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EVALUATING THE RESPONSE OF *ANOPHELES COLUZZII* AND *CULEX QUINQUEFASCIATUS* MOSQUITO LARVAE TO SODIUM CHLORIDE AND DETERGENT POWDERS IN SEMI-FIELD CONDITION IN NIGERIA

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Abstract

Sodium chloride (NaCl) and detergent solution have been evaluated for mosquito larvae in laboratory studies but there is no evaluation for powdery salt and detergent. Therefore, this current study evaluated the response of *Anopheles coluzzii* and *Culex quinquifasciatus* larvae to salt and detergent powders in semi-field experiments. *Anopheles* and *Culex* larvae were collected from the wild fields. Twenty larvae were introduced into 100ml of water with 1g, 2.5g, 5g, 10g & 15g of salt and detergent. These corresponded to 0.01g/ml, 0.025g/ml, 0.05g/ml, 0.1g/ml &0.15g/ml respectively. The experiment was replicated into ten. Analysis of variance test was used for significance and Probit model analysis was used to predict lethal concentrations for 50% and 95%. Mean toxicity of larvae increased as concentration increased in all the treatments. *Cx. quinquifasciatus* and exposed to 10g and 15g of salt and detergent recorded complete toxicity. The differences between *Culex* and *Anopheles* mosquitoes exposed to salt and detergent were significant ($p<0.05$). Complete mortality was recorded in all but at different time. Complete mortality in *Culex* and *Anopheles* exposed to 15g of salt and detergent was after 10 minutes. Irrespective of the species, lethal time for 50% of mosquitoes ranged from 5.0 to 1560.8 minutes whereas LT95 range from 6.3 to 1893.3 minutes for salt and detergent exposure. *Culex* exposed to detergent recorded the lowest lethal time. So, the salt and detergent at best concentrations were effective and treated abandoned mosquito breeding site.

Keywords: Nigeria, Detergent, Efficacy, Mosquitoes, Powders, Salt, Sodium chloride

Introduction

Mosquitoes are vectors for numerous diseases, posing significant public health challenges globally. Mosquitoes, notably *Anopheles coluzzii* and *Culex quinquifasciatus*, are known carriers of diseases such as malaria and many arboviruses. The management of mosquito populations, rather than their eradication has been the key focus of public health initiatives, with the use of insecticides being a typical strategy. However, the emergence of insecticide resistance has led to the search for alternate approaches for mosquito control. The control of mosquito populations, especially in their larval stages, has become a focal point in strategies aimed at curbing the spread of mosquito-borne diseases. Chemical insecticides have been extensively applied in laboratory trials, semi-field and even field trials on mosquitoes (Marcombe *et al,* 2018; Derua *et al,* 2022; Sakka *et al,* 2023). However, the toxicological impact on the environment and the development of insecticide resistance underscores their potentials as valid alternative and sustainable control measures (Singh *et al,* 2012). More so, botanicals have been extensively applied on mosquitoes without any issues of environmental toxicology (Şengül and Canpolat, 2022). Studies have magnified the problems about the use of recommended control interventions, the sustainable adoption of plant materials with promising resultsin vector control (El-Hela *et al,* 2013), treating parasitic infections (Abouel-Nour *et al*, 2016) and even as anti-aging (El Fiky *et al,* 2022). The insecticide management, insecticide resistance assays, drug discoveries and advances in molecular approaches were reported as well (Benelli *et al,* 2016; Sougoufara *et al,* 2020; Ojianwuna *et al,* 2021).

