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INTERACTIVE EFFECTS OF SALINITY AND MOSQUITO LARVICIDES TOXICITY TO LARVAE OF *AEDES TAENIORHYNCHUS*

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ABSTRACT. Understanding the influence of salinity on the efficacy of mosquito larvicides in brackish water habitats is crucial for effective salt-marsh *Aedes taeniorhynchus* control. This study investigated the interactive effects of salinity on the toxicity of 3 commonly used mosquito larvicides: *Bacillus thuringiensis israelensis* (VectoBac® 12AS), spinosad (Natular® SC), and S-methoprene (Altosid® 12AS) against *Ae. taeniorhynchus* larvae. Four salinity levels (0 ppt [parts per thousand], 8 ppt, 16 ppt, and 32 ppt) were tested in laboratory bioassays. The results revealed distinct responses of these larvicides to varying salinity levels. VectoBac 12AS displayed consistent efficacy across all salinity levels, indicating its suitability for brackish water habitats. In contrast, Natular 2EC exhibited increased effectiveness with higher salinity, making it a preferable choice for saline environments. Altosid 12AS showed its highest efficacy in freshwater, with reduced effectiveness as salinity increased. These findings underscore the need to consider salinity levels when selecting and applying mosquito larvicides in diverse aquatic habitats. Understanding the complex interplay between salinity and larvicide performance is essential for optimizing mosquito control strategies and mitigating mosquito-borne diseases in various environments.

KEY WORDS *Aedes taeniorhynchus*, *Bacillus thuringiensis*, salinity, S-methoprene, spinosad, toxicity

INTRODUCTION

Aedes taeniorhynchus (Wiedemann), colloquially recognized as the black salt-marsh mosquito, represents a globally dispersed mosquito species, notably prevalent within coastal and marshland regions. This species assumes a pivotal role within diverse ecosystems, albeit concurrently posing substantive concerns for human populations. While serving as a vital nutritional source for various predators, including avian and aquatic species, *Ae. taeniorhynchus* concurrently establish themselves as vectors for a range of diseases encompassing dog heartworm disease (Nayar and Rutledge 2008) and arboviruses such as the eastern equine encephalitis virus (EEEV) and Venezuelan equine encephalitis virus (VEEV), both capable of instigating severe maladies in both human and animal subjects (Drew 2001). The remarkable adaptation of *Ae. taeniorhynchus* to their native habitats—such as salt marshes, mangrove thickets, tidal flats, coastal dunes, and swampy terrain—is evident. Their larval stages flourish in brackish waters characterized by substantial fluctuations in salinity levels (Bradley 1987), thereby forming an intrinsic association with wetland and coastal ecosystems wherein their life cycle intimately synchronizes with tidal oscillations. Consequently, their encroachment into densely populated localities engenders apprehensions necessitating efficacious control protocols.

Managing mosquito larvae bred within brackish environments such as *Ae. taeniorhynchus* presents heightened challenges compared to their freshwater counterparts due to the intricate interplay between water salinity and the efficacy of mosquito larvicides. Elevated salinity levels impact not only the physical attributes of water—density and viscosity—but also impede the penetration of larvicides, potentially compromising their effectiveness against target larvae. Furthermore, chemical properties, such as solubility and stability, are subject to alteration, thereby diminishing the larvicides' potency. Biological processes of mosquito larvae, inclusive of metabolism and immune response, also bear the influence of heightened salinity, thereby influencing susceptibility to larvicidal agents. Presently, two primary categories of mosquito larvicides have garnered widespread usage: bacterial larvicides, comprising *Bacillus thuringiensis israelensis* deBarjac (*Bti*), *Lysinibacillus sphaericus* Myer and Neid (*Ls*), and *Saccharopolyspora spinosa* (spinosad), along with insect growth regulators like methoprene and pyriproxyfen. The interactive effects of water salinity and *Bti* larvicide toxicity have been reported extensively. Nayar et al. (1999) elucidated the gradual decline in *Bti* (VectoBac® 12AS) efficacy against *Ae. taeniorhynchus* larvae with escalating water salinity levels up to 50% seawater. Jude et al. (2012) concurred, highlighting the adverse impact of water salinity on *Bti* toxin toxicity to *Aedes aegypti* (L.) larvae, particularly within salinity ranges correlating to *Ae. aegypti* habitats. In contrast, Osborn et al. (2007) found that *Bti* efficacy (VectoBac 12AS) remained unaffected by escalating water salinity concerning *Anopheles aquasalis* Curry. This observation was held for two *Bti* formulations, VectoBac 12AS and Bactive®, against *An. aquasalis* larvae. Intriguingly, the increase in salinity negatively impacted Bactive's effectiveness, while VectoBac 12AS remained unaffected. Conversely, the influence of salinity on other larvicides such as temephos toxicity in early instars of *Aedes*

