LARVICIDAL EFFECT OF SELECTED SALTS AGAINST THE DENGUE VECTOR MOSQUITO AEDES AEGYPTI (DIPTERA: CULICIDAE) IN BANGLADESH

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Abstract

The container breeding mosquito Aedes aegypti (Linnaeus) is the major universal vector of dengue viruses that cause dengue fever (DF), dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). Vector control has an important role in reducing human cases of DHF/DSS. The purpose of this work was to evaluate the larvicidal effect of salt solutions against the larvae of Ae. aegypti. Freshly collected larvae were transferred to the laboratory and reared using rainwater as the rearing medium, with yeast granules as larval food. Five salts, AgNO3, HgCl₂, CdCl₂, CuSO4, and CuCl₂, were tested to assess the larvicidal effect on both the 1st and 3rd instar larvae of Ae. aegypti. Serial concentrations (0, 1, 3, 5, 7 and 10 ppm) of each salt were prepared using distilled water as the solvent. Silver nitrate (AgNO₃) was noted as the most effective larvicide, followed by HgCl₂, CdCl₂, CuSO₄, and CuCl₂. The LC₅₀ values of AgNO₃ against 1st and 3rd instar larvae were 0.12 and 1.66 ppm, and the LC₉₀ values were 2 and 3.35 ppm, respectively. The LT₅₀ values of AgNO₃ (1 ppm) against 1st and 3rd instar larvae were 0.58 and 30.42 h, and the LT₉₀ values were 6.00 and 67.49 h, respectively. All 1st instar larvae died, failing to pupate in every salt concentration (1-10 ppm) within 7 days. Third instar larvae were also unable to pupate entirely in AqNO₃ and HqCl₂ solutions but a very few (5.00-36.66%) pupations were found at 7 ppm, with higher concentrations of CdCl₂, CuSo₄ and CuCl₂ salts. In addition, CdCl₂, CuSo₄ and CuCl₂ prevented 66.66%, 57.14% and 50% of adult emergences from pupae at 5 ppm concentration, respectively. The order of decrease of toxicity for larval mortality was AqNO₃>HgCl₂>CdCl₂>CuSO₄>CuCl₂, and in preventing pupation and adult emergence it was AqNO₃≥HqCl₂>CdCl₂>CuSO₄>CuCl₂. AqNO₃ was found to have very good potential in the killing of Aedes aegypti larvae, prevention of pupae formation and adult emergence. Therefore, the results obtained could be considered a contribution to the search for eco-friendly larvicides of natural origin. Further studies are needed to understand the residual aquatic toxicity of this salt in the field.

KEY WORDS: Vector, larvicidal effect, dengue, organic salts

Introduction

Aedes aegypti (L.) is the predominant vector of dengue virus, a mosquito-borne arbovirus belonging to the family Flaviviridae (Gubler & Kuno, 1997). It is also a recognized vector of the Zika, Chikungunya and yellow fever viruses. Like Plasmodium species, the causative agents of malaria, dengue virus has become a global threat (Pinheiro & Corber, 1997; Gubler, 1998). Dengue fever (DF) is an acute and painful disease that is often not lethal, but dengue hemorrhagic fever (DHF) may lead to dengue shock syndrome (DSS), i.e., circulatory failure that is often lethal within 12 to 24 h. Dengue virus is transmitted almost always from man to mosquito and vice versa (Rudnick & Lim, 1986). Dengue virus spreads rapidly and can develop into pandemic proportions (Jelinek, 2000). A recent study estimated that there were more people at risk of dengue infection, calculating up to 3.97 billion living in 128 countries (Brady et al., 2013). Both species of Aedes (Ae. aegypti and Ae. albopictus) mosquitoes frequently breed in artificial containers such as waterstorage vessels, flower vases, water accumulations, discarded tins, automobile tires and blocked gutters. These provide excellent larval habitats and adult resting sites (Curtis, 1991). Though DF has been reported as an urban disease (Gubler, 2004), recently dengue infection and the vector mosquitoes have been detected in rural areas of Thailand and India (Tewari et al., 2004; Mammen et al., 2008). Unplanned urbanization in cities and technological advancements in villages might have played a significant role in dengue transmission (Samuel et al., 2007; Pongsumpun et al., 2008).

The World Health Organization described mosquitoes as public enemy no. 1 (WHO, 1996). Mosquito control is a worldwide problem due to the vector nature and revival of various infectious diseases. The control of dengue transmission can be achieved by different strategies against the immature stages of *Ae. aegypti*. Application of control measures during these stages would increase the mortality of this vector before it reaches the adult stage (Bhat & Krishnamoorthy, 2017). The best way to manage dengue vectors is level control because breeding habitats are confined to limited stagnant water bodies. The most frequently used method of mosquito control worldwide is the use of synthetic insecticides (Burdick *et al.*, 1964). Several insecticides have been indiscriminately used against mosquito larvae and as a result they have developed, over the time, resistance to a wide variety of insecticides. *Ae. aegypti* has developed resistance to malathion, temephos, permethrin, propoxur (Brown, 1986; Lee & Lime, 1989) and fenitrothion (Wattanachai *et al.*, 1994). Although conventional insecticides have rapid effects, their adverse effects on humans and the environment are well known. Burdick *et al.* (1964) observed that residual toxicity may be magnified biologically in the food chain. On the other hand, salts are generally safer and more environmentally friendly than chemical pesticides. Therefore, the present study was conducted to determine the effects of salt solutions on the larvae of *Ae. aegypti*.

Materials and Methods

Mosquito rearing

The period from July to September has been reported as the peak dengue season in Bangladesh. *Ae. aegypti* larvae were collected from breeding sources at Amin Bazar Bus stops in Dhaka, Bangladesh. Freshly collected *Ae. aegypti* larvae were brought to the laboratory and kept for a short time in distilled water to remove dirty water from the breeding sites. The larvae were reared using rainwater (previously stored) as a rearing medium at the IRES (Insect Rearing and Experimental Station), Department of Zoology, Jahangirnagar University, Savar, Dhaka, at ambient temperature and relative humidity (28±2°C and 70±5% RH). In each rearing container (10x10x5 cm³), about 500 ml rainwater was added and 500 larvae were released. An amount of 0.10 g of yeast granules as larval food was added to each rearing container once a

day. Each container was placed inside a rearing cage (30x30x30 cm³) made of iron wire and covered with fine-mesh mosquito netting to prevent egg-laying by other mosquitoes. To avoid fungal contamination, the larvae were transferred to clean container every three days. Inspections were made in 6-h intervals to measure the water temperature, relative humidity, larval molting and wastage of food, if any. Periodically, surface film or scum food was removed with a syringe or brush and yeast granules (<0.02 g/100 ml) were added to the rearing medium, if needed. As the larvae developed, pupae were selected using a pipette and transferred to a glass jar (covered with gauze net). About 500 pupae were transferred to a new plastic bowl containing rainwater for adult emergence. No food was supplied for the non-feeding pupal stages. Adult mosquitoes were identified morphologically under stereomicroscopes using taxonomic keys (Barraud, 1934; Mattingly, 1971; Rattanarithikul, 1982; Amerasinghe, 1995; Rueda 2004) within a few hours after sampling.

A batch of 100 males and 300 females was housed together in a cage (30x30x30 cm³) for about 5-6 days to mate. Cotton pads soaked with 10% glucose solutions were placed inside the cage as a food supplement for the adults. Gravid females were then placed in another cage and a rock pigeon (*Columba livia*) was fastened to the roof of rearing cage for about an hour to provide a sucking blood meal for the adult mosquitoes. Five plastic cups (125 ml) filled with distilled water were lined with a 7-cm-wide strip of filter paper and placed inside each cage for egg-laying. Eggs were removed at regular intervals and kept in air-dried conditions for subsequent use. When required, these eggs were released into rainwater in a plastic bowl for hatching. During the period of hatching, 0.02 g of glucose or yeast granules were added to each 100 ml of distilled water as larval food.

Preparation of stock solutions

Salt solutions of different concentrations were prepared by serial dilution. Serial dilution is a method used to stepwise dilute a substance into a solution with a constant dilution factor at each step. In this method, 0.1 g of salt was mixed with 100 ml of water for a stock solution of 1000 ppm. After that, 1 ml, 0.7 ml, 0.5 ml, 0.3 ml and 0.1 ml of stock solution were mixed with 99, 99.3, 99.5, 99.7 and 99.9 ml of water, respectively, to prepare 10, 7, 5, 3 and 1 ppm salt solutions.

Larvicidal testing procedure

To test each of the salt solutions, 36 plastic cups (125 ml) were cleaned with distilled water. They were then air-dried and arranged in groups numbered 1, 2, 3, 4, 5 and 6, with six cups in each group. Each cup in groups 2, 3, 4, 5 and 6 was filled with 99.9, 99.7, 99.5, 99.3 and 99 ml of rainwater, respectively. Afterward, 0.1, 0.3, 0.5, 0.7 and 1 ml of the stock solutions of salt were added to each cup of groups 2, 3, 4, 5 and 6, respectively. The cups in group 1, to which no salt solutions were added, were used as the control. The 1st and 3rd instar larvae were selected for the study because they are easily differentiated, and separate sets of experiments were carried out for each instar. Fourth instar larvae were avoided due to the possibility of immediate pupation. Thus, a batch of ten larvae of each instar stage was collected from the rearing container and released into each cup of groups 2, 3, 4, 5 and 6. An amount of 0.2g of yeast granules was added regularly to each cup as larval food. All the plastic cups along with larvae were placed inside the rearing cage to prevent any contamination and egg-laying by other mosquitoes. Cups with a particular type of salt were kept in separate cages. The larvae that showed no signs of motion were considered dead. Their number was counted and recorded at each inspection. Inspections were made at 6-h intervals. Decomposing larval food was removed with a syringe from each cup during the last inspection of the day to eliminate any possible impact. A small amount (<0.02 gm/100 ml) of larval food was added to each cup if needed. The mortality of larvae (after 24 h), as well as the duration of larval instar stages, number of pupae, mortality of pupae and adult emergence were recorded.

Statistical analysis

LC₅₀, LC₉₀, LT₅₀, LT₉₀ at a 95% confidence interval (CI) were generated through probit analysis using the Statistical Package for Social Sciences (SPSS®) version 20 (SPSS 2007). Mean mortality of larvae, standard error, % of pupation, % of pupal mortality and % of adult emergence of mosquitoes were calculated using MS Excel (2013).

Results

Effects of salts on 1st and 3rd instar larvae

Data showing the LC₅₀ and LC₉₀ values along with a 95% CI of the different salts are presented in Table I. The LC₅₀ and LC₉₀ values of salts against 1st instar larvae ranged from 0.12 to 5.85 ppm and 2.00 to 11.50 ppm, respectively. The LC₅₀ values of AgNO₃, HgCl₂, CdCl₂, CuSO₄ and CuCl₂ were 0.12, 0.81, 1.47, 1.99 and 5.85 ppm respectively, whereas the LC₉₀ values were 2.00, 4.06, 5.69, 7.31 and 11.50 ppm. AgNO₃ had the lowest LC₅₀ (0.12 ppm) and LC₉₀ (2 ppm) values, and CuCl₂ registered the highest LC₅₀ (5.85 ppm) and LC₉₀ (11.50 ppm) values of the five salts tested.

The LC₅₀ values of AgNO₃, HgCl₂, CdCl₂, CuSO₄ and CuCl₂ against 3^{rd} instar larvae were 1.66, 1.84, 2.56, 3.29 and 7.84 ppm, respectively, and the LC₉₀ values were 3.35, 4.22, 6.33, 8.08 and 19.38 ppm, respectively (Table I).

Type of salt	LC values (ppm)						
		1 st instar	3 rd instar				
	LC ₅₀	LC ₉₀	LC ₅₀	LC ₉₀			
AgNO₃	0.12(0.09-0.14)	2.00(1.20-3.40)	1.66 (1.55-1.77)	3.35 (3.08-3.70)			
HgCl₂	0.81(0.52-1.05)	4.06(3.15-6.42)	1.84 (1.72-1.96)	4.22 (3.89-4.64)			
CdCl ₂	1.47(1.23-1.68)	5.69(4.70-7.49)	2.56(2.39-2.74)	6.33(5.62-7.37)			
CuSO₄	1.99(1.80-2.19)	7.31(6.01-9.79)	3.29 (3.08-3.54)	8.08 (6.96-9.88)			
CuCl ₂	5.85(5.51-6.30)	11.50(10.08-13.64)	7.84 (7.01-9.17)	19.38(15.31-26.85)			

Table I. Lethal concentrations (LC) of 1st and 3rd instar larvae of *Ae. aegypti* at 95% confidence interval of the salt solutions. Data in brackets represent the 95% of LC values against 1st and 3rd instar larvae.

Results on the LT₅₀ and LT₉₀ values for 1st instar larvae at 1 ppm concentration of all salts are shown in Table II. Of all the salts, the lowest LT₅₀ (0.58 h) and LT₉₀ (6.00 h) values were found at 1 ppm concentration of AgNO₃. In contrast, higher LT₅₀ values were 6.86, 23.37, 28.39 and 37.14 h at 1 ppm concentration for HgCl₂, CdCl₂, CuSO₄ and CuCl₂, respectively, whereas the higher LT₉₀ values for the salts were 14.06, 140.98, 177.36 and 220.63 h, respectively.

Of all the tested salts, the lowest LT₅₀ (30.42 h) and LT₉₀ (67.49 h) values were found at 1 ppm concentration of the AgNO₃ solution; on the other hand, the highest LT₅₀ (82.53 h) and LT₉₀ (213.17 h) values were found at 1 ppm concentration of the CuSO₄ solution (Table II). The higher LT₅₀ values (33.27, 62.07 and 82.53 h) and LT₉₀ values (73.99, 149.36 and 213.17 h) were found at 1 ppm concentration of HgCl₂, CdCl₂ and CuSO₄, respectively. Furthermore, it was observed that out of the five salts, AgNO₃ showed the lowest LC₅₀ and LC₉₀ values and the lowest LT₅₀ values at each concentration.

Type of salt			Lethal time (LT) against each concentration of a salt								
	LT Values	1 ppm		3 ppm		5 ppm		7 ppm		10 ppm	
		1 st instar	3 rd instar	1 st instar	3 rd instar	1 st instar	3 rd instar	1 st instar	3 rd instar	1 st instar	3 rd instar
AgNO3 LT50 LT90	LT ₅₀	0.58	30.42	0.30	3.11	0.18	2.14	0.09	1.32	0.02	1.31
	LT ₉₀	6.00	67.49	3.01	11.28	2.84	6.83	2.21	5.32	1.06	3.48
HgCl ₂ LT ₅₀ LT ₉₀	LT ₅₀	6.86	33.27	3.17	6.29	2.86	4.18	2.32	2.26	1.23	1.99
	LT ₉₀	14.06	73.99	12.04	24.55	7.52	20.64	3.26	8.90	2.08	7.73
CdCl ₂	LT ₅₀	23.37	62.07	9.49	10.55	7.49	10.31	4.97	6.13	3.68	3.69
	LT ₉₀	140.98	149.36	31.79	36.57	17.89	33.39	12.20	20.60	10.00	10.74
CuSO ₄	LT ₅₀	28.39	82.53	11.15	21.96	7.99	18.42	5.57	9.75	3.57	5.32
	LT ₉₀	177.36	213.17	46.41	36.90	31.22	35.37	17.02	25.16	10.92	13.34
CuCl ₂	LT ₅₀	37.11	93.14	21.88	30.14	13.21	22.65	10.45	16.54	5.64	13.61
	LT90	220.63	257.09	165.96	57.10	60.57	43.90	40.69	42.16	13.94	38.79

Table II. Lethal time of 1st and 3rd instar larvae (*Ae. aegypti*) for different concentrations of salt solutions.

Effects of salts on pupation and adult emergence

It was noted that 100% of 1st instar larvae died at all concentrations (1-10 ppm) of each salt within 7 days. All larvae died within 9, 6 and 3 h when exposed to 1 ppm, 3 ppm, and 5 ppm solutions of AgNO₃, respectively. On the other hand, 46%, 93% and 100% of 3rd instar larvae died within 24, 24 and 9 h when exposed to 1, 3 and 10 ppm solutions of AgNO₃, respectively. No pupation and adult emergence were observed of 3rd instar larvae at 7 and 10 ppm concentrations of the CdCl₂, CuSO₄ and CuCl₂ salt solutions (Table III).

