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Laboratory studies on the effect of inorganic fertilizers on survival and development of immature *Culex quinquefasciatus* (Diptera: Culicidae)

Ephantus J. Muturi[a], Benjamin G. Jacob[a], Josephat Shililu[b] & Robert Novak[a]

[a]*Department of Medicine, William C. Gorgas Center for Geographic Medicine, Birmingham, Alabama, USA;* [b]*Human Health Division, International Centre of Insect Physiology and Ecology, Nairobi, Kenya*

Abstract

Background & objectives: Studies were conducted to determine the effect of ammonium sulfate (AM) and muriate of potash (MOP) fertilizers on survival and development of immature stages of *Culex quinquefasciatus* Say, a major vector of Bancroftian filariasis in Africa.

Methods: Twenty I instar larvae each were added in four doses of each fertilizer dissolved in one litre of deionised water and in one litre of deionised water as a control in replicates of five. The larvae were monitored every morning throughout their life and the numbers of each instar surviving were recorded. The experiments were discontinued when all the larvae had died or emerged into adults.

Results: An analysis of variance test and Tukey's HSD test revealed a significant impact of fertilizers on survival and development of aquatic stages of *Cx. quinquefasciatus*. Ammonium sulfate accounted for up to 40% mortality rate, and one week delay in development time and this effect was both instar and dose dependent. None of the MOP dosages had significant impact on survival of immatures of *Cx. quinquefasciatus* and only the higher dosages showed significant impact on development time but in significantly lower magnitudes compared with similar dosages of ammonium sulfate.

Interpretation & conclusion: These findings demonstrate the toxic effect of fertilizers on immature stages of *Cx. quinquefasciatus* contrary to field observations.

Key words *Culex quinquefasciatus* – development time – fertilizers – survival

Introduction

Culex quinquefasciatus Say (Diptera: Culicidae) is one of the dominant mosquito species in irrigated rice agro-ecosystems in Africa[1,2] and has been linked with an increase in transmission and prevalence of Bancroftian filariasis in these areas[3,4]. *Cx. quinquefasciatus* is also considered a potential vector of arboviruses and an important pest species in irrigated rice agro-ecosystems[2]. Important factor promoting the abundance of *Cx. quinquefasciatus* in these areas is its ability to utilise inundated rice fields for larval development[5]. Proper understanding of the factors contributing to the distribution and relative abundance of *Cx. quinquefasciatus* in rice fields is therefore an important pre-requisite of vector control operations. Unfortunately, little is known about the factors that regulate spatio-temporal distribution of rice land mosquitoes. Where studies have been done, the focus has mainly been on malaria vectors at the expense of other species[6–8].

A large number of variables act interdependently to influence the abundance of rice land mosquitoes.

These include; physico-chemical and biotic factors, quality and amount of available food, and several agricultural practices. These parameters fluctuate greatly during the course of rice growing cycle affecting distribution and abundance of mosquito larvae. High algal productivity and associated photosynthesis result in an increase in dissolved oxygen concentration favouring the breeding of *Cx. vishnui* and their predators[9]. The level of organic matter and temperature of the water are important determinants of the amount of food available for mosquito larvae[10, 11]. An increase in rice height has direct effect on some mosquito species by obstructing gravid females from ovipositing and supporting greater diversity of aquatic predators[12,13]. Rice canopy cover may also reduce the amount of sunlight reaching the water surface resulting in lower temperatures. This in turn causes a decline in microbial growth upon which mosquito larvae depend on and increases larval development time exposing them to greater risks of contact with potential predators and competitors[14].

The use of agricultural inputs such as pesticides and fertilizers also produce significant impact on larval abundance in rice fields. Service[15] observed a resