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Methionine as an effective mosquito larvicide in natural water sources

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Abstract

Methionine is a naturally occurring amino acid that has demonstrated toxic properties for control of larval mosquitoes in laboratory experiments. Methionine offers many desirable qualities for an effective, biorational pesticide, including its minimal effects on non-target species. Because previous studies regarding this amino acid's toxicity were laboratory based, the next step is to establish if methionine is likely to have similar effects in natural water bodies before attempting costly field trials. Therefore, the goal of this study was to test the effectiveness of DL-methionine applied to various water sources. Concentration response experiments conducted in glass jars used larval *Aedes aegypti* (L.) (Diptera: Culicidae) as a model organism. Well, deionized, and pond water were evaluated in the study. In general, increased mortality of *Ae. aegypti* larvae occurred with increasing concentrations of DL-methionine at 48 h. However, larval DL-methionine LC_{50} values were not different between water sources. This study has shown that DL-methionine can be added to various water sources as a possible biorational larvicide when applied to natural water sources such as ponds or water-holding containers that often are preferred larval developmental sites for a variety of mosquito disease vectors.

Key Words: *Aedes aegypti*; larvae; Culicidae; pesticide; biorational; pond; lethal concentration

Resumo

La metionina es un aminoácido natural que ha demostrado propiedades tóxicas para el control de larvas de mosquitos en experimentos de laboratorio. La metionina ofrece muchas cualidades deseables para un pesticida eficaz biorracional, incluido sus efectos mínimos en especies no objetivo. Debido a que los estudios previos sobre la toxicidad de este aminoácido se realizaron en laboratorio, el siguiente paso es establecer la probabilidad de que la metionina tenga efectos similares en los cuerpos de agua naturales antes de intentar costosos ensayos de campo. Por lo tanto, el objetivo de este estudio fue probar la efectividad de la DL-metionina aplicada a diversas fuentes de agua. Los experimentos de respuesta a la concentración realizados en frascos de vidrio utilizaron larvas de *Aedes aegypti* (L.) (Diptera: Culicidae) como organismo modelo. En el estudio se evaluó el agua de pozo, desionizada y de estanque. En general, el aumento de la mortalidad de las larvas de *Ae. aegypti* se presentaron con concentraciones crecientes de DL-metionina a las 48 h. Sin embargo, los valores de CL_{50} de DL-metionina de las larvas no fueron diferentes entre las fuentes de agua. Este estudio ha demostrado que se puede agregar DL-metionina a varias fuentes de agua como un posible larvicida biorracional cuando se aplica a fuentes de agua naturales como estanques o recipientes con agua que a menudo son los sitios preferidos de desarrollo larvario para una variedad de mosquitos vectores de enfermedades.

Palabras Clave: *Aedes aegypti*; larvas; Culicidae; pesticida; biorracional; estanque; concentración letal

Mosquitoes present an important threat to public health, transmitting pathogens to people worldwide. For example, *Aedes aegypti* (L.) (Diptera: Culicidae) transmits multiple arboviruses of importance throughout the world such as dengue fever, yellow fever, and Zika virus. Vaccines are unavailable for most mosquito-borne diseases. However, preventing contact between mosquitoes and human hosts is the best method to reduce pathogen transmission risk in the absence of a vaccine. Reducing the number of adult female mosquitoes should reduce the biting frequency and, therefore, the transmission rate. Application of larvicides to developmental sites substantially reduces subsequent adult mosquito populations, thereby preventing the transmission of pathogens by female mosquitoes.

There is a growing demand for biorational mosquito adulticides and larvicides to fill the gap of control options due to issues such as cancellation of product registrations, non-target effects, and pesticide resistance. Currently, mosquito larval populations are controlled using insect growth regulators and microbial agents. Insect growth regulators, although effective, are not mosquito specific and can have negative effects on non-target arthropods including beneficial insects and crustaceans that play essential roles in the environment (Magagula & Samways 2000). Microbial agents currently in use for controlling mosquito larvae are *Bacillus thuringiensis israelensis* Barjac and *Lysinibacillus sphaericus* (Meyer & Neide) Ahmed et al. (both Bacillaceae). Both bacterial species are more specific than insect growth regulators with no long-term effects on species richness, abundance, or non-target diversity (Derua et al. 2018). Unfortunately, there has been some evidence that mosquitoes can develop resistance to *Lysinibacillus* toxins (Wirth et al. 2000; Paul et al. 2005).

