40 µg/L Ag		80 µg/L Ag		Control	
t = 24 h	94.75 [91.50, 96.92]		91.51 [87.19, 94.63]		89.85 [85.30, 93.27]		99.24 [97.52, 99.81]	
t = 48 h	79.20 [72.29, 84.97]		63.89 [55.72, 71.47]		50.09 [41.91, 58.26]		94.24 [90.01, 96.91]	
t = 72 h	58.66 [49.92, 66.99]		36.87 [28.85, 45.52]		8.38 [4.90, 13.45]		82.43 [75.48, 87.98]	
Copper Treatment	300 µg/L Cu		600 µg/L Cu		1200 µg/L Cu		Control	
t = 24 h	88.71 [75.01, 95.97]		85.46 [69.89, 94.42]		74.39 [54.91, 88.25]		99.42 [96.88, 99.93]	
t = 48 h	53.40 [32.80, 73.11]		44.86 [25.47, 65.60]		26.94 [12.57, 46.72]		95.02 [85.93, 98.66]	
t = 72 h	20.67 [8.66, 39.21]		16.82 [6.59, 33.90]		11.38 [3.92, 25.69]		83.63 [66.76, 93.64]	
Chlorine Treatment	500 µg/L Free Cl		1000 µg/L Free Cl		2000 µg/L Free Cl		Control	
t = 24 h	77.29 [71.79, 82.13]		72.55 [66.53, 77.97]		41.02 [35.19, 47.05]		94.55 [91.65, 96.59]	
t = 48 h	25.37 [20.28, 31.05]		14.57 [10.53, 19.56]		3.34 [2.02, 5.29]		80.39 [75.26, 84.82]	
t = 72 h	15.24 [11.04, 20.38]		8.92 [5.75, 13.23]		0.80 [0.18, 2.87]		75.44 [69.54, 80.66]	

3.2.2. Copper Sulfate Pentahydrate Exposure to Younger Instar

The results suggest that copper sulfate pentahydrate was effective in controlling first instar *Ae. aegypti*. The larvae’s survival rates after exposure to 300, 600, and 1200 µg/L treatments for 72 h were 21.85 ± 13.71%, 17.71 ± 7.18%, and 12.81 ± 8.79%, respectively. The predicted probability of survival, presented in Figure 12, for first instar *Ae. aegypti* exposed to 300, 600, and 1200 µg/L treatments for 72 h were 20.67% [8.66, 39.21], 16.82% [6.59, 33.90], and 11.38% [3.92, 25.69], respectively. By the end of the experimental period, all treatments

performed similarly in terms of producing larvicidal effects ($p_{\text{CuTreatments-Control}} \leq 0.001$; $p_{600\text{Cu}-300\text{Cu}} = 0.744$, $p_{1200\text{Cu}-600\text{Cu}} = 0.430$, $p_{1200\text{Cu}-300\text{Cu}} = 0.122$).

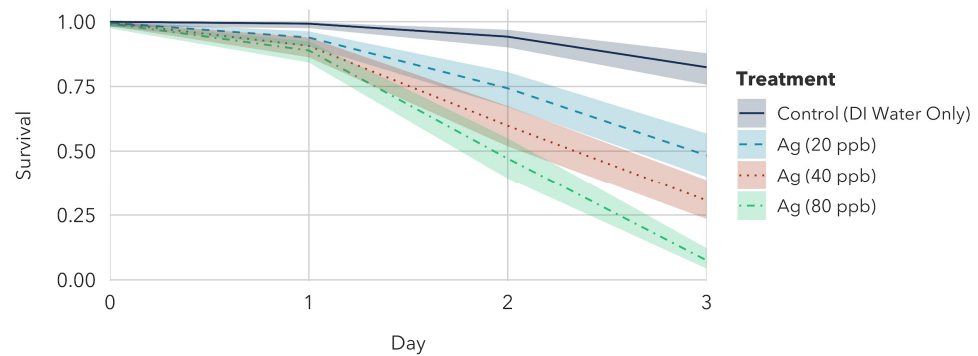


Figure 11. Predicted probabilities of survival of *Ae. aegypti* larvae in contact with silver nitrate concentrations of 20, 40, and 80 $\mu\text{g}/\text{L}$ Ag.

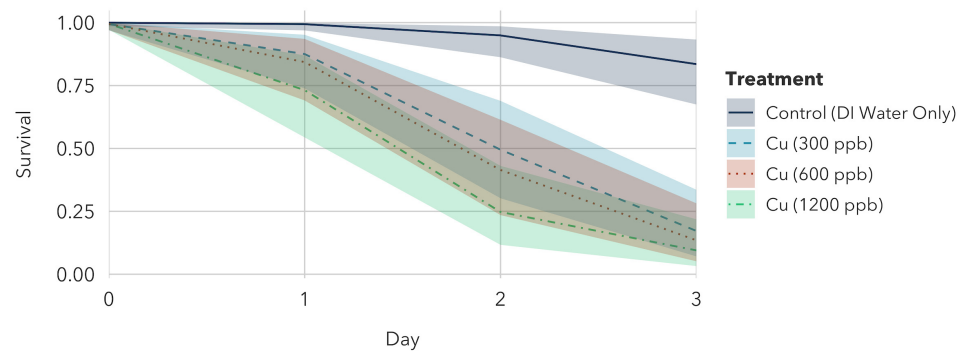


Figure 12. Predicted probabilities of survival of *Ae. aegypti* larvae in contact with copper sulfate pentahydrate concentrations of 300, 600, and 1200 $\mu\text{g}/\text{L}$ Cu.

3.2.3. Sodium Hypochlorite Exposure to Younger Instar

This study finds that sodium hypochlorite shows great efficacy in managing young *Ae. aegypti* larvae. After being subjected to treatments of 500, 1000, and 2000 $\mu\text{g}/\text{L}$ for 72 h, the larvae exhibited survival rates of $15.40 \pm 0.69\%$, $8.60 \pm 6.89\%$, and $0 \pm 0\%$, respectively. The survival rates of the larvae following exposure to the sodium hypochlorite treatments for 24, 48, and 72 h are shown in Table 5. As illustrated in Figure 13, the first instar *Ae. aegypti* that were exposed to 500, 1000, and 2000 $\mu\text{g}/\text{L}$ free Cl treatments for 72 h have a predicted probability of survival of 15.24% [11.04, 20.38], 8.92% [5.75, 13.23], and 0.80% [0.18, 2.87], respectively. While the 500 $\mu\text{g}/\text{L}$ and 1000 $\mu\text{g}/\text{L}$ free chlorine treatments were not statistically significant on day 3 ($p_{1000\text{Cl}-500\text{Cl}} = 0.161$), both treatments were statistically different from the better performance of the 2000 $\mu\text{g}/\text{L}$ treatment ($p_{(2000\text{Cl}-500\text{Cl})} \leq 0.001$; $p_{(2000\text{Cl}-1000\text{Cl})} \leq 0.001$).

3.3. Comparing and Contextualizing the Results

To compare differences across water disinfectants, Figure 14 provides a summary of the model generated IE% data for older instar on day 16. Based on the model output, it was determined that within the low dosage range, the treatment of 20 $\mu\text{g}/\text{L}$ Ag was notably more effective than both the 300 $\mu\text{g}/\text{L}$ Cu treatment and the 500 $\mu\text{g}/\text{L}$ free Cl treatment. The interpretation of the model data also demonstrated that as the concentrations of the water disinfectants increased into the mid-range and high range, the differences in efficacy between the various treatments became less pronounced. The analysis also considered the statistical significance across the treatments in order to determine if significant dose

responses were occurring. The key findings of this analysis are summarized below for each of the three disinfectants for the late third instar *Ae. aegypti*:

- Silver nitrate treatments: The 80 µg/L Ag treatment was significantly different from the 20 µg/L treatment, but not significantly different from the 40 µg/L treatment.
- Copper sulfate pentahydrate treatments: The model found that all copper treatments were statistically significant from each other.
- Free chlorine treatments: The 500 µg/L free chlorine treatment was statistically significant from the 1000 µg/L and 2000 µg/L treatments; however, the 1000 µg/L and 2000 µg/L treatments were not statistically different from each other.