sollicitans (Walker) was explored by Huang and Brattsten (2007). Larvae raised in freshwater exhibited altered toxicity levels at varying salinity levels, whereas saltwater-raised larvae displayed consistent toxicity patterns. Akin findings were reported by Song and Brown (1998), with *Ae. taeniorhynchus* displaying enhanced survival under isosmotic conditions, yet demonstrating variable tolerance under hyperosmotic conditions, when treated with four distinct insecticides: tebufenozide, imidacloprid, aldicarb, and dimethoate. High salinity can also increase the toxicity of the carbamate insecticide aldicarb and low salinity can reduce the aldicarb toxicity to euryhaline fish (Daikoku et al. 1988, Schlenk et al. 1996, Schlenk 1998). Notably, the direct impact of spinosad and methoprene on mosquito larvicides toxicity within diverse water salinity levels remains sparsely documented.

This study aims to investigate the interactive effects of salinity on the effectiveness of three commonly used mosquito larvicides' (*Bti,* spinosad, and methoprene) toxicity to *Ae. taeniorhynchus* larvae. By comprehensively examining the impact of different salinity levels on larvicide activity, we seek to contribute valuable insights to the field of mosquito control and enhance our ability to combat mosquito-borne diseases effectively. The findings from this study will aid in the development of evidence-based mosquito control programs, optimizing the use of larvicides in diverse aquatic habitats, while considering the varying challenges posed by salinity levels.

MATERIALS AND METHODS

Test materials

Three formulated products were tested in this study: VectoBac 12AS (Valent BioSciences, Libertyville, IL, USA), which consists of 11.60% *Bacillus thuringiensis israelensis*, strain AM 65-52, fermentation solids, and solubles; Natular® SC (provided by Clarke Mosquito Control Products, Inc., Roselle, IL, USA), containing 22.5% spinosad (a mixture of Spinosyn A and Spinosyn D) with Lot # 2301050004; and Altosid® 12AS (ZOËCON, Schaumburg, IL, USA), containing 5% S-methoprene (EPA REG NO. 2724-392).

Laboratory bioassay

Eggs of *Aedes taeniorhynchus* were generously provided by the USDA-Agricultural Research Services (ARS), Center for Medical, Agricultural, and Veterinary Entomology (CMAVE) located in Gainesville, FL. This salt marsh mosquito colony, originally derived from a collection made in Orlando, FL, in 1952, has been meticulously maintained within our laboratory setting. Our rearing protocol closely adhered to the guidelines outlined in the USDA-ARS-CMAVE mosquito rearing handbook, with only minor adjustments. In particular, we deviated from the recommended 0.5% saltwater concentration specified in the USDA handbook. Instead, we employed a 1% (10 parts per thousand [ppt]) saltwater solution, prepared by combining 200 g of Instant Ocean® Sea Salt (Blacksburg, VA), with 15 liters of RO (reversed osmosis) water, for the purpose of colony maintenance.

To investigate the impact of salinity on the efficacy of three mosquito larvicides (VectoBac, Natular, and Altosid), we conducted experiments using four different salinity levels: representing 100%, 50%, 25%, and 0% seawater salt concentrations. Seawater for these experiments was collected from a boat ramp at Oslo Road, Vero Beach, FL, with coordinates 27° 35′12.11″N, 80°21′54.31″W, possessing a salinity of 32 ppt (parts per thousand), equivalent to 3.2% (parts per hundred). To create the desired 50% and 25% seawater concentrations, we mixed 500 ml and 250 ml of seawater, respectively, with 500 ml and 750 ml of RO water, resulting in salinities of 16 ppt and 8 ppt. Pure RO water was utilized to prepare the 0% seawater (0 ppt).

For VectoBac and Natular, we followed standard bioassay protocols (Estrada and Mulla 1986, Su et al. 2018) to assess their effectiveness against 3rd stage USDA *Ae. taeniorhynchus* larvae. We created a 1% stock suspension of these formulated materials and prepared dilutions using distilled water. The desired dilution strengths were added to 100 ml of water within 116-ml polystyrene foam food cups (Dart J Cup, Dart Container Corporation, Mason, Michigan), each containing 25 mosquito larvae. Each test consisted of 4–5 concentrations spanning the activity range, with 4 replicates for each concentration. In addition, 4 cups were designated as untreated controls. For each salinity level, the same salinity water was used as control in the experiments. These experiments were repeated 2–3 times on different occasions, resulting in 8–12 replicates per test. We added 3 drops of larval food (10 g ground-up Rabbit pellets [Menu Care Complex, Vitakraft Sun Seed, Inc., Bowling Green, OH] in 100 ml of distilled water) to each cup. Following treatment, the treated larvae were kept in an insectary maintained at a constant temperature of 27°C with a photoperiod of 16 h of light and 8 h of darkness. We assessed larval mortality after both 24 and 48 h, including moribund larvae as deceased.

The evaluation of Altosid 12AS involved a protocol differing from standard bioassays, as per Mulla et al. 1989, Su et al. 2021. We prepared a 0.1% stock solution of Altosid and created dilutions using distilled water. Each test involved subjecting 25 late-4th-stage larvae (Methoprene disrupts the balance of juvenile hormone during the transition from late-4th instars to pupae and adults) to 4 different salinity concentrations within 116-ml (6 fl. oz) polystyrene foam food cups. For each concentration, we conducted 4 replicates, and 4 untreated cups served as controls, totaling 5 concentrations per test. These

Table 1. Susceptibility of *Aedes taeniorhynchus* 3rd stage larvae exposed for 24 h and 48 h to VectoBac® 12AS in different water salinities in the lab.*

	24 h		48 h	
Water salinity (ppt)	LC_{50} (ppm) (95% CI)	LC_{90} (ppm) (95% CI)	LC_{50} (ppm) (95% CI)	LC_{90} (ppm) (95% CI)
0	0.10 a (0.054–0.897)	0.21 a (0.111–0.345)	0.09 a (0.045–0.789)	0.18 a (0.106–0.028)
8	0.08 a (0.069–0.100)	0.13 a (0.107–0.237)	0.07 a (0.044–0.096)	0.10 a (0.081–0.626)
16	0.09 a (0.058–0.122)	0.15 a (0.110–1.133)	0.08 a (0.069–0.083)	0.11 a (0.100–0.136)
32	0.08 a (0.069–0.096)	0.14 a (0.122–0.189)	0.07 a (0.068–0.080)	0.13 a (0.116–0.160)

* Mortality data were corrected if mortality in control was > 5%, using the Abbott formula (Abbott 1925) before probit analysis. The LC_{50} and LC_{90}: concentrations causing 50% and 90% mortality. CI, confidence intervals; ppm, parts per million; ppt, part per thousand. TukeyHSD post-hoc test was preformed to analyze the significant differences ($P < 0.05$) in susceptibility between different water salinities at each exposure duration by using R statistical program.

bioassays were carried out 2–3 times on different occasions. We added a small quantity of rabbit pellets (less than 100 mg) to each bioassay cup. The cups were covered with clear plastic domes featuring a 1-in. diam fine-screened top, which confined emerging adults. We maintained the cups in the laboratory at a constant temperature of 24°C with a photoperiod of 16 h of light and 8 h of darkness. Mortality was documented for dead larvae, pupae, or incompletely emerged adults with wings and/or legs attached to the exuviae. Only free exuviae were considered indicative of a successful emergence for adults. The counting process continued until all larvae were accounted for in each cup, with dead larvae, pupae, or adults removed after each count.