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We have shown previously that the amino acid methionine is an effective mosquito larvicide for control of multiple genera using laboratory assays (Long 2004; Weeks et al. 2018a). In those studies, larval *Aedes albopictus* Skuse, *Culex tarsalis* Coquillett, and *Anopheles quadrimaculatus* Say (all Diptera: Culicidae) were sensitive to the toxic effects of DL-methionine, with *An. quadrimaculatus* having a 10-fold greater sensitivity than the other species (Weeks et al. 2018a). L-methionine was shown to be highly toxic to *Ae. aegypti* larvae when exposed to levels that exceed insect dietary requirements (Long 2004). This effectiveness, coupled with the fact that resistance is unlikely to develop because it is an essential amino acid in mosquito diets at trace levels (Singh & Brown 1957), demonstrate its potential as a novel tool for mosquito management. DL-methionine is used globally as a livestock and aquaculture nutritional supplement (Nunes et al. 2014), therefore the industrial economies of scale favor the racemic DL form over the L stereoisomer for commercial use in mosquito control.

The toxic action of methionine on mosquitoes is likely that of a midgut toxin. Methionine-modulated cation channels have been documented in the midgut epithelium of *Manduca sexta* L. (Lepidoptera: Sphingidae) (Feldman et al. 2000; Quick & Stevens 2001; Stevens et al. 2002), that results in toxicity when provided at levels greater than physiological requirements. The mechanism of toxicity is limited to alkaline gut conditions of pH > 9.5 (Feldman et al. 2000; Quick & Stevens 2001; Stevens et al. 2002; Liu et al. 2003). Mosquito larvae are known to have an anterior midgut pH of approximately 10 (Dadd 1975). Furthermore, the mosquito gut expresses these same cation channels (Boudko et al. 2005a, b) and, therefore, it is likely that the concentration-induced mortality observed by Weeks et al. (2018a) is due to the effect of methionine on this channel.

Mortality is induced through ingestion of the amino acid by an insect with an alkaline gut. Black flies and cranes flies have an alkaline gut pH of 11.4 and 11.6, respectively, so they likely would be affected by DL-methionine toxicity (Undeen 1979; Martin et al. 1980). Conversely, non-target effects from methionine exposure are unlikely to occur in those insects that do not possess this gut pH level (Long 2004; Weeks et al. 2018b). For example, methionine was reported to be "practically nontoxic" to the western honey bee (*Apis mellifera* L.; Hymenoptera: Apidae) (Weeks et al. 2018b), which is believed to have an acidic gut pH. Moreover, aquatic insects with a neutral gut pH such as stone flies, caddis flies, and chironomids, probably would be unaffected (Martin et al. 1981a, b; Frouz et al. 2007). The goal of our study was to compare the effectiveness of DL-methionine at reducing mosquito larval survival when applied to various water sources as a larvicide.

Materials and Methods

INSECTS

Aedes aegypti eggs were obtained from the Veterinary Entomology Laboratory in the Entomology and Nematology Department at the University of Florida in Gainesville, Florida, USA. The colony was established > 20 yr ago, with no exposure to pesticides. Larvae were provided ground tropical fish food (0.3 g TetraMin® Tropical Fish Flakes, Tetra, Blacksburg, Virginia, USA) and reared in deionized water inside an incubator at 30 °C, 80% RH, and a 12:12 h (L:D) photoperiod. Second instars were used for all tests based on previous research by Weeks et al. (2018a).

BIOASSAYS

DL-methionine (≥ 99.0% AI; Sigma Aldrich, CAS Reg. No. 59-51-8, St. Louis, Missouri, USA) was prepared as a 1% stock solution and diluted in the appropriate water source to provide the following concentrations: 1.00%, 0.50%, 0.10%, and 0.05%. The physicochemical properties of DL-methionine include its maximal water solubility of 30 g per L at 20 °C (3% w/v), with pKa = 2.13.

DL-methionine was applied to 3 different water sources: deionized water, well water, and pond water. Deionized and well water were obtained from the University of Florida Entomology and Nematology Department. Pond water was acquired from a small pond in the University of Florida Natural Area Teaching Laboratory (29.633723°N, 82.367652°W). This water source is a frequently monitored healthy urban pond with a high diversity of plants, insects (including mosquitoes), fish, amphibians, and reptiles. At least 29 mosquito species have been reported from the Natural Area Teaching Laboratory (NATL 2013). Individual surface water samples (about 1 L) from each source were collected in Nalgene™ high density polyethylene bottles (Thermo Scientific™, Fisher Scientific, Hampton, New Hampshire, USA) on 5 Aug 2019 and delivered to the Florida LAKEWATCH laboratory (University of Florida, Gainesville, Florida). Samples were analyzed for total phosphorus, total nitrogen, specific conductance, and color following Florida LAKEWATCH standard operating procedures (Canfield et al. 2002; Hoyer et al. 2012).