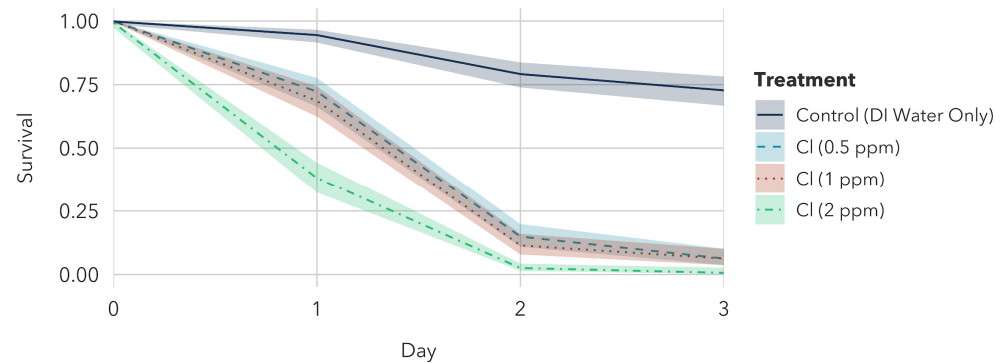


Figure 13. Predicted probabilities of survival of *Ae. aegypti* larvae in contact with chlorine at concentrations of 500, 1000, and 2000 µg/L of free chlorine.

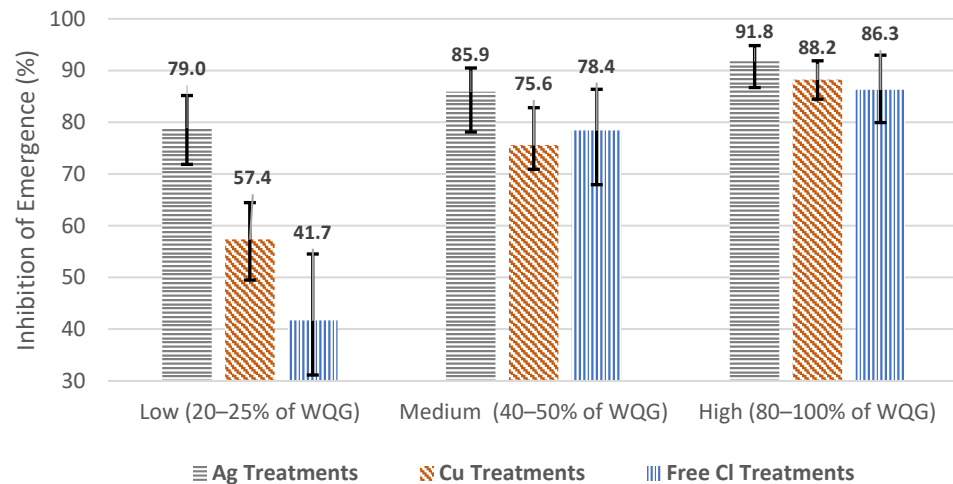


Figure 14. Summary of predicted probabilities of inhibition of emergence results regarding the efficacy of water disinfectants as larval control for older instar *Ae. aegypti*. IE% calculated from the model generated data from day 16. Error bars represent the 95% confidence interval.

While the study design revealed that the first instar larvae were highly vulnerable to all of the water disinfectants, the treatment that yielded the best results was 2000 µg/L Cl, with no larvae surviving the 72 h exposure period. Figure 15 displays the summary of the predicted probability of survival of the late first instar *Ae. aegypti* after 72 h of exposure to the water disinfectants. The relatively large error bars representing the 95% confidence interval in Figure 15 reflect the biological variability inherent in working with live mosquito larvae, particularly at concentrations that require extended time to achieve lethal effects. Each batch of *Ae. aegypti* mosquitoes may exhibit slight differences in susceptibility, as evidenced by variability seen even in the controls. This can be expected at such young and vulnerable stages and may suggest that *Ae. aegypti* egg batches tested were not equally fit or there were minor inconsistencies in environmental conditions or handling.

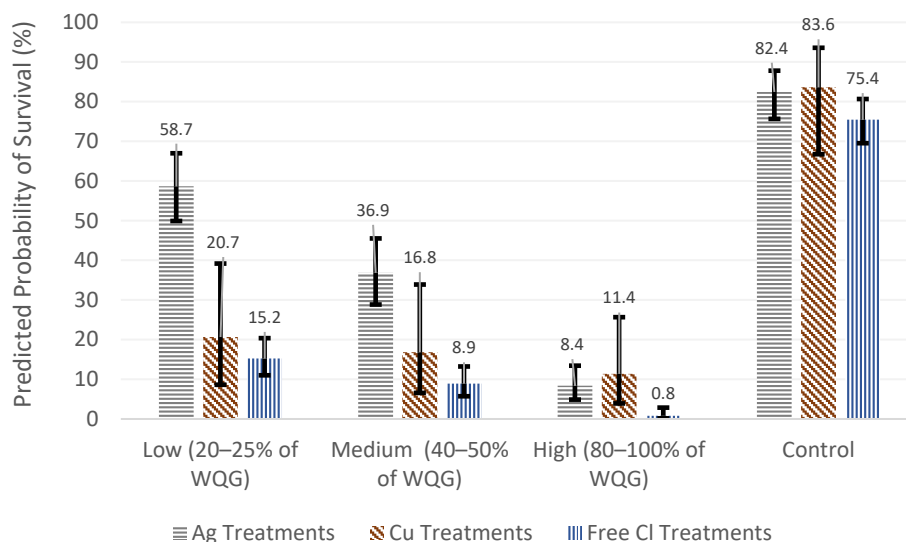


Figure 15. Summary of predicted probability of survival at 72 h regarding the efficacy of water disinfectants as larval control for younger instar *Ae. aegypti*. Error bars represent the 95% confidence interval.

The results of this study are consistent with previous research demonstrating that small aquatic invertebrates (relevant to the egg and larval stages of mosquito development) experience toxicity to metals silver and copper [127–138] and to various formulations of chlorine [139–142].

The effectiveness of these water disinfectants as vector control agents is highly dependent on contact time with the larvae and the developmental stage at which the larvae encounter the disinfectant. This has been observed in other studies examining mosquito larvae development in metal stressed environments [127,128,135] and chlorinated environments [139,140]. For example, a study utilizing household bleach with 5.25% sodium hypochlorite as the active ingredient found that the lethal dose for first instar *Ae. aegypti* larvae in the presence of food was 16 mg/L, whereas the lethal dose for third and fourth instar larvae jumped up to 250 mg/L [141], illustrating the significant impact of instar age on treatment efficacy. Building on the concept that age at which mosquito larvae come into contact with a disinfectant matters, a study that dosed first instar *Ae. aegypti* larvae with a copper sulfate treatment of 1250 µg/L observed that most mortality occurred during the first instar stage. Larvae that reached the second instar were generally able to progress to the pupal stage, suggesting mosquitoes are particularly sensitive to metal exposure as first instar. The findings also suggest the concept that surviving larvae rapidly develop tolerance to metal exposure [127]. In another study examining the effects of copper sulfate on third instar *Ae. aegypti*, researchers found that exposure to the disinfectant at concentrations of 1500 and 15,000 µg/L reduced the survival of *Ae. aegypti* during the immature stages, specifically citing that the development of *Ae. aegypti* was impaired both in the transition from larvae to pupae and from pupae to adult; however, in that study, the larvae were exposed to copper sulfate for only 24 h, which may explain why larvicidal effects were not observed at concentrations below 1500 µg/L [134]. In contrast, the larvicidal effects presented in this paper reflect the cumulative effects of prolonged exposure to the disinfectant as the larvae are in contact with the disinfectant during the duration of the observation period.

Many prior studies investigating the effects of silver, copper, and chlorine on mosquitoes did not specifically focus on the application of these chemicals in treating water storage containers for both vector control and drinking water purposes; thus, higher

concentrations of the chemicals were often used in these studies yielding increases in larvicidal efficacy in shorter exposure periods [130,131,138,141]. These studies typically aimed to determine LC₅₀ or LC₉₀ values, which represent the concentration of a chemical required to kill 50% or 90% of the exposed population, respectively, within a period of 24 to 48 h. In contrast, the experiments presented in this paper involved observing older instar larvae over a 16-day exposure period and younger instar larvae over a 72 h exposure period. For example, one study found that the LC₅₀ for copper sulfate at 24 h was 33 mg/L for late third instar *Ae. Aegypti*, which is over 25 times that of EPA's drinking water standard for copper. Within the same study, researchers observed 7% mortality at 3200 µg/L and 1% mortality at 320 µg/L after 24 h [132]. For comparison, in the experiments described in this paper, we observed a $6.6 \pm 4.0\%$ and $5.8 \pm 3.2\%$ mortality at 24 h for third instar *Ae. aegypti* exposed to 1200 µg/L and 300 µg/L, respectively. The discrepancy in the mortality outcomes between the two studies can likely be attributed to differences in environmental conditions, such as the use of tap water versus deionized (DI) water; *Ae. aegypti* strains; culturing methods; and feeding protocols.