The search for environment-friendly alternative substance from the plants and animals as well as inorganic compounds has been on the rise. This became necessary as insecticide resistance raised concerns worldwide and became a challenge for reducing mosquito population and practical solutions towards curing larval breeding sites. Many substances have been tried in the control of insect-vectors especially the mosquitoes larvae (Ojianwuna and Enwemiwe, 2022; Ojianwuna *et al,* 2021) Sodium chloride (NaCl) and detergents are essential substances for human use. NaCl as it is known as table salt has been used in seasoning, preservatives, food additives as well as laboratory applications. Salt has been reported for their efficacy in preserving dried plantain chips (Ojianwuna and Enwemiwe, 2021). Similarly, detergents are used as substance for laundry and other washing. Among the various uses, they have been spotted as key substances capable of causing a change in the water chemistry of mosquito breeding sites. NaCl and detergents are cost-effective substances common globally. Some studies have reported the potentials of salt in causing physiological changes such as disruption of osmoregulation and so on leading to desiccation in larvae (Kumar et al., 2017; Peng et al., 2018). Table salt have been tried on *Aedes* mosquitoes in laboratory subculture by Mukhopadhyay et al. (2010) with varied mortality at different time as with stages of development. *Aedes* mosquito adaptation to salt water suggested the need to examine the underlying mechanisms. The study of de Brito Arduino et al. (2015) opined that *Aedes* mosquitoes exposed to salt, adjusted in order to bypass the hurdle created by the presence of salt. Lukwa et al. (2017) reported that mortality in *Anopheles* mosquitoes was almost complete and salt content in water is a determinant for the occurrence of mosquito larvae. Even a more recent study by Guo et al., 2022 has reported the laboratory efficacy of salt in causing disruption in larval, egg and hatching in *Aedes* mosquitoes. No mortality was reported with salt exposure in adult mosquitoes (Yee et al. 2021).

Detergent powder, containing surfactants and other active compounds, has shown promise in disrupting the larval cuticle and interfering with respiratory processes, presenting an alternative avenue for mosquito control (Suleman et al., 2019; Umar et al., 2020). Numerous studies have outlined the efficacy of detergent solution in the interruption of mosquito larvae development (Antonio-Nkondjio *et al,* 2014; Mdoe *et al,* 2014). The study of VanderGiessen *et al.* (2023) opined that the ability of adult mosquitoes to locate host is altered by soap application. Despite these numerous studies conducted on salt and detergent, there exists a considerable knowledge gap regarding the potential efficacy of salt and detergent in their powdery form and in semi-field condition, their specific impact on the larvae of *Anopheles coluzzii* and *Culex quinquifasciatus*. More so, investigating the response of these mosquito species to sodium chloride and detergent powder is crucial for assessing the feasibility and practicality of these substances as larvicidal agents. As the global community seeks innovative and eco-friendly approaches to mosquito control, understanding the responses of specific mosquito species to unconventional larvicidal agents becomes imperative.

Materials and methods

Study area: This semi-field study was conducted around the Insectary, Department of Animal and Environmental Biology, Delta State University, Abraka. Temperature and relative humidity of the environment were measured and read 28.3±3.2^{o}C and 81±3%. Mosquito larvae were collected from Ethiope East LGA, Delta State. The immature stages were left in the lab. for 24 hours before exposure to the treatments. Salt (product name: Mr. Chef) and Detergent (product name: Viva detergent) were the treatment acquired from the local market in the area.

Sample collection and identification: Immature stage of mosquitoes was collected using ladles and scooping spoon. Ponds, puddles, tyre marks and ditches were prevalent natural breeding sites for the collection of mosquitoes (El-Bahnasawy *et al,* 2013). The emerged mosquitoes were morphologically identified as *Anopheles coluzzii* and *Culex quinquifasciatus* using the manual (Rueda, 2004; Coetzee (2020). Molecular was confirmed by identification of protocol outlined in the studies of Egedegbe *et al*. (2023) and Ojianwuna *et al.* (2022).

Experimental design: Twenty larvae from each of *Anopheles coluzzii* and *Culex quinquifasciatus* were put into 100ml of water contained 1g, 2.5g, 5g, 10g & 15g of salt and detergent (Ojianwuna and Enwemiwe, 2021). These corresponded to 0.01g/ml, 0.025g/ml, 0.05g/ml, 0.1g/ml & 0.15g/ml of tested materials. The experiment was replicate into ten. Acute mortality time was read for 5, 10, 15, 20, 30, 40, 50 and 60 minutes while chronic mortality was taken for 6 hours, 24 hours, and 48 hours. The environmental data was measured using thermo-hygrometer. Mortality reading was taken after 30 minutes to monitor acute toxicity using the Centre for disease Control timing for mosquitoes.