Ten to 15 second instar *Ae. aegypti* were placed in glass jars (946 mL) that already contained 500 mL DL-methionine solution. As a negative control, mosquito larvae were placed in the corresponding water source without a DL-methionine treatment. Four replicates of each of the 5 treatments (4 DL-methionine concentrations and 1 control) and 3 water sources were assayed. Therefore, per treatment tested there was a total of 12 jars, 4 replicates, and 160 to 240 mosquitoes. Jars were placed in a controlled environmental chamber at 27 °C and a 14:10 h (L:D) photoperiod. Larvae were provided about 0.05 g of flaked tropical fish food daily. Mortality data was collected at 48 h. Larvae were scored as dead if they displayed no signs of movement, floated to the top, or displayed darker pigmentation. To ensure that the lack of larval movement meant the larvae were dead, jars were swirled in a circular motion because this action often caused live larvae to react and swim to the bottom of the jar. Between experiments, a 3-step cleaning procedure consisting of bleach, detergent, and rinsing with water was performed for each jar.

STATISTICAL ANALYSIS

Mortality data at 48 h collected from the assays were subjected to probit analysis using PoloPlus (PoloPlus 1.0; LeOra Software, El Cerrito, California, USA) (Throne et al. 1995). Mortality in controls was not corrected because it averaged < 5%, but control data were included in analyses. Concentration response curves were plotted, and lethal concentration values were calculated at 50%, 90%, and 99% for all 3 water sources. Nonoverlapping confidence intervals (95%) were used to determine significant treatment effects ($P < 0.05$).

Results

For all water sources, *Ae. aegypti* larval mortality consistently showed a positive correlation with DL-methionine concentration (Fig. 1; Table 1). The lethal concentration values varied slightly between the 3 water sources with well water and deionized water typically being closer in value and higher than pond water (Table 2). The LC_{90} and LC_{99} followed the same trend with DL-methionine treated pond water being the most toxic. However, the confidence intervals overlapped for all water sources at each lethal concentration value, indicating no significant difference between water sources on larval mortality in the pres-

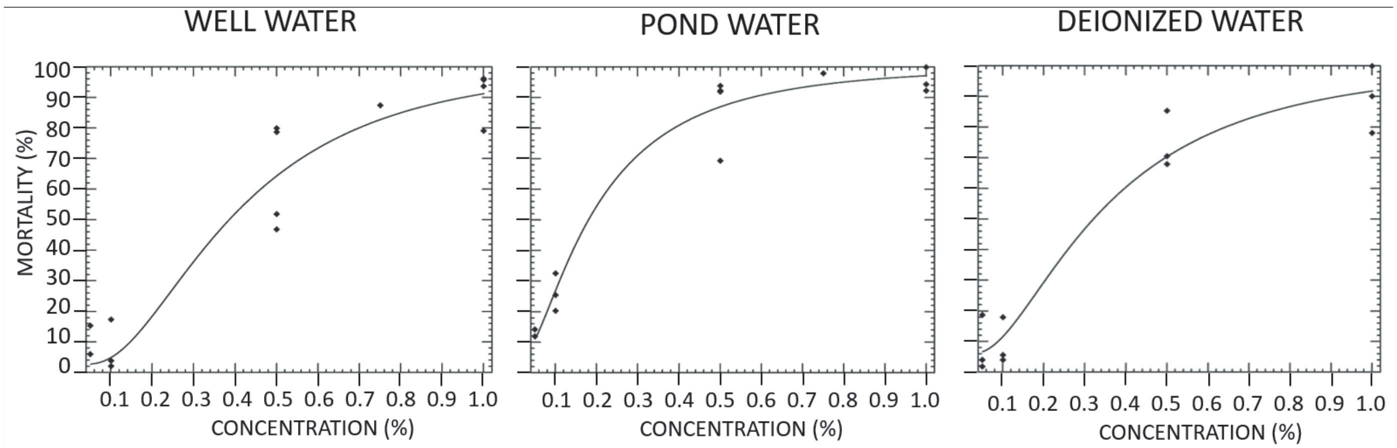

Fig. 1. Fitted percent concentration-mortality response curves of larval *Aedes aegypti* exposed to DL-methionine in well water, pond water, and deionized water.

ence of DL-methionine. Larval sensitivity (i.e., concentration-response slopes) to methionine treated deionized and pond water was similar (Table 2). However, larval sensitivity to this amino acid in treated well water was greater as indicated by a steeper slope compared with the other water sources. In addition, we observed reduced growth and development of treated larvae when compared with controls (EAR, NOA, personal observation).

The water analysis revealed that the total nitrogen values, as an indicator of organic matter content, were higher in well water (4,400 µg per L) and pond water (1,080 µg per L) compared with deionized water (190 µg per L) (Table 3). Well water contained the highest pH (7.5), and the lowest was deionized water (5.0).