Data analysis

For VectoBac and Natular, we estimated dose-response lines LC_{50} and LC_{90} along with their 95% confidence intervals (CI) using POLO-PC (LeOra Software 1987). These estimates were based on the readings taken in both 24 and 48 h. For Altosid, we estimated the inhibition of adult emergence (IE%) IE_{50} and IE_{90} values and their corresponding 95% confidence intervals using POLO-PC.

To assess the proportion of stage-specific mortality (%), we calculated the percentage by employing the following formula: 100 × (total number of deceased individuals at a specific stage of larvae, pupae, or incomplete adult emergence/total number of deceased individuals) across all test concentrations. In cases where the total mortality in the control group exceeded 5%, we applied the Abbott formula (Abbott 1925) for correction. Significant differences in LC and IE levels were determined by separate 95% CIs, following the methodology outlined by Su et al. (2021). Additionally, Tukey's Honestly-Significant Difference (TukeyHSD) post-hoc test was preformed to analyze the significant differences ($P < 0.05$) in susceptibility between different water salinities at each exposure duration by using R statistical program.

RESULTS

The laboratory bioassays yielded distinct responses to varying salinity levels for each tested larvicide toxicity against *Ae. taeniorhynchus* larvae. For VectoBac 12AS, it became evident that salinity had no discernible impact on its efficacy. The susceptibility of 3rd instar *Ae. taeniorhynchus* to VectoBac 12AS remained consistent across different salinity levels (Table 1). Specifically, the 24-h LC_{50} and 24-h LC_{90} values were 0.1, 0.08, 0.09, 0.08 ppm (parts per million), and 0.21, 0.13, 0.15, 0.14 ppm at 0 ppt, 8 ppt, 16 ppt, and 32 ppt salinity levels, respectively. Comparing the 24-h results with those at 48 h, it was observed that the extent of larval mortality (LC_{50} and LC_{90} values) slightly increased after 48 h of exposure, indicating an increase in the potency of VectoBac 12AS over time. This time-dependent effect is likely due to the gradual action of *Bti* on the mosquito larvae. The 48-h LC_{50} and 48-h LC_{90} values were 0.09, 0.07, 0.08, 0.07 ppm, and 0.18, 0.10, 0.11, 0.13 ppm at 0 ppt, 8 ppt, 16 ppt, and 32 ppt salinity levels, respectively.

Conversely, the impact of salinity on the toxicity of Natular 2EC differed significantly from that of VectoBac 12AS. Third instars of *Ae. taeniorhynchus* displayed varying susceptibility to Natular 2EC at different salinity levels. At 0, 8, 16, and 32 ppt salinity, the 24-h LC_{50} values stood at 0.07, 0.03, 0.04, and 0.02 ppm, respectively. Compared to larvae exposed to freshwater (0 ppt), the susceptibility of third stage larvae significantly increased by 2.3 and 1.8-fold at 8 and 16 ppt salinity, respectively. This increase was more pronounced at 32 ppt salinity, reaching a 3.5-fold rise (refer to Table 2). Concurrently, the 24-h LC_{90} values reflected a similar trend, escalating by 2.3, 2, and 3.6-fold at 8, 16, and 32 ppt,

Table 2. Susceptibility of *Aedes taeniorhynchus* 3rd stage larvae exposed for 24 h and 48 h to Natular® SC in different water salinities in the lab.*