Statistical analysis**:** Toxicity value was collected, tabulated, computerized, and analyzed. Analysis of variance (ANOVA) test was used to check the significant difference within the treatment. Probit model was used to analyze the lethal concentration of 50% (LC_{50}) & 95% (LC_{95}) of the mosquitoes.

Results

Mean toxicity of mosquito larvae exposed to salt and detergent (Tab.1). Mean toxicity of larvae increased as concentration increased in all the treatments. *Cx. quinquifasciatus* and *An. coluzzii* exposed to 10g and 15g of salt and detergent recorded complete toxicity and this was followed by *Cx. quinquifasciatus* exposed to 5g of salt. *Anopheles* species exposed to 1g of salt caused lowest mortality. Differences between *Culex* and *Anopheles* mosquitoes exposed to salt and detergent were significant (p<0.05).

Toxicity time of mosquito larvae to salt and detergent was given. Mortality generally increased with time as concentration increased at 48 hours. Acute toxicity was highest in *Culex* exposed to salt (15g) and *Anopheles* mosquitoes exposed to detergent (15g). Complete mortality was recorded in all but at different time. *Culex* mosquitoes exposed to 15g of salt and detergent recorded complete mortality from 10 to 20 minutes respectively. Similar trend was observed for 10g of detergent exposed to same mosquitoes but that of salt was from 15 minutes. *Anopheles* mosquitoes exposed to salt and detergent recorded complete mortality from 40 minutes and 10 minutes respectively. Similar trend was observed in mosquitoes exposed to 10g of salt, but in mosquitoes exposed to 10g of detergent, complete mortality was recorded at 20 minutes post exposure period. There was no mortality recorded in Anopheles mosquitoes exposed to 1g and 2.5g of salt from 10 to 60 minutes. However, in Anopheles exposed to detergent there were no mortality recorded in 1g in less than 50 minutes and 2.5g in less than 20 minutes. This trend for Anopheles mosquitoes exposed to detergent compared favorably with *Culex* mosquitoes exposed to both treatments.

The lethal time of larvae exposed to salt and detergent is shown in Table 2. Lethal time for 50% in *Culex* mosquitoes exposed to salt & detergent ranged from 5.0 to 56.4 minutes whereas LT_{95} ranged from 6.3 to 73.6 minu-tes. *Anopheles* mosquitoes exposed to both treatments gave LT_{50} ranged from 7.6 to 1560.8 minutes and LT_{95} of 8.2 to 1893.3 minutes. Lowest lethal time was in *Culex* exposed to 15g of salt and closely followed by *Anopheles* exposed to 15g of detergent. Lethal times of mosquitoes were all significant (p<0.05)

Details were given in tables (1, 2, 3, & 4).

Table 1: Mean mortality of mosquito larvae exposed to salt and detergent in various concentration after 30 minutes

Mosquito	Conc. (grams)	Log dosage	Mean mortality in salt exposure	Mean mortality in detergent exposure
Culex	0.00	0.000	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
	1.00	0.000	$21.73\pm6.04d^{e}$	21.46 ± 5.44^{b}
	2.50	0.398	29.18 ± 5.58^{e}	32.60 ± 5.58^{d}
	5.00	0.699	59.50 ± 5.24^{f}	55.00 ± 4.58^{e}
	10.0	1.000	60.00 ± 4.53^{f}	60.00 ± 4.24^{f}
	15.0	1.176	60.00 ± 1.73^{f}	60.00 ± 4.28^{f}
Anopheles	0.00	0.000	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
	1.00	0.000	6.96 ± 3.67^{b}	21.83 ± 6.09^{b}
	2.50	0.398	11.33±4.85c	25.37 ± 5.69^{c}
	5.00	0.699	18.54 ± 5.49^{d}	57.19 ± 4.06^{e}
	10.0	1.000	60.00 ± 5.93^{f}	60.00 ± 3.93^{f}
	15.0	1.176	60.00 ± 4.99^{f}	60.00 ± 3.93^{f}

Concentrations in grams, mortality in mean ± standard error, mean values with different superscripts significance ($p<0.05$).

Table 2: Mean time mortality of *Anopheles coluzzii* mosquito larvae exposed to salt and detergent.