Table III. Efficacy of salts on pupation, pupal mortality and adult emergence from 3rd instar larvae of Ae. aegypti

Type of salt	Concentrations (ppm)	Larval mortality (%)	Pupation (%)	Pupal mortality (%)	Adult emergence (%)
	1	78.34	21.66	53.84	46.16
	3	88.34	11.66	57.14	42.86
CdCl ₂	5	95	05	66.66	33.33
	7	100	00	00	00
	10	100	00	00	00
	1	73.34	26.66	50	50
	3	86.67	15	55.55	44.45
CuSO4	5	88.34	11.66	57.14	42.86
	7	100	00	00	00
	10	100	00	00	00
	1	63.34	36.66	36.36	63.64
CuCl ₂	3	81.67	18.33	45.45	54.55
	5	86.67	13.33	50	50
	7	100	00	00	00
	10	100	00	00	00

					Table III – continued
Type of salt	Concentrations (ppm)	Larval mortality (%)	Pupation (%)	Pupal mortality (%)	Adult Emergence (%)
AgNO₃	1	100	00	00	00
	3	100	00	00	00
	5	100	00	00	00
	7	100	00	00	00
	10	100	00	00	00
HgCl ₂	1	100	00	00	00
	3	100	00	00	00
	5	100	00	00	00
	7	100	00	00	00
	10	100	00	00	00

Discussion

The efficacy of salt solutions on the development/mortality of mosquito larvae might be due to their impact on the cells of the anal gill. Parallel result was reported by Wigglesworth (1933a), who found that cells of the anal gills of *Ae. argenteus* (Poir.) became swollen, perhaps due to the diffusion of hypertonic NaCl into the cells that was caused by differences in the concentrations of hemolymph and the external medium. He also stated that the concentration of NaCl in the cells rises above that in the hemolymph and water from the latter moves into the cells by osmosis. This difference in osmotic pressure between the body fluids and the external medium is the main factor behind the swelling of cells. MacFie (1914) also found that the destructive action of a 2% salt solution or higher on the larvae of *Ae. aegypti* (synonym *Stegomyia fasciata*) is due to the hypertonicity of the solution. Bradley (1994) stated that only 5% of all extant species of the family Culicidae are capable of surviving in saltwater and those species of *Aedes* that do not possess a segment on the rectum for the regulation of ions cannot survive salty water. In the present study, it was also observed that the anal gills of *Aedes* larvae became swollen by absorbing salt solutions, after which the larvae died. It was noted that all the salt solutions were able to kill 1st instar larvae of *Ae. aegypti* at a lower concentration. It is pertinent to mention here the findings of Suzuki (1959), who found that the LC₅₀ values of AgNO₃ and HgCl₂ were 1.7 ppm (0.00017%) and 1 ppm (0.0001%), respectively, against 4th instar larvae of *Culex pipiens pallens*.

In the present study, it was noted that AgNO₃ kills 60% of 3rd instar larvae at 1 ppm (0.0001%) concentration and when the concentration increased to 3 ppm (0.0003%), mortality also increased to 98.3%. Riaz *et al.* (2013) reported that a 10% NaCl solution killed the majority of *Ae. aegypti* larvae in the laboratory and 100% mortality was achieved within the minimum time when they were exposed to a 20% salt solution. MacFie (1922) reported that undiluted seawater killed the larvae of *Stegomyia fasciata* within 2 to 4 h, and 50% or more diluted seawater caused death after 24 h. Wigglesworth (1993) conducted experiments with NaCl and obtained good results with a 1.25% solution against *Aedes* species within 4-7 days. Wigglesworth (1933) also stated that 1% of NaCl is effective in killing larvae within a week and mortality increased with the increase in salt concentration. Suzuki (1959) studied the relation of the required time for death of 50% of larvae (LT₅₀ or TD₅₀) to the concentration of the salts and calculated the order of decreasing toxicity of the heavy-metal salts. He also stated that TD₅₀ increases in parallel to the decreases in salt concentration. A high larval toxicity of AgNO₃ against *Ae. aegypti* larvae was found in the present study. A similar result was found by Suzuki (1959) in Japan.

There was no pupation or adult emergence of 3rd instar larvae in solutions of AgNO₃ and HgCl₂. Though very few pupations (5.00-36.66%) were found in the other three salt solutions, they could not develop into adults (they died in the pupal stage) or the time of emergence might be prolonged. Our results support those of

Pappas & Pappas (1983) from Peru who reported that less than 50% of *Culiseta inornata* larvae reached the pupal stage at NaCl concentrations above 0.01 ppm.