Studies considering these water disinfectants have also tested various mosquito species such as the *Ae. albopictus*, *Anopheles (An.) subpictus*, *An. quadrimaculatus*, *An. farauti*, *Culex (Cx.) quinquefasciatus*, and *Cx. pipiens* [128,130,131,133,136–138,140,142]. Among the studies considering other species, some included test concentrations of the disinfectants at levels considered safe for human consumption. In one study, first instar larvae of *Ae. albopictus*, *Cx. quinquefasciatus*, and *Cx. pipiens* were exposed to copper sulfate at concentrations between 1100 and 10,000 µg/L, with *Cx. pipiens* found to be the most sensitive to the treatments, highlighting that different mosquito species exhibit varying levels of sensitivity to the same treatments. Focusing on the most affected species, *Cx. pipiens*, at 3300 µg/L, 50% of larvae died within 24 h, whereas no significant mortality was observed at 1100 µg/L with the same contact time; however, after 72 h of exposure, approximately 50% mortality was recorded at 1100 µg/L, and 80% mortality was observed at 3300 µg/L [136]. Comparing these results with our study, at 24 h, approximately 30% mortality was observed in first instar *Ae. aegypti*, and at 72 h, roughly 87% mortality was recorded at 1200 µg/L.

The mechanisms of action underlying the developmental effects of silver, copper, and chlorine treatments on mosquito larvae reveal a complex interplay of physiological and biochemical disruptions, with many details still largely understudied. Sodium hypochlorite treatments have been observed to significantly prolong the larval development period, with adverse effects on the integument (the outer covering of the larvae) and abnormalities in the siphon (the breathing tube). Many treated larvae exhibited an inability to shed their exoskeleton, ultimately failing to complete the metamorphosis process, suggesting that hypochlorite disrupts key developmental processes essential for molting and metamorphosis [140]. For copper, one proposed mechanism of action involves its negative impact on the larval gut microbiota, with some studies suggesting that this can lead to gut dysfunction, impairing nutrient absorption. This, in turn, could reduce the amount of energy available for larval development, including molt, metamorphosis, and adult development [113,114,120,121]. Other mechanisms of action for the effects of copper on mosquito larvae include direct toxicity to the larvae, interference with physiological processes such as respiration or ion transport, or disruption of key enzymes or proteins involved in development [143]. The mechanisms of action underlying the effects of silver nitrate on mosquito larvae are not well understood. While silver's antimicrobial properties suggest potential disruptions in cellular processes, specific studies detailing its effects on mosquito larval development are lacking.

4. Conclusions

The presence of water storage containers near and within households provides an opportunity for mosquitoes to breed in close proximity to humans, thus increasing the risk for the spread of vector-borne diseases. The increase in cases and burden of mosquito-borne diseases has been exacerbated by the emergence of resistance to common chemical interventions, such as temephos, currently utilized in large global mosquito mitigation efforts. As a result, there is a need for innovative and creative approaches to manage and control mosquito populations, including the treatment of water storage containers. This research seeks to provide communities, organizations, and governments with guidance regarding chemical water treatment alternatives that may serve as viable options for addressing both vector control and water treatment management. The findings of this laboratory study suggest that using water disinfectants in water storage containers can serve two purposes simultaneously—making the water safe to drink and preventing the proliferation of disease-carrying mosquitoes.

In this study, alternative methods for treating water storage containers were examined, focusing on the use of silver nitrate, copper sulfate pentahydrate, and sodium hypochlorite. These treatments, tested at concentrations deemed safe for human consumption, were assessed based on their ability to reduce the survival of juvenile *Ae. aegypti* and prevent the emergence of larvae into adult mosquitoes capable of disease transmission. The findings demonstrate that low concentrations of commonly used water disinfectants exhibit significant larvicidal effects, offering a viable option for treating water storage containers. While the ideal vector control management approach would involve rapidly and completely eliminating all larvae, this study represents an initial step toward understanding the potential for water treatment disinfectants as a viable solution. Future work could explore the use of these treatments in tandem with other strategies to achieve the ultimate goal of comprehensive larval elimination.

By addressing two important issues simultaneously, public health projects that incorporate this approach may be more appealing to funding agencies and donors who prioritize a holistic approach to health and environmental concerns. Additionally, the use of water treatment chemicals for vector control can be seen as a cost-effective strategy for controlling disease transmission, as it targets the mosquito larvae at the source, which may be more efficient than other control methods. Other necessary ingredients for a successful integrated vector management approach include integrated vector and disease surveillance, education to promote community awareness, social mobilization (e.g., commitment from governments and community engagement), and a multisectoral approach.

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References

- World Health Organization (WHO). *Global Vector Control Response 2017–2030*; World Health Organization: Geneva, Switzerland, 2017; ISBN 978-92-4-151297-8.
- World Health Organization (WHO). Vector-Borne Diseases. Available online: <https://www.who.int/news-room/fact-sheets/detail/vector-borne-diseases> (accessed on 3 March 2022).
- Rocklöv, J.; Dubrow, R. Climate Change: An Enduring Challenge for Vector-Borne Disease Prevention and Control. *Nat. Immunol.* **2020**, *21*, 479–483. [[CrossRef](#)]
- El-Sayed, A.; Kamel, M. Climatic Changes and Their Role in Emergence and Re-Emergence of Diseases. *Environ. Sci. Pollut. Res.* **2020**, *27*, 22336–22352. [[CrossRef](#)] [[PubMed](#)]
- Githeko, A.K.; Lindsay, S.W.; Confalonieri, U.E.; Patz, J.A. Climate Change and Vector-Borne Diseases: A Regional Analysis. *Bull. World Health Organ.* **2000**, *78*, 1136–1147.
- Boisson, S.; Wohlgemuth, L.; Yajima, A.; Peralta, G.; Obiageli, N.; Matendechero, S.; Baayenda, G.; Seife, F.; Hamilton, H.; Chase, C.; et al. Building on a Decade of Progress in Water, Sanitation and Hygiene to Control, Eliminate and Eradicate Neglected Tropical Diseases. *Trans. R. Soc. Trop. Med. Hyg.* **2021**, *115*, 185–187. [[CrossRef](#)] [[PubMed](#)]
- Tidman, R.; Abela-Ridder, B.; de Castañeda, R.R. The Impact of Climate Change on Neglected Tropical Diseases: A Systematic Review. *Trans. R. Soc. Trop. Med. Hyg.* **2021**, *115*, 147–168. [[CrossRef](#)] [[PubMed](#)]
- Fazeli-Dinan, M.; Nikookar, S.H.; Azarnoosh, M.; Jafari, A.; Daneshpour, E.; Enayati, A.; Zaim, M. An Overview of Different Control Methods of Invasive *Aedes Aegypti* and *Aedes Albopictus*. *J. Maz. Univ. Med. Sci.* **2024**, *34*, 260–286.
- Facchinelli, L.; Badolo, A.; McCall, P.J. Biology and Behaviour of *Aedes Aegypti* in the Human Environment: Opportunities for Vector Control of Arbovirus Transmission. *Viruses* **2023**, *15*, 636. [[CrossRef](#)]
- Wilson, A.L.; Courtenay, O.; Kelly-Hope, L.A.; Scott, T.W.; Takken, W.; Torr, S.J.; Lindsay, S.W. The Importance of Vector Control for the Control and Elimination of Vector-Borne Diseases. *PLoS Negl. Trop. Dis.* **2020**, *14*, e0007831. [[CrossRef](#)]
- World Health Organization (WHO). *Dengue Guidelines, for Diagnosis, Treatment, Prevention and Control*; World Health Organization: Geneva, Switzerland, 2009; ISBN 978-92-4-154787-1.
- de Almeida, J.P.; Aguiar, E.R.; Armache, J.N.; Olmo, R.P.; Marques, J.T. The Virome of Vector Mosquitoes. *Curr. Opin. Virol.* **2021**, *49*, 7–12. [[CrossRef](#)]
- European Centre for Disease Prevention and Control *Aedes Aegypti*-Factsheet for Experts. Available online: <https://www.ecdc.europa.eu/en/disease-vectors/facts/mosquito-factsheets/aedes-aegypti> (accessed on 6 February 2023).
- Leta, S.; Beyene, T.J.; De Clercq, E.M.; Amenu, K.; Revie, C.W.; Kraemer, M.U.G. Global Risk Mapping for Major Diseases Transmitted by *Aedes Aegypti* and *Aedes Albopictus*. *Int. J. Infect. Dis. IJID Off. Publ. Int. Soc. Infect. Dis.* **2018**, *67*, 25–35. [[CrossRef](#)]
- World Health Organization (WHO). Chikungunya Fact Sheet. Available online: <https://www.who.int/news-room/fact-sheets/detail/chikungunya> (accessed on 13 May 2024).
- U.S. Food and Drug Administration FDA Approves First Vaccine to Prevent Disease Caused by Chikungunya Virus. Available online: <https://www.fda.gov/news-events/press-announcements/fda-approves-first-vaccine-prevent-disease-caused-chikungunya-virus> (accessed on 13 May 2024).
- Costa, L.B.; Barreto, F.K.d.A.; Barreto, M.C.A.; dos Santos, T.H.P.; de Andrade, M.d.M.O.; Farias, L.A.B.G.; de Freitas, A.R.R.; Martinez, M.J.; Cavalcanti, L.P.d.G. Epidemiology and Economic Burden of Chikungunya: A Systematic Literature Review. *Trop. Med. Infect. Dis.* **2023**, *8*, 301. [[CrossRef](#)]
- World Health Organization Dengue and Severe Dengue. Available online: <https://www.who.int/news-room/fact-sheets/detail/dengue-and-severe-dengue> (accessed on 15 May 2024).
- Yang, X.; Quam, M.B.M.; Zhang, T.; Sang, S. Global Burden for Dengue and the Evolving Pattern in the Past 30 Years. *J. Travel. Med.* **2021**, *28*, taab146. [[CrossRef](#)]
- World Health Organization. Yellow Fever. Available online: <https://www.who.int/news-room/fact-sheets/detail/yellow-fever> (accessed on 15 May 2024).
- Gaythorpe, K.A.; Hamlet, A.; Jean, K.; Garkauskas Ramos, D.; Cibrelus, L.; Garske, T.; Ferguson, N. The Global Burden of Yellow Fever. *eLife* **2021**, *10*, e64670. [[CrossRef](#)]
- United States Food and Drug Administration (FDA) Yellow Fever Vaccine: YF-Vax. Available online: <https://www.fda.gov/vaccines-blood-biologics/vaccines/yf-vax> (accessed on 15 May 2024).
- Centers for Disease Control and Prevention (CDC). What We Know About Zika. Available online: <https://www.cdc.gov/zika/about/index.html> (accessed on 15 May 2024).
- Guo, Z.; Jing, W.; Liu, J.; Liu, M. The Global Trends and Regional Differences in Incidence of Zika Virus Infection and Implications for Zika Virus Infection Prevention. *PLoS Negl. Trop. Dis.* **2022**, *16*, e0010812. [[CrossRef](#)]