Treatment	Conc.	5	10	15	20	30	40	50	60	6hr	24hr	48hr
Salt	1	0.00^{A}	0.00^{A}	0.00^{A}	0.00^{A}	0.00^{A}	0.00^{A}	0.00^{A}	0.00^{A}	0.00^{A}	16.50^{B}	60.00^{D}
	2.5	0.00^{A}	0.00^{A}	0.00^{A}	0.00^{A}	0.00^{A}	0.00^{A}	0.00^{A}	0.00^{A}	4.50^{A}	60.00^{D}	60.00^{D}
	5	0.00^{A}	0.00^{A}	0.00^{A}	0.00^{A}	0.00^{A}	0.00^{A}	3.00^{A}	21.00^{B}	60.00^{D}	60.00^{D}	60.00^{D}
	10	0.00^{A}	0.00^{A}	0.00^{A}	0.00^{A}	28.50^{C}	60.00^{D}	60.00^{D}	60.00^{D}	60.00^{D}	60.00^{D}	60.00^{D}
	15	0.00^{A}	3.00^{A}	18.00^{A}	30.00^{C}	57.00^{D}	60.00^{D}	60.00^{D}	60.00^{D}	60.00^{D}	60.00^{D}	60.00^{D}
detergent	1	0.00^{A}	0.00^{A}	0.00^{A}	0.00^{A}	0.00^{A}	0.00^{A}	0.00^{A}	60.00^{H}	60.00^{H}	60.00^{H}	60.00^{H}
	2.5	0.00^{A}	0.00^{A}	0.00^{A}	0.00^{A}	3.00^{A}	15.00^{B}	21.00^{C}	60.00^{H}	60.00^{H}	60.00^{H}	60.00^{H}
	5	0.00^{A}	21.00^{C}	36.00^{E}	48.00^{F}	54.00^{G}	60.00^{H}	60.00^{H}	60.00^{H}	60.00^{H}	60.00^{H}	60.00^{H}
	10	0.00^{A}	27.00^{D}	51.00^{FG}	60.00^{H}	60.00^{H}	60.00^{H}	60.00^{H}	60.00^{H}	60.00^{H}	60.00^{H}	60.00^{H}
	15	0.00^{A}	60.00^{H}	60.00^{H}	60.00^{H}	60.00^{H}	60.00^{H}	60.00^{H}	60.00^{H}	60.00^{H}	60.00^{H}	60.00^{H}

Mean values with different superscript letter significance ($P<0.05$). Treatment concentration in grams, mortality in mean. Standard error for all= ± 0.64 for salt and 0.53 for detergent.

Table 3: Mean time mortality of *Culex quinquefasciatus* mosquito larvae exposed to salt and detergent.

Treatment	Conc.	5	10	15	20	30	40	50	60	6hr	24hr	48hr
Salt	1	0.00^{A}	0.00^{A}	0.00^{A}	0.00^{A}	0.00^{A}	0.00^{A}	3.00^{A}	60.00^{D}	60.00^{D}	60.00^{D}	60.00^{D}
	2.5	0.00^{A}	0.00^{A}	0.00^{A}	0.00^{A}	10.50^{AB}	25.50^{ABC}	40.50^{C}	60.00^{D}	60.00^{D}	60.00^{D}	60.00^{D}
	5	0.00^{A}	0.00^{A}	24.00^{ABC}	37.5^{BCD}	51.00^{CD}	60.00^{D}	60.00^{D}	60.00^{D}	60.00^{D}	60.00^{D}	60.00^{D}
	10	0.00^{A}	9.00^{AB}	60.00^{D}	60.00^{D}	60.00^{D}	60.00^{D}	60.00^{D}	60.00^{D}	60.00^{D}	60.00^{D}	60.00^{D}
	15	46.50^{CD}	60.00^{D}	60.00^{D}	60.00^{D}	60.00^{D}	60.00^{D}	60.00^{D}	60.00^{D}	60.00^{D}	60.00^{D}	60.00^{D}
Detergent	1	0.00^{A}	0.00^{A}	0.00^{A}	0.00^{A}	0.00^{A}	3.00^{A}	19.50^{B}	36.00^{D}	60.00^{F}	60.00^{F}	60.00^{F}
	2.5	0.00^{A}	0.00^{A}	0.00^{A}	15.00^{B}	30.00^{C}	46.50^{E}	55.50^{F}	57.00^{F}	60.00^{F}	60.00^{F}	60.00^{F}
	5	0.00^{A}	0.00^{A}	33.00^{CD}	43.50^{E}	55.50^{F}	60.00^{F}	60.00^{F}	60.00^{F}	60.00^{F}	60.00^{F}	60.00^{F}
	10	0.00^{A}	19.50^{B}	34.50^{CD}	60.00^{F}	60.00^{F}	60.00^{F}	60.00^{F}	60.00^{F}	60.00^{F}	60.00^{F}	60.00^{F}
	15	0.00^{A}	15.00^{B}	46.50^{E}	60.00^{F}	60.00^{F}	60.00^{F}	60.00^{F}	60.00^{F}	60.00^{F}	60.00^{F}	60.00^{F}