	24 h		48 h	
Water salinity (ppt)	LC_{50} (ppm) (95% CI)	LC_{90} (ppm) (95% CI)	LC_{50} (ppm) (95% CI)	LC_{90} (ppm) (95% CI)
0	0.07 a (0.056–0.090)	0.18 a (0.125–0.436)	0.03 (–) **	0.07 (0.001–0.034)
8	0.03 b (0.031–0.037)	0.08 b (0.068–0.094)	0.01 (–) **	0.03 (–) **
16	0.04 b (0.037–0.049)	0.09 b (0.078–0.121)	0.02 (–) **	0.03 (–) **
32	0.02 b (0.017–0.028)	0.05 b (0.036–0.082)	0.01 (–) **	0.02 (–) **

* Mortality data were corrected if mortality in control was > 5%, using Abbott formula (Abbott 1925) before probit analysis. The LC_{50} and LC_{90}: concentrations causing 50% and 90% mortality; CI = confidence intervals; ppm: parts per million. ppt: part per thousand. ** No CT was calculated. TukeyHSD post-hoc test was preformed to analyze the significant differences ($P < 0.05$) in susceptibility between different water salinities at each exposure duration by using R statistical program.

respectively, mirroring the LC_{50} values. Moreover, the extent of larval mortality at 48 h demonstrated significant escalation, rising by 2.3, 3, and 2-fold, respectively at LC_{50}, and 2.7, 3, and 2.5-fold, respectively at LC_{90}, at 8, 16, and 32 ppt salinity levels compared to the 24-h exposure.

In contrast, the effect of salinity on Altosid 12AS toxicity exhibited a different pattern from the other 2 tested larvicides. It displayed its highest efficacy at the lowest salinity level (0 ppt) and significantly decreased in effectiveness as water salinity increased to 8 ppt, 16 ppt, and 32 ppt (Table 3.). The IE_{50} and IE_{90} values for 4th stage *Ae. taeniorhynchus* larvae to Altosid were 0.015, 0.044, 0.036, 0.053 ppb, and 0.55, 0.794, 0.661, 0.862 ppb, respectively, at 0, 8, 16, and 32 ppt water salinities. The IE_{50} and IE_{90} values displayed a 2.9, 2.4, and 1.4, 1.2-fold decrease when compared to the larvae exposed to 0 ppt at 8 and 16 ppt seawater, with a more significant decrease (3.5 and 1.6-fold) in larvae exposed to 32 ppt seawater. There were no significant differences in IE_{50} and IE_{90} values among salinity levels of 8, 16, and 32 ppt.

DISCUSSION

Most mosquito larvicides are primarily designed and tested for use in freshwater habitats, where they have consistently demonstrated high effectiveness in reducing mosquito larval populations. Freshwater environments provide an optimal setting for larvicides to exert their maximum impact on mosquito larvae. However, it is essential to recognize that the efficacy of larvicides can be influenced by various environmental factors, with salinity playing a pivotal role in affecting mosquito larvicidal activity. Previous studies have documented reduced efficacy of organophosphate and bacterial larvicides in saline waters (Nayar and Sauerman 1974, Song and Brown 1998, Nayar et al. 1999, Huang and Brattsten 2007, Jude et al. 2012, Osborn et al. 2007, Kengne et al. 2019) and their impact on larval growth and development (Clark et al. 2004). Conversely, some studies have reported that certain larvicides can still provide moderate control in saline environments (Osborn et al. 2007). However, the impact of water salinity on the efficacy of spinosad and methoprene has not been extensively explored. Therefore, it is crucial to consider the salinity level of the target water body when selecting and applying larvicides, such as spinosad and methoprene, to effectively control mosquito populations.