Conclusion

In the present study, it was recorded that all the tested salt solutions were effective in killing both tested larval instars (1st and 3rd) of *Ae. Aegypti.* The salt solution with AgNO₃ showed the highest efficacy. Among the five salts, AgNO₃ had the lowest LT₅₀ and LT₉₀ values (0.58 and 6 h for 1st instar, and 30.42 and 67.49 h for 3rd instar, respectively). AgNO₃ and HgCl₂ solutions successfully prevented pupation and adult emergence of both instar (1st and 3rd) larvae. These promising results could be useful in the search for a more effective and eco-friendlier larvicidal product against *Ae. aegypti*, especially in regions where mosquitoes are highly resistant to chemical insecticides. However, the findings of this laboratory-based study need to be evaluated in the field. Additionally, further investigation into the residual toxicity on non-target organisms is very important and deserves attention.

Author contributions

KB and AJH designed the study. MZHI carried out the laboratory and field work and computed data and analysis. MZHI, KB and AJH collaborated in writing the manuscript. All authors read and approved the final manuscript.

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ЛАРВИЦИДНИ ЕФЕКАТ ОДАБРАНИХ СОЛИ НА КОМАРЦЕ ВЕКТОРЕ ДЕНГА ГРОЗНИЦЕ *AEDES AEGYPTI* (DIPTERA: CULICIDAE) У БАНГЛАДЕШУ

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Извод

Aedes aegypti (Linnaeus) је главни универзални вектор вируса који изазива денга грозницу (DF), денга хеморагичну грозницу (DHF) и синдром денга шока (DSS). Контрола вектора има важну улогу у смањењу случајева DHF/DSS код људи. Сврха овог рада је била да се процени ларвицидни ефекат раствора соли на ларве Ae. aegypti. Свеже сакупљене ларве су пребачене у лабораторију и узгајане користећи кишницу као медијум за узгој, са гранулама квасца којима се хране ларве. Пет соли, AgNO₃, HgCl₂, CdCl₂, CuSO₄, и CuCl₂, тестирано је да би се проценио ларвицидни ефекат на ларве 1. и 3. ступња Ae. aegypti. Серијске концентрације (0, 1, 3, 5, 7 и 10 ppm) сваке соли су припремљене коришћењем дестиловане воде као растварача. Сребро нитрат (AgNO₃) је забележен као најефикаснији ларвицид, а затим следе HgCl₂, CdCl₂, CuSO₄, и CuCl₂. Вредности LC₅₀ AgNO₃ у односу на ларве 1. и 3. ступња биле су 0,118 и 1,659 ррт, а вредности LC₉₀ биле су 2 и 3,347 ррт. Вредности LT₅₀ AgNO₃ (1 ppm) у односу на ларве 1. и 3. ступња биле су 0,575 и 30,42 h, а вредности LT₉₀ биле су 6,00 и 67,49 h. Све ларве 1. ступња су угинуле, не успевши да се улуткају у свакој концентрацији соли (1-10 ppm) у року од 7 дана. Ларве 3. ступња такође нису биле у стању да се улуткају у потпуности у растворима AgNO₃ и HgCl₂, али је пронађено врло мало (5,00-36,66%) лутки при 7 ppm, са вишим концентрацијама соли CdCl₂, CuSo₄ и CuCl₂. Поред тога, CdCl₂, CuSo₄ и CuCl₂ спречили су 66,66%, 57,14% и 50% изласка адулта из лутки при концентрацији од 5 ppm. Редослед смањења токсичности за морталитет ларви био је AqNO3>HqCl2>CdCl2>CuSO4>CuCl2, а у спречавању улуткавања и изласка одраслих AqNO₃≥HqCl₂>CdCl₂>CdCl₂>CuSO₄>CuCl₂. Утврђено је да AqNO₃ има веома добар потенцијал у убијању ларви Aedes aegypti, спречавању улуткавања и изласка одраслих. Стога се добијени резултати могу сматрати доприносом у потрази за еколошки прихватљивим ларвицидима природног порекла. Потребна су даља истраживања да би се разумела резидуална токсичност ове соли за воду на терену.

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