Mean values with different superscript letter significance ($P<0.05$). Treatment concentration in grams; mortality in mean.

Table 4: Lethal time of mosquito larvae exposed to salt, detergent and ashes of some plants

Larvae	Treatments	Log Conc. (gm)	Regression Line	R^2	LT_{50}	LT_{95}
Culex	salt	0.000	y=0.75x-39.2	0.97	52.2	54.4
		0.398	y=0.10x-4.16	0.73	42.3	59.0
		0.699	y=0.14x-2.72	0.68	19.5	31.3
		1.000	y=1.36x-14.6	0.91	10.8	12.0
		1.176	y=1.21x-5.98	0.64	5.0	6.3
	detergent	0.000	y=0.10x-5.38	0.75	56.4	73.6
		0.398	y=0.08x-2.70	0.64	32.4	52.1
		0.699	y= 0.13x-1.99	0.59	15.7	28.6
		1.000	y= 0.26x-3.35	0.72	13.0	19.3
		1.176	y= 0.33x-4.08	0.78	12.4	17.4
Anopheles	salt	0.000	y= 0.01x-7.72	0.53	1560.8	1893.3
		0.398	y= 0.02x-7.16	0.92	450.6	554.2
		0.699	y= 0.13x-8.23	0.85	62.9	75.5
		1.000	y= 0.61x-18.43	0.71	30.1	32.8
		1.176	y= 0.16x-3.15	0.73	19.5	29.7
	detergent	0.000	y=1.21x-66.3	1.00	54.9	56.2
		0.398	y=0.10x-5.14	0.75	48.2	63.7
		0.699	y= 0. 21x-1.79	0.57	14.8	28.4
		1.000	y= 0.32x-3.50	0.75	11.1	16.3
		1.176	y= 2.70x-20.57	1.00	7.6	8.2

Note: 50% & 95% Lethal time, LT_{50} & LT_{95}, in minutes; Adjusted R: R^2 all showed significance at $p< 0.0001$

Discussion

In modern day, it is a must to try and incorporate effective affordable substances with potentials to improve mosquitoes' control, with a focus on reducing both the economic cost and insecticide resistance. To help with this, powders of sodium chloride and detergent were tried for possible recommendation. Many studies focused on the solution of salt and detergent on mosquitoes based on different concentrations (Mukhopadhyay *et al,* 2010; Prasantong, 2010; Mdoe *et al,* 2014; Sudarmaja and Swastika, 2015; Oji-anwuna and Enwemiwe, 2021). But this is the first study that has evaluated the response of mosquitoes to the powdery form of the treatment under study. Moreover, our data set included higher concentrations in grams which have not been reported. In these several concentrations of the experiments, the best concentrations conferred total mortality on the mosquitoes. The total mortality with salt may be due to that salt concentration affected the regulatory surfaces of the larvae because of reduced water volume. Mortality due to detergent may be due to the active components and that the powdery droplets might have blocked the spiracle of the mosquito larvae.