25. World Health Organization. Countries and Territories with Current or Previous Zika Virus Transmission (Data as of Feb 2022). Available online: <https://www.who.int/publications/m/item/countries-and-territories-with-current-or-previous-zika-virus-transmission> (accessed on 13 May 2024).
26. McGregor, B.L.; Connelly, C.R. A Review of the Control of *Aedes Aegypti* (Diptera: Culicidae) in the Continental United States. *J. Med. Entomol.* **2021**, *58*, 10–25. [[CrossRef](#)]
27. Roiz, D.; Wilson, A.L.; Scott, T.W.; Fonseca, D.M.; Jourdain, F.; Müller, P.; Velayudhan, R.; Corbel, V. Integrated *Aedes* Management for the Control of *Aedes*-Borne Diseases. *PLoS Negl. Trop. Dis.* **2018**, *12*, e0006845. [[CrossRef](#)]
28. Smith, D.L.; Perkins, T.A.; Reiner, R.C.; Barker, C.M.; Niu, T.; Chaves, L.F.; Ellis, A.M.; George, D.B.; Le Menach, A.; Pulliam, J.R.C.; et al. Recasting the Theory of Mosquito-Borne Pathogen Transmission Dynamics and Control. *Trans. R. Soc. Trop. Med. Hyg.* **2014**, *108*, 185–197. [[CrossRef](#)]
29. Jansen, C.C.; Beebe, N.W. The Dengue Vector *Aedes Aegypti*: What Comes Next. *Microbes Infect.* **2010**, *12*, 272–279. [[CrossRef](#)]
30. Carvalho, F.D.; Moreira, L.A. Why Is *Aedes Aegypti* Linnaeus so Successful as a Species? *Neotrop. Entomol.* **2017**, *46*, 243–255. [[CrossRef](#)]
31. Pruszyński, C.A.; Stenn, T.; Acevedo, C.; Leal, A.L.; Burkett-Cadena, N.D. Human Blood Feeding by *Aedes Aegypti* (Diptera: Culicidae) in the Florida Keys and a Review of the Literature. *J. Med. Entomol.* **2020**, *57*, 1640–1647. [[CrossRef](#)]
32. Christophers, S.R. *Aedes Aegypti: The Yellow Fever Mosquito: Its Life History, Bionomics and Structure*; Cambridge University Press: Cambridge, MA, USA, 1960; ISBN 978-0-521-04638-1.
33. Faull, K.J.; Williams, C.R. Intraspecific Variation in Desiccation Survival Time of *Aedes Aegypti* (L.) Mosquito Eggs of Australian Origin. *J. Vector Ecol.* **2015**, *40*, 292–300. [[CrossRef](#)] [[PubMed](#)]
34. Lima, E.P.; Goulart, M.O.F.; Rolim Neto, M.L. Meta-Analysis of Studies on Chemical, Physical and Biological Agents in the Control of *Aedes Aegypti*. *BMC Public Health* **2015**, *15*, 858. [[CrossRef](#)] [[PubMed](#)]
35. Erlanger, T.E.; Keiser, J.; Utzinger, J. Effect of Dengue Vector Control Interventions on Entomological Parameters in Developing Countries: A Systematic Review and Meta-Analysis. *Med. Vet. Entomol.* **2008**, *22*, 203–221. [[CrossRef](#)]
36. Barrera, R.; Harris, A.; Hemme, R.R.; Felix, G.; Nazario, N.; Muñoz-Jordan, J.L.; Rodriguez, D.; Miranda, J.; Soto, E.; Martinez, S.; et al. Citywide Control of *Aedes Aegypti* (Diptera: Culicidae) during the 2016 Zika Epidemic by Integrating Community Awareness, Education, Source Reduction, Larvicides, and Mass Mosquito Trapping. *J. Med. Entomol.* **2019**, *56*, 1033–1046. [[CrossRef](#)]
37. van den Berg, H.; da Silva Bezerra, H.S.; Al-Eryani, S.; Chanda, E.; Nagpal, B.N.; Knox, T.B.; Velayudhan, R.; Yadav, R.S. Recent Trends in Global Insecticide Use for Disease Vector Control and Potential Implications for Resistance Management. *Sci. Rep.* **2021**, *11*, 23867. [[CrossRef](#)]
38. Metcalf, R.L. Insect Resistance to Insecticides. *Pestic. Sci.* **1989**, *26*, 333–358. [[CrossRef](#)]
39. Dusfour, I.; Vontas, J.; David, J.-P.; Weetman, D.; Fonseca, D.M.; Corbel, V.; Raghavendra, K.; Coulibaly, M.B.; Martins, A.J.; Kasai, S.; et al. Management of Insecticide Resistance in the Major *Aedes* Vectors of Arboviruses: Advances and Challenges. *PLoS Negl. Trop. Dis.* **2019**, *13*, e0007615. [[CrossRef](#)]
40. Guedes, R.N.C.; Beins, K.; Navarro Costa, D.; Coelho, G.E.; Bezerra, H.S.d.S. Patterns of Insecticide Resistance in *Aedes Aegypti*: Meta-Analyses of Surveys in Latin America and the Caribbean. *Pest Manag. Sci.* **2020**, *76*, 2144–2157. [[CrossRef](#)]
41. Bharati, M.; Saha, D. Insecticide Resistance Status and Biochemical Mechanisms Involved in *Aedes* Mosquitoes: A Scoping Review. *Asian Pac. J. Trop. Med.* **2021**, *14*, 52. [[CrossRef](#)]
42. Antonio, G.E.; Sánchez, D.; Williams, T.; Marina, C.F. Paradoxical Effects of Sublethal Exposure to the Naturally Derived Insecticide Spinosad in the Dengue Vector Mosquito, *Aedes Aegypti*. *Pest Manag. Sci.* **2009**, *65*, 323–326. [[CrossRef](#)]
43. Sulaiman, S.; Fadhila, K.; Hidayatulfathi, O. Evaluation of Pyrethrin Formulations on Dengue/Dengue Haemorrhagic Fever Vectors in the Laboratory and Sublethal Effects. *J. Arthropod-Borne Dis.* **2007**, *1*, 1–6.
44. Shaalan, E.A.-S.; Canyon, D.V.; Younes, M.W.F.; Abdel-Wahab, H.; Mansour, A.-H. Effects of Sub Lethal Concentrations of Synthetic Insecticides and *Callitris Glaucophylla* Extracts on the Development of *Aedes Aegypti*. *J. Vector Ecol.* **2005**, *30*, 295–298. [[PubMed](#)]
45. Silva, I.M.; Martins, G.F.; Melo, C.R.; Santana, A.S.; Faro, R.R.; Blank, A.F.; Alves, P.B.; Picanço, M.C.; Cristaldo, P.F.; Araújo, A.P.A.; et al. Alternative Control of *Aedes Aegypti* Resistant to Pyrethroids: Lethal and Sublethal Effects of Monoterpene Bioinsecticides. *Pest Manag. Sci.* **2018**, *74*, 1001–1012. [[CrossRef](#)]
46. Salokhe, S.G.; Mukherjee, S.N.; Deshpande, S.G.; Ghule, V.P.; Mathad, J.R. Effect of Sub-Lethal Concentrations of Insect Growth Regulator, Lufenuron on Larval Growth and Development of *Aedes Aegypti*. *Curr. Sci.* **2010**, *99*, 1256–1259.
47. da Silva, J.J.; Mendes, J.; Lomônaco, C. Effects of Sublethal Concentrations of Diflubenzuron and Methoprene on *Aedes Aegypti* (Diptera: Culicidae) Fitness. *Int. J. Trop. Insect Sci.* **2009**, *29*, 17–23. [[CrossRef](#)]
48. Aldridge, R.L.; Alto, B.W.; Connelly, C.R.; Okech, B.; Siegfried, B.; Linthicum, K.J. Lethal and Sublethal Concentrations of Formulated Larvicides Against Susceptible *Aedes Aegypti*. *J. Am. Mosq. Control Assoc.* **2022**, *38*, 250–260. [[CrossRef](#)]