Discussion

Before investing in time consuming and laborious operational field trials, it was desirable to determine if previous laboratory studies investigating DL-methionine toxicity for control of mosquito larvae by Long (2004) and Weeks et al. (2018a) likely would translate to natural water sources. Our study demonstrated that DL-methionine is effective at reducing survival of *Ae. aegypti* larvae regardless of the water source in which larvae were exposed. Although the lethal concentration values of methionine in the treated pond water were consistently lower than the other 2 water sources, their differences were not statistically significant. This finding indicates there is potential for DL-methionine to be slightly more effective in natural settings versus laboratory settings. Moreover, results of the present study imply that methionine is capable of killing mosquitoes under a variety of water quality conditions. For example, DL-methionine was effective within a pH range of 5.0 to 7.5, and from a total nitrogen content of 190 to 4,400 µg per L. If we consider total nitrogen to be an indicator of organic matter, then the water analysis results in our study combined with the mortality data indicate that organic matter does not influence the ability of DL-methionine to induce mortality in mosquitoes. Once ingested by the larvae, methionine's pKa of 2.31 favors high availability to its putative binding sites, which are the cation transporters of the highly alkaline (pH about 10) midgut epithelium (Feldman et al. 2000; Quick & Stevens 2001; Stevens et al. 2002; Boudko et al. 2005a, b). DL-methionine exposures in treated pond water resulted in the lowest lethal concentration values. The water quality analysis revealed that the levels measured in our pond water occurred between those of deionized water and well water. This indicated that DL-methionine performed best under the conditions provided by pond water. However, further research would be needed to confirm this hypothesis.

Although DL-methionine toxicity for control of *Ae. albopictus* has been demonstrated previously (Weeks et al. 2018a), this was the first study to test the mixture of methionine enantiomers effectiveness for control of *Ae. aegypti*. This latter species was selected for use in our study due to its importance as a vector known to be associated with human dwellings (Jansen & Beebe 2010). The LC_{50} for DL-methionine in well water to control second instar *Ae. aegypti* (0.39%) was comparable to the previously reported value for *Ae. albopictus* (0.37%) (Weeks et al. 2018a).

We also observed additional sublethal effects of DL-methionine that could limit the larval development of field populations of *Ae. aegypti*, such as delayed mortality, failure to pupate, and extended development times, possibly leading to reduced fecundity and fertility in adults. Future research could examine the effects of sublethal exposure to methionine. A limitation of our study is that we evaluated only 3 water sources, whereas many other natural aquatic habitats could have been tested as well as other mosquito species. However, our

Table 1. Percent mean mortality (± SE) of second instar *Aedes aegypti* when exposed to increasing DL-methionine concentrations after 48 h in each of the 3 water sources: deionized, well, and pond.

	% Mean mortality (± SE)		
Concentration (%)	Deionized water	Well water	Pond water
Control	5.98 ± 3.01	2.53 ± 0.98	4.12 ± 1.83
0.05	8.24 ± 5.29	10.75 ± 4.63	13.14 ± 1.14
0.10	9.25 ± 4.40	7.81 ± 4.82	26.16 ± 3.55
0.50	74.68 ± 5.44	64.40 ± 8.70	86.89 ± 5.85
1.00	89.46 ± 6.31	91.24 ± 4.06	95.58 ± 1.99

Table 2. Percent lethal concentration (LC; 95% confidence intervals) values of second instar *Aedes aegypti* when exposed to DL-methionine for 48 h in well water, pond water, and deionized water. *N* is the number of mosquitoes tested in 4 replicates.

Water source	*N*	Slope ± SE	LC_{50}	LC_{90}	LC_{99}	χ^2
Well water	884	3.309 ± 0.489	0.394 (0.117–0.523)	0.960 (0.744–2.483)	1.987 (1.217–24.345)	55.192
Pond water	872	2.599 ± 0.178	0.189 (0.138–0.243)	0.590 (0.461–0.808)	1.488 (1.041–2.541)	30.320
Deionized water	768	2.897 ± 0.290	0.341 (0.188–0.471)	0.944 (0.678–1.787)	2.165 (1.296–7.884)	44.482

Table 3. Water analysis of deionized water, well water, and pond water prior to treatment with DL-methionine.

Water quality	Deionized	Well	Pond
Color	Clear	Clear	Light yellow
pH	5.0	7.5	6.8
Total alkalinity (mg per L)	0.0	126.0	11.2
Cloride (mg per L)	0.0	17.5	5.5
Total phosphorus (µg per L)	5	159	80
Total nitrogen (µg per L)	190	4,400	1,080
Specific conductance (µS per cm at 25 °C)	1.5	358.0	47.6

results support additional investigations for the use of DL-methionine as a biorational larvicide when applied to natural water sources such as ponds or water-holding containers, which often are preferred larval developmental sites for a variety of mosquito disease vectors.

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