In this study, lowest concentration caused above 20% mortality was in *Culex* species. The mortality in *Anopheles* mosquitoes exposed to the lowest concentration of detergent compared favorably to *Culex* mosquitoes exposed to lowest concentration of both treatments. *Anopheles* mosquitoes exposed to 1.00g of salt recorded the least in this experiment. The differences in mortality may be ascribed to the larvae positioning in the natural breeding site (Prasantong, 2010). Although studies explored the larvicidal potential of detergent solutions against *Aedes aegypti* larvae (Mukhopadhyay *et al*, 2010), yet it was noteworthy that, to the best of present authors knowledge that the investingations specifically focused on the detergent powder remain absent in the existing literature. The present study contributes to the gap by assessing the detergent powder efficacy, providing novel insights into its larvicidal capabilities. The absence of prior study on detergent powder for the mosquito larvae underscores the significance of the present results and thus opens avenues for more exploration in this critical domain.

In the present study, the toxicity of mosquito larvae increased with the increased in concentration of detergent in all treatments. Complete toxicity was recorded in both the mosquito species when exposed to 10g & 15g of detergent. Sudarmaja and Swastika (2015) reported that even though the concentrations were different from those reported in this present study. The time toxicity of mosquito larvae to detergent increased with concentration, and complete mortality was observed at different times for different concentrations.

The mean toxicity of mosquito larvae exposed to salt increased as concentration rose in each treatment. *An. coluzzii* and *Cx. quinquifasciatus* subjected to 10g and 15g of salt reported total toxicity, and *Cx. quinquifasciatus* mosquitoes subjected to 5g of salt followed favorably. The lowest mortality was seen in *Anopheles* mosquitoes breeding container treated with 1 gram of salt. Toxicity of mosquitoes increased with increasing time. *Culex* exposure treated with 15g of salt showed the highest level of acute time toxicity. Mortality in mosquito larvae could be due to dehydration caused by osmotic pressure caused by increased salt concentration. It is also possible that the larvae were killed by the toxicity of the salt instead of the osmotic pressure, most likely via the gut. Patrick *et al.* (2001) reported that concentration-related responses were evident in the study on the effects of various NaCl concentrations on *Anopheles gambiae* s.l. larvae. But, Jude *et al.* (2012) showed 100% larval mortality at lower NaCl concentrations and thus lower salinity concentrations didn't kill *A. gambiae* s.l. larvae completely in the present study. This suggested that the mosquitoes in Nigeria are predisposed to the natural salt ions that might cause salt resistance in terms of toxicity. However, de Brito *et al.* (2015) indicated a larger margin of larval survival (up to 14ppt salinity). Also, the present res-ult agreed with Sanchez-Ribas *et al.* (2015), who have taken into account fluctuations in salt levels the prevalence impact of mosquito larvae. But, in the present study, didn't ex-amine this feature, and de Brito *et al.* (2015), who found that salt levels in breeding locat-ions didn't affect *Ae. aegypti* egg-laying ten-dencies. These findings, however, rule out the use of salt in disease control programs to prevent mosquito egg lying even while the larvae were killed, as vector mosquitoes would continue to lay eggs regardless of salinity levels. Similar to the present results, de Brito *et al.* (2015) reported a decline in *Ae. aegypti* larvae when salt content rose in breeding water areas significant in understanding larval ecology for disease prevention.

The measure of lethality in time of these mosquito larvae exposed to salt and detergent is crucial for understanding the practical implications of using these substances for mosquito control. Irrespective of the mosquito species exposed to detergent powder, the lethal time for 50% and 95% was between 7.6 and 56.4 minutes, and 8.2 and 73.6 minutes respectively. Similarly, exposure to salt recorded ranges between 5.0 and 1560 minutes, 6.3 and 1893.3 minutes. This show that detergent powders performed better probably due to the chemical components incorporated unlike salts that acted probably based on the ions. The study provides valuable insights into the potential use of salt and detergent as larvicidal agents for mosquito control in semi-field conditions, as it bridges the gap between laboratory and field trials, providing valuable insights into the practicality and feasibility in field settings.

Conclusion

Data significantly reported the potential of salt and detergent as the alternative larvicidal agents for both mosquitoes control.

The toxicity increased with the higher cencrations and time of salt and detergent, with significant differences in mortality between the two mosquito species; highlight the effectiveness of these substances in the higher concentration for controlling mosquitoes. More study is ongoing to understand the effectiveness of these substances, and environmental impact on non-target species.

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