49. Belinato, T.A.; Valle, D. The Impact of Selection with Diflubenzuron, a Chitin Synthesis Inhibitor, on the Fitness of Two Brazilian *Aedes Aegypti* Field Populations. *PLoS ONE* **2015**, *10*, e0130719. [CrossRef]
50. Liu, W.; Todd, R.G.; Gerberg, E.J. Effect of Three Pyrethroids on Blood Feeding and Fecundity of *Aedes Aegypti*. *J. Am. Mosq. Control Assoc.* **1986**, *2*, 310–313.
51. Agyemang-Badu, S.Y.; Awuah, E.; Oduro-Kwarteng, S.; Dzamesi, J.Y.W.; Dom, N.C.; Kanno, G.G. Environmental Management and Sanitation as a Malaria Vector Control Strategy: A Qualitative Cross-Sectional Study Among Stakeholders, Sunyani Municipality, Ghana. *Environ. Health Insights* **2023**, *17*, 11786302221146890. [CrossRef]
52. Keiser, J.; Singer, B.H.; Utzinger, J. Reducing the Burden of Malaria in Different Eco-Epidemiological Settings with Environmental Management: A Systematic Review. *Lancet Infect. Dis.* **2005**, *5*, 695–708. [CrossRef]
53. United Nations Children’s Fund (UNICEF). 2.1 Billion People Lack Safe Drinking Water at Home, More than Twice as Many Lack Safe Sanitation. Available online: <https://www.who.int/news/item/12-07-2017-2-1-billion-people-lack-safe-drinking-water-at-home-more-than-twice-as-many-lack-safe-sanitation> (accessed on 2 May 2022).
54. United Nations-Water Summary Progress Update 2021: SDG 6—Water and Sanitation for All. Available online: <https://www.unwater.org/publications/summary-progress-update-2021-sdg-6-water-and-sanitation-all> (accessed on 10 May 2024).
55. Deshpande, A.; Miller-Petrie, M.K.; Lindstedt, P.A.; Baumann, M.M.; Johnson, K.B.; Blacker, B.F.; Abbastabar, H.; Abd-Allah, F.; Abdelalim, A.; Abdollahpour, I.; et al. Mapping Geographical Inequalities in Access to Drinking Water and Sanitation Facilities in Low-Income and Middle-Income Countries, 2000–2017. *Lancet Glob. Health* **2020**, *8*, e1162–e1185. [CrossRef]
56. Venkataramanan, V.; Collins, S.M.; Clark, K.A.; Yeam, J.; Nowakowski, V.G.; Young, S.L. Coping Strategies for Individual and Household-Level Water Insecurity: A Systematic Review. *WIREs Water* **2020**, *7*, e1477. [CrossRef]
57. Barrera, R.; Avila, J.; González-Téllez, S. Unreliable Supply of Potable Water and Elevated *Aedes Aegypti* Larval Indices: A Causal Relationship? *J. Am. Mosq. Control Assoc.* **1993**, *9*, 189–195. [PubMed]
58. Pinchoff, J.; Silva, M.; Spielman, K.; Hutchinson, P. Use of Effective Lids Reduces Presence of Mosquito Larvae in Household Water Storage Containers in Urban and Peri-Urban Zika Risk Areas of Guatemala, Honduras, and El Salvador. *Parasites Vectors* **2021**, *14*, 167. [CrossRef]
59. Akanda, A.S.; Johnson, K.; Ginsberg, H.S.; Couret, J. Prioritizing Water Security in the Management of Vector-Borne Diseases: Lessons From Oaxaca, Mexico. *GeoHealth* **2020**, *4*, e2019GH000201. [CrossRef]
60. Vannavong, N.; Seidu, R.; Stenström, T.-A.; Dada, N.; Overgaard, H.J. Effects of Socio-Demographic Characteristics and Household Water Management on *Aedes Aegypti* Production in Suburban and Rural Villages in Laos and Thailand. *Parasites Vectors* **2017**, *10*, 170. [CrossRef]
61. Quintero, J.; Brochero, H.; Manrique-Saide, P.; Barrera-Pérez, M.; Basso, C.; Romero, S.; Caprara, A.; De Lima Cunha, J.C.; Beltrán-Ayala, E.; Mitchell-Foster, K.; et al. Ecological, Biological and Social Dimensions of Dengue Vector Breeding in Five Urban Settings of Latin America: A Multi-Country Study. *BMC Infect. Dis.* **2014**, *14*, 38. [CrossRef]
62. Overgaard, H.J.; Alexander, N.; Mátiz, M.I.; Jaramillo, J.F.; Olano, V.A.; Vargas, S.; Sarmiento, D.; Lenhart, A.; Seidu, R.; Stenström, T.A. Diarrhea and Dengue Control in Rural Primary Schools in Colombia: Study Protocol for a Randomized Controlled Trial. *Trials* **2012**, *13*, 182. [CrossRef]
63. George, L.; Lenhart, A.; Toledo, J.; Lazaro, A.; Han, W.W.; Velayudhan, R.; Ranzinger, S.R.; Horstick, O. Community-Effectiveness of Temephos for Dengue Vector Control: A Systematic Literature Review. *PLoS Negl. Trop. Dis.* **2015**, *9*, e0004006. [CrossRef]
64. Garelli, F.M.; Espinosa, M.O.; Weinberg, D.; Trinelli, M.A.; Gürtler, R.E. Water Use Practices Limit the Effectiveness of a Temephos-Based *Aedes Aegypti* Larval Control Program in Northern Argentina. *PLoS Negl. Trop. Dis.* **2011**, *5*, e991. [CrossRef]
65. Tawatsin, A.; Thavara, U.; Chompoonsri, J.; Bhakdeenuan, P.; Asavadachanukorn, P. Larvicidal Efficacy of New Formulations of Temephos in Non-Woven Sachets against Larvae of *Aedes Aegypti* (L.) (Diptera: Culicidae) in Water-Storage Containers. *Southeast Asian J. Trop. Med. Public Health* **2007**, *38*, 641–645. [PubMed]
66. Chen, C.D.; Nazni, W.A.; Lee, H.L.; Sofian-Azirun, M. Weekly Variation on Susceptibility Status of *Aedes* Mosquitoes against Temephos in Selangor, Malaysia. *Trop. Biomed.* **2005**, *22*, 195–206. [PubMed]
67. Pinheiro, V.C.S.; Tadei, W.P. Evaluation of the Residual Effect of Temephos on *Aedes Aegypti* (Diptera, Culicidae) Larvae in Artificial Containers in Manaus, Amazonas State, Brazil. *Cad. Saude Publica* **2002**, *18*, 1529–1536. [CrossRef] [PubMed]
68. Laws, E.R.; Sedlak, V.A.; Miles, J.W.; Joseph, C.R.; Lacomba, J.R.; Diaz Rivera, A. Field Study of the Safety of Abate for Treating Potable Water and Observations on the Effectiveness of a Control Programme Involving Both Abate and Malathion. *Bull. World Health Organ.* **1968**, *38*, 439–445.
69. Marcombe, S.; Chonephetsarath, S.; Thammavong, P.; Brey, P.T. Alternative Insecticides for Larval Control of the Dengue Vector *Aedes Aegypti* in Lao PDR: Insecticide Resistance and Semi-Field Trial Study. *Parasites Vectors* **2018**, *11*, 616. [CrossRef]
70. Bellinato, D.F.; Viana-Medeiros, P.F.; Araújo, S.C.; Martins, A.J.; Lima, J.B.P.; Valle, D. Resistance Status to the Insecticides Temephos, Deltamethrin, and Diflubenzuron in Brazilian *Aedes Aegypti* Populations. *BioMed Res. Int.* **2016**, *2016*, 8603263. [CrossRef]