Our findings reveal that water salinity significantly impacts the effectiveness of the tested larvicides against *Ae. taeniorhynchus* larvae. Each larvicide displayed distinct responses to varying salinity conditions. VectoBac exhibited consistent efficacy across salinity levels, while Natular demonstrated increased effectiveness in higher salinity environments, making it a preferable choice for brackish habitats. In contrast, Altosid displayed its highest efficacy in freshwater, with reduced effectiveness as water salinity increased. These differential responses may be attributed to the diverse modes of action of these larvicides. For instance, VectoBac acts solely through ingestion (toxins produced by bacteria *Bacillus thuringiensis* bind to different cell receptors in the midgut of mosquito larvae after ingestion), while Natular act both by contact and ingestion (Spinsyns A and D involve disrupting/inhibiting the binding of acetylcholine at the nicotinic acetylcholine receptors located at the post-synaptic cell junctures as well as bind partially to the receptor and changes the shape). Altosid, on the other hand, primarily acts through ingestion contact, interfering with larval insect growth and development. Additionally, our study highlights that all three larvicides tend to become more potent with longer exposure times, suggesting the potential for enhanced efficacy with repeated applications.

The mechanisms underlying the influence of salinity on mosquito larvicide toxicity are multifaceted.

Table 3. Susceptibility of *Aedes taeniorhynchus* 4th stage larvae exposed to Altosid® 12AS in different water salinities in the lab.*

Water salinity (ppt)	IE_{50} (ppb) (95% CI)	IE_{90} (ppb) (95% CI)
0	0.015 a (0.0093–0.024)	0.55 a (0.245–2.002)
8	0.044 b (0.037–0.052)	0.794 a (0.588–1.128)
16	0.036 b (0.021–0.716)	0.661 b (0.089–2.133)
32	0.053 b (0.039–0.070)	0.862 b (0.506–1.86)

* Mortality data were corrected if mortality in control was > 5%, using the Abbott formula (Abbott 1925) before probit analysis. The IE_{50} and IE_{90}: concentrations causing 50% and 90% inhibition of adult emergence respectively; CI = confidence intervals; ppb: parts per billion. TukeyHSD post-hoc test was preformed to analyze the significant differences ($P < 0.05$) in susceptibility between different water salinities at each exposure duration by using R statistical program.

Previous research has suggested that salinity can impact the biological processes of mosquito larvae, including their metabolism and immune system, potentially reducing their susceptibility to larvicides. In cases where mosquito larvae are metabolically stressed due to suboptimal salinity conditions, the addition of larvicides may synergistically stress the larvae, increasing their susceptibility to toxins (Song and Brown, 1998, 2006). Furthermore, salinity can disrupt the osmoregulation process of mosquito larvae, diminishing their overall fitness and potentially affecting the uptake and absorption of larvicides, particularly in brackish and seawater habitats.

Salinity may also influence the rate of larvicide degradation or dissipation, as well as the chemical nature of degradation products (Huang and Brattsten, 2007, Bourquin, 1977). Similar observations have been documented in other organisms, such as the fish *Hypomesus transpacificus* McAllister (Hobbs et al. 2019; DeCourten et al. 2019) and shrimp (Wang et al. 2013). Moreover, salinity can alter the chemical properties of larvicides, including solubility and stability, potentially reducing their effectiveness in controlling mosquito larvae (Saranjampour et al. 2017). Changes in mosquito egg surface features and larval cuticle properties in response to salinity could also contribute to variations in larvicide efficacy, as seen in increased temephos resistance in brackish water (Sivabalakrishnan et al. 2023).

Our study emphasizes the pivotal role of salinity considerations in mosquito control programs. The nuanced relationship between salinity and larvicide effectiveness is fundamental in crafting precise and efficient strategies aimed at diminishing mosquito populations and countering mosquito-borne diseases. Larvicide selection based on specific salt marsh salinity levels is essential. This knowledge empowers a more informed and targeted approach. For instance, in areas with lower salinity, Altosid 12AS remains a viable option, while Natular 2EC proves more effective in saline environments. Meanwhile, VectoBac 12AS emerges as a versatile choice adaptable across a broad salinity spectrum.

Integrating salinity-specific insights into salt marsh mosquito control plans optimizes the efficacy of control measures. However, ongoing research is crucial to pinpoint and enhance larvicide products suitable for varying salinity conditions. This could involve developing new formulations or modifying existing larvicides to bolster their effectiveness across diverse salinity ranges, ultimately fortifying mosquito control efforts in various aquatic environments.

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