71. Lau, K.W.; Chen, C.D.; Lee, H.L.; Norma-Rashid, Y.; Sofian-Azirun, M. Evaluation of Insect Growth Regulators Against Field-Collected *Aedes Aegypti* and *Aedes Albopictus* (Diptera: Culicidae) from Malaysia. *J. Med. Entomol.* **2015**, *52*, 199–206. [[CrossRef](#)]
72. Neto, L.; Pamplona, L.; José Soares Pontes, R.; Oliveira Lima, J.W. Influence of Water Replacement on Diflubenzuron Duration Effect in the Control of *Aedes Aegypti* in Simulated Field Conditions, in Northeastern Brazil. *J. Health Biol. Sci.* **2013**, *1*, 21–26. [[CrossRef](#)]
73. World Health Organization (WHO). *Diflubenzuron in Drinking-Water: Use for Vector Control in Drinking-Water Sources and Containers Background Document for Development of WHO Guidelines for Drinking-Water Quality*; World Health Organization: Geneva, Switzerland, 2008.
74. Thavara, U.; Tawatsin, A.; Chansang, C.; Asavadachanukorn, P.; Zaim, M.; Mulla, M.S. Simulated Field Evaluation of the Efficacy of Two Formulations of Diflubenzuron, a Chitin Synthesis Inhibitor against Larvae of *Aedes Aegypti* (L.) (Diptera: Culicidae) in Water-Storage Containers. *Southeast Asian J. Trop. Med. Public Health* **2007**, *38*, 269–275.
75. Juarez, J.G.; Garcia-Luna, S.M.; Roundy, C.M.; Branca, A.; Banfield, M.G.; Hamer, G.L. Susceptibility of South Texas *Aedes Aegypti* to Pyriproxyfen. *Insects* **2021**, *12*, 460. [[CrossRef](#)]
76. Hustedt, J.C.; Boyce, R.; Bradley, J.; Hii, J.; Alexander, N. Use of Pyriproxyfen in Control of *Aedes* Mosquitoes: A Systematic Review. *PLoS Negl. Trop. Dis.* **2020**, *14*, e0008205. [[CrossRef](#)] [[PubMed](#)]
77. Oo, S.Z.M.; Thaug, S.; Maung, Y.N.M.; Aye, K.M.; Aung, Z.Z.; Thu, H.M.; Thant, K.Z.; Minakawa, N. Effectiveness of a Novel Long-Lasting Pyriproxyfen Larvicide (SumiLarv[®]2MR) against *Aedes* Mosquitoes in Schools in Yangon, Myanmar. *Parasites Vectors* **2018**, *11*, 16. [[CrossRef](#)] [[PubMed](#)]
78. Maoz, D.; Ward, T.; Samuel, M.; Müller, P.; Runge-Ranzinger, S.; Toledo, J.; Boyce, R.; Velayudhan, R.; Horstick, O. Community Effectiveness of Pyriproxyfen as a Dengue Vector Control Method: A Systematic Review. *PLoS Negl. Trop. Dis.* **2017**, *11*, e0005651. [[CrossRef](#)] [[PubMed](#)]
79. Seng, C.M.; Setha, T.; Nealon, J.; Socheat, D.; Nathan, M.B. Six Months of *Aedes Aegypti* Control with a Novel Controlled-Release Formulation of Pyriproxyfen in Domestic Water Storage Containers in Cambodia. *Southeast Asian J. Trop. Med. Public Health* **2008**, *39*, 822–826.
80. Sihuíncha, M.; Zamora-Perea, E.; Orellana-Rios, W.; Stancil, J.D.; López-Sifuentes, V.; Vidal-Oré, C.; Devine, G.J. Potential Use of Pyriproxyfen for Control of *Aedes Aegypti* (Diptera: Culicidae) in Iquitos, Perú. *J. Med. Entomol.* **2005**, *42*, 620–630. [[CrossRef](#)]
81. Ritchie, S.A.; Rapley, L.P.; Benjamin, S. *Bacillus Thuringiensis* Var. *Israelensis* (Bti) Provides Residual Control of *Aedes Aegypti* in Small Containers. *Am. J. Trop. Med. Hyg.* **2010**, *82*, 1053–1059. [[CrossRef](#)] [[PubMed](#)]
82. Setha, T.; Chantha, N.; Socheat, D. Efficacy of *Bacillus Thuringiensis* *Israelensis*, VectoBac WG and DT, Formulations against Dengue Mosquito Vectors in Cement Potable Water Jars in Cambodia. *Southeast Asian J. Trop. Med. Public Health* **2007**, *38*, 261–268.
83. Mulla, M.S.; Thavara, U.; Tawatsin, A.; Chompoosri, J. Procedures for the Evaluation of Field Efficacy of Slow-Release Formulations of Larvicides against *Aedes Aegypti* in Water-Storage Containers. *J. Am. Mosq. Control Assoc.* **2004**, *20*, 64–73.
84. Marcombe, S.; Darriet, F.; Agnew, P.; Etienne, M.; Yp-Tcha, M.-M.; Yébakima, A.; Corbel, V. Field Efficacy of New Larvicide Products for Control of Multi-Resistant *Aedes Aegypti* Populations in Martinique (French West Indies). *Am. J. Trop. Med. Hyg.* **2011**, *84*, 118–126. [[CrossRef](#)]
85. World Health Organization. *Spinosaad DT in Drinking-Water: Use for Vector Control in Drinking-Water Sources and Containers Background Document for Development of WHO Guidelines for Drinking-Water Quality*; World Health Organization: Geneva, Switzerland, 2010.
86. Njoroge, T.M.; Berenbaum, M.R. Laboratory Evaluation of Larvicidal and Oviposition Deterrent Properties of Edible Plant Oils for Potential Management of *Aedes Aegypti* (Diptera: Culicidae) in Drinking Water Containers. *J. Med. Entomol.* **2019**, *56*, 1055–1063. [[CrossRef](#)]
87. Amer, A.; Mehlhorn, H. Larvicidal Effects of Various Essential Oils against *Aedes*, *Anopheles*, and *Culex* Larvae (Diptera, Culicidae). *Parasitol. Res.* **2006**, *99*, 466–472. [[CrossRef](#)] [[PubMed](#)]
88. Vontas, J.; Kioulos, E.; Pavlidi, N.; Morou, E.; della Torre, A.; Ranson, H. Insecticide Resistance in the Major Dengue Vectors *Aedes Albopictus* and *Aedes Aegypti*. *Pestic. Biochem. Physiol.* **2012**, *104*, 126–131. [[CrossRef](#)]
89. Saeung, M.; Ngoen-Klan, R.; Thanispong, K.; Muenworn, V.; Bangs, M.J.; Chareonviriyaphap, T. Susceptibility of *Aedes Aegypti* and *Aedes Albopictus* (Diptera: Culicidae) to Temephos in Thailand and Surrounding Countries. *J. Med. Entomol.* **2020**, *57*, 1207–1220. [[CrossRef](#)]
90. Valle, D.; Bellinato, D.F.; Viana-Medeiros, P.F.; Lima, J.B.P.; Martins, A.d.J. Resistance to Temephos and Deltamethrin in *Aedes Aegypti* from Brazil between 1985 and 2017. *Mem. Inst. Oswaldo Cruz* **2019**, *114*, e180544. [[CrossRef](#)]
91. Khan, H.A.A.; Akram, W. Resistance Status to Deltamethrin, Permethrin, and Temephos Along With Preliminary Resistance Mechanism in *Aedes Aegypti* (Diptera: Culicidae) From Punjab, Pakistan. *J. Med. Entomol.* **2019**, *56*, 1304–1311. [[CrossRef](#)]
92. Morales, D.; Ponce, P.; Cevallos, V.; Espinosa, P.; Vaca, D.; Quezada, W. Resistance Status of *Aedes Aegypti* to Deltamethrin, Malathion, and Temephos in Ecuador. *J. Am. Mosq. Control Assoc.* **2019**, *35*, 113–122. [[CrossRef](#)]

93. United States Environmental Protection Agency (EPA) National Primary Drinking Water Regulations. Available online: <https://www.epa.gov/ground-water-and-drinking-water/national-primary-drinking-water-regulations> (accessed on 5 January 2025).
94. World Health Organization (WHO). *Alternative Drinking-Water Disinfectants: Bromine, Iodine and Silver*; World Health Organization (WHO): Geneva, Switzerland, 2018; ISBN 978-92-4-151369-2.
95. World Health Organization (WHO). Copper in Drinking-Water: Background Document for Development of WHO Guidelines for Drinking-Water Quality. Available online: https://cdn.who.int/media/docs/default-source/wash-documents/wash-chemicals/copper.pdf?sfvrsn=194c0f12_4s://www.who.int/teams/environment-climate-change-and-health/water-sanitation-and-health/chemical-hazards-in-drinking-water/copper (accessed on 12 April 2023).
96. World Health Organization (WHO). Silver in Drinking-Water: Background Document for Development of WHO Guidelines for Drinking-Water Quality. Available online: https://cdn.who.int/media/docs/default-source/wash-documents/wash-chemicals/silver.pdf?sfvrsn=195cf8b3_4 (accessed on 12 April 2023).
97. Estrella-You, A.; Harris, J.; Singh, R.; Smith, J. Inactivation of Waterborne Pathogens by Copper and Silver Ions, Free Chlorine, and N-Chloramines in Point-of-Use Technology: A Review. In *Water Purification: Processes, Applications and Health Effects*; LeBlanc, P., Ed.; Water Purification; Nova Science Publishers, Inc.: Hauppauge, NY, USA, 2022; pp. 1–88, ISBN 978-1-68507-622-1.
98. Arnold, B.F.; Colford, J.M. Treating Water with Chlorine at Point-of-Use to Improve Water Quality and Reduce Child Diarrhea in Developing Countries: A Systematic Review and Meta-Analysis. *Am. J. Trop. Med. Hyg.* **2007**, *76*, 354–364. [[CrossRef](#)]
99. Collivignarelli, M.C.; Abbà, A.; Benigna, I.; Sorlini, S.; Torretta, V. Overview of the Main Disinfection Processes for Wastewater and Drinking Water Treatment Plants. *Sustainability* **2018**, *10*, 86. [[CrossRef](#)]
100. Crider, Y.; Sultana, S.; Unicomb, L.; Davis, J.; Luby, S.P.; Pickering, A.J. Can You Taste It? Taste Detection and Acceptability Thresholds for Chlorine Residual in Drinking Water in Dhaka, Bangladesh. *Sci. Total Environ.* **2018**, *613–614*, 840–846. [[CrossRef](#)]
101. Albert, J.; Luoto, J.; Levine, D. End-User Preferences for and Performance of Competing POU Water Treatment Technologies among the Rural Poor of Kenya. *Environ. Sci. Technol.* **2010**, *44*, 4426–4432. [[CrossRef](#)]
102. Kim, J.; Chung, Y.; Shin, D.; Kim, M.; Lee, Y.; Lim, Y.; Lee, D. Chlorination By-Products in Surface Water Treatment Process. *Desalination* **2003**, *151*, 1–9. [[CrossRef](#)]
103. Thurman, R.B.; Gerba, C.P.; Bitton, G. The Molecular Mechanisms of Copper and Silver Ion Disinfection of Bacteria and Viruses. *Crit. Rev. Environ. Control* **1989**, *18*, 295–315. [[CrossRef](#)]
104. Ngwenya, N.; Ncube, E.J.; Parsons, J. Recent Advances in Drinking Water Disinfection: Successes and Challenges. In *Reviews of Environmental Contamination and Toxicology*; Whitacre, D.M., Ed.; Springer: New York, NY, USA, 2013; pp. 111–170, ISBN 978-1-4614-4717-7.
105. Lucier, K.J.; Dickson-Anderson, S.E.; Schuster-Wallace, C.J. Effectiveness of Silver and Copper Infused Ceramic Drinking Water Filters in Reducing Microbiological Contaminants. *J. Water Supply Res. Technol.-AQUA* **2017**, *66*, 528–536. [[CrossRef](#)]
106. Pooi, C.K.; Ng, H.Y. Review of Low-Cost Point-of-Use Water Treatment Systems for Developing Communities. *Npj Clean Water* **2018**, *1*, 1–8. [[CrossRef](#)]
107. Singh, R.; Edokpayi, J.N.; Odiyo, J.O.; Smith, J.A. *E. Coli* Inactivation by Metals and Effects of Changes in Water Chemistry. *J. Environ. Eng.* **2019**, *145*, 04018136. [[CrossRef](#)]
108. Ehdaie, B.; Su, Y.-H.; Swami, N.S.; Smith, J.A. Protozoa and Virus Disinfection by Silver- and Copper-Embedded Ceramic Tablets for Water Purification. *J. Environ. Eng.* **2020**, *146*, 04020015. [[CrossRef](#)]
109. Ndebele, N.; Edokpayi, J.N.; Odiyo, J.O.; Smith, J.A. Field Investigation and Economic Benefit of a Novel Method of Silver Application to Ceramic Water Filters for Point-Of-Use Water Treatment in Low-Income Settings. *Water* **2021**, *13*, 285. [[CrossRef](#)]
110. Estrella-You, A.; Smith, J.A. Synergistic Bacterial Inactivation by Silver Ions and Free Chlorine in Natural Waters. *J. Environ. Eng.* **2022**, *148*, 04022072. [[CrossRef](#)]
111. Hill, C.L.; Harris, J.D.; Turner, S.S.; Wason, K.L.; Gaylord, A.P.; Hatley, M.G.; Hardcastle, L.T.; Roberts, I.T.; You, J.Y.; Renneker, K.O.; et al. Field and Laboratory Assessment of a New Electrolytic Point-of-Use Water Treatment Technology. *Water* **2022**, *14*, 1077. [[CrossRef](#)]
112. Alherek, M.; Basu, O.D. Impact of Low Levels of Silver, Zinc and Copper Nanoparticles on Bacterial Removal and Potential Synergy in Water Treatment Applications. *J. Chem. Technol. Biotechnol.* **2023**, *98*, 1137–1146. [[CrossRef](#)]
113. Harris, J.D.; Homola, L.M.J.; Estrella-You, A.; Smith, J.A. Enhancing Microbial Disinfection in Household Water Treatment by Combining a Silver–Ceramic Tablet with Copper and Chlorine Technologies. *J. Environ. Eng.* **2024**, *150*, 04024026. [[CrossRef](#)]
114. Silvestry-Rodriguez, N.; Sicairos-Ruelas, E.E.; Gerba, C.P.; Bright, K.R. Silver as a Disinfectant. In *Reviews of Environmental Contamination and Toxicology*; Springer: New York, NY, USA, 2007; pp. 23–45, ISBN 978-0-387-69163-3.
115. Ehdaie, B.; Rento, C.T.; Son, V.; Turner, S.S.; Samie, A.; Dillingham, R.A.; Smith, J.A. Evaluation of a Silver-Embedded Ceramic Tablet as a Primary and Secondary Point-of-Use Water Purification Technology in Limpopo Province, S. Africa. *PLoS ONE* **2017**, *12*, e0169502. [[CrossRef](#)] [[PubMed](#)]
116. Armstrong, A.M.; Sobsey, M.D.; Casanova, L.M. Disinfection of *Escherichia Coli* and *Pseudomonas Aeruginosa* by Copper in Water. *J. Water Health* **2016**, *14*, 424–432. [[CrossRef](#)] [[PubMed](#)]

117. World Health Organization. *Guidelines for Drinking-Water Quality, 4th Edition, Incorporating the 1st Addendum*; World Health Organization: Geneva, Switzerland, 2017; ISBN 978-92-4-154995-0.
118. World Health Organization. *Guidelines for Laboratory and Field Testing of Mosquito Larvicides*; World Health Organization: Geneva, Switzerland, 2005.
119. Ngonzi, A.J.; Muyaga, L.L.; Ngowo, H.; Urrio, N.; Vianney, J.-M.; Lwetoijera, D.W. Susceptibility Status of Major Malaria Vectors to Novaluron, an Insect Growth Regulator South-Eastern Tanzania. *Pan Afr. Med. J.* **2022**, *41*, 273. [CrossRef]
120. Abbott, W.S. A Method of Computing the Effectiveness of an Insecticide. *J. Econ. Entomol.* **1925**, *18*, 265–267. [CrossRef]
121. Bliss, C.I. The Method of Probits. *Science* **1934**, *79*, 38–39. [CrossRef]
122. Finney, D.J. *Probit Analysis*; Cambridge University Press: Cambridge, MA, USA; References-Scientific Research Publishing: Wuhan, China, 1971; 333p. Available online: [https://www.scirp.org/\(S\(i43dyn45teexjx455qlt3d2q\)\)/reference/ReferencesPapers.aspx?ReferenceID=1493267](https://www.scirp.org/(S(i43dyn45teexjx455qlt3d2q))/reference/ReferencesPapers.aspx?ReferenceID=1493267) (accessed on 4 April 2023).
123. Postelnicu, T. Probit Analysis. In *International Encyclopedia of Statistical Science*; Lovric, M., Ed.; Springer: Berlin/Heidelberg, Germany, 2011; pp. 1128–1131, ISBN 978-3-642-04898-2.
124. Ong, S.-Q.; Jaal, Z. Larval Age and Nutrition Affect the Susceptibility of *Culex Quinquefasciatus* (Diptera: Culicidae) to Temephos. *J. Insect Sci.* **2018**, *18*, 38. [CrossRef]
125. Nartey, R.; Owusu-Dabo, E.; Kruppa, T.; Baffour-Awuah, S.; Annan, A.; Oppong, S.; Becker, N.; Obiri-Danso, K. Use of *Bacillus Thuringiensis* Var *Israelensis* as a Viable Option in an Integrated Malaria Vector Control Programme in the Kumasi Metropolis, Ghana. *Parasites Vectors* **2013**, *6*, 116. [CrossRef]
126. Patil, C.D.; Borase, H.P.; Patil, S.V.; Salunkhe, R.B.; Salunke, B.K. Larvicidal Activity of Silver Nanoparticles Synthesized Using *Pergularia Daemia* Plant Latex against *Aedes Aegypti* and *Anopheles Stephensi* and Nontarget Fish *Poecilia Reticulata*. *Parasitol. Res.* **2012**, *111*, 555–562. [CrossRef]
127. Perez, M.H.; Noriega, F.G. *Aedes Aegypti* Pharate 1st Instar Quiescence Affects Larval Fitness and Metal Tolerance. *J. Insect Physiol.* **2012**, *58*, 824–829. [CrossRef]
128. Miah, M.A. Effect of Copper Sulphate Pentahydrate on Mosquito Larval *Aedes Aegypti*, *Culex Quinquefasciatus*, and *Anopheles Quadrimaculatus* in Laboratory and under Semi-Field Conditions. *J. Fla. Mosq. Control. Assoc.* **2021**, *68*, 79–85. [CrossRef]
129. Ratte, H.T. Bioaccumulation and Toxicity of Silver Compounds: A Review. *Environ. Toxicol. Chem.* **1999**, *18*, 89–108. [CrossRef]
130. Shanmugasundaram, T.; Balagurunathan, R. Mosquito Larvicidal Activity of Silver Nanoparticles Synthesised Using *Actinobacterium*, *Streptomyces* Sp. M25 against *Anopheles Subpictus*, *Culex Quinquefasciatus* and *Aedes Aegypti*. *J. Parasites Dis. Off. Organ Indian Soc. Parasitol.* **2015**, *39*, 677–684. [CrossRef] [PubMed]
131. Velayutham, K.; Ramanibai, R.; Umadevi, M. Green Synthesis of Silver Nanoparticles Using *Manihot Esculenta* Leaves against *Aedes Aegypti* and *Culex Quinquefasciatus*. *J. Basic Appl. Zool.* **2016**, *74*, 37–40. [CrossRef]
132. Rayms-Keller, A.; Olson, K.E.; McGaw, M.; Oray, C.; Carlson, J.O.; Beaty, B.J. Effect of Heavy Metals on *Aedes Aegypti* (Diptera: Culicidae) Larvae. *Ecotoxicol. Environ. Saf.* **1998**, *39*, 41–47. [CrossRef]
133. Reza, M.; Ilmiawati, C. Laboratory Testing of Very Low-Copper-Treated Water to Prolong Pupation and Emerging Time of Mosquito Larvae: An Alternative Method to Delay Mosquito Breeding Capability. *PLoS ONE* **2020**, *15*, e0226859. [CrossRef]
134. Miranda, F.R.; Fernandes, K.M.; Farder-Gomes, C.F.; Bernardes, R.C.; de Oliveira, A.H.; Arthidoro de Castro, M.B.; Dourado, L.A.; Oliveira, L.L.; Martins, G.F.; Serrão, J.E. Exposure to Copper Sulfate Impairs Survival, Post-Embryonic Midgut Development and Reproduction in *Aedes Aegypti*. *Infect. Genet. Evol.* **2022**, *97*, 105185. [CrossRef]
135. Neff, E.; Dharmarajan, G. The Direct and Indirect Effects of Copper on Vector-Borne Disease Dynamics. *Environ. Pollut.* **2021**, *269*, 116213. [CrossRef]
136. Reza, M.; Yamamoto, D.S.; Matsuoka, H. Larvicidal and Ovipositional Preference Test of Copper Solution for Mosquitoes. *Med. Entomol. Zool.* **2014**, *65*, 147–150. [CrossRef]
137. El-Sheikh, E.-S.M.Y.; Fouda, M.A.; Hassan, M.I.; Abd-Elghaphar, A.-E.A.; Hasaballah, A.I. Toxicological Effects of Some Heavy Metal Ions on *Culex Pipiens* L. (Diptera: Culicidae). *Egypt. Acad. J. Biol. Sci. F Toxicol. Pest Control* **2010**, *2*, 63–76. [CrossRef]
138. Salunkhe, R.B.; Patil, S.V.; Patil, C.D.; Salunke, B.K. Larvicidal Potential of Silver Nanoparticles Synthesized Using Fungus *Cochliobolus Lunatus* against *Aedes Aegypti* (Linnaeus, 1762) and *Anopheles Stephensi* Liston (Diptera; Culicidae). *Parasitol. Res.* **2011**, *109*, 823–831. [CrossRef]
139. Sherman, C.; Fernandez, E.A.; Chan, A.S.; Lozano, R.C.; Leontsini, E.; Winch, P.J. La Untadita: A Procedure for Maintaining Washbasins and Drums Free of *Aedes Aegypti* Based on Modification of Existing Practices. *Am. J. Trop. Med. Hyg.* **1998**, *58*, 257–262. [CrossRef]
140. Shahan, M.; El-Wahsh, H.; Ramadan, H.; Hegazi, M.; Al-Sharkawi, I.; Seif, A. A Comparison Of The Toxicity Of Calcium And Sodium Hypochlorite Against *Culex Pipiens* (Diptera: Culicidae) Larvae. *Environ. Sci. Curr. Res.* **2020**, *3*, 1–9. [CrossRef]
141. Barrera, R.; Amador, M.; Clark, G.G. The Use of Household Bleach to Control *Aedes Aegypti*. *J. Am. Mosq. Control Assoc.* **2004**, *20*, 444–448.

142. Brown, M.D.; Walker, D.O.; Hendrikz, J.K.; Cabral, C.P.; Araujo, D.B.; Ribeiro, Z.M.; Kay, B.H. Chlorine Tolerance of Mesocyclops (Cyclopoida: Cyclopidae) Copepods and Three Container-Breeding Species of Mosquitoes. *Environ. Entomol.* **1994**, *23*, 1245–1249. [[CrossRef](#)]
143. Muttkowski, R.A. Copper: Its Occurrence and Rôle in Insects and Other Animals. *Trans. Am. Microsc. Soc.* **1921**, *40*, 144–157. [[CrossRef](#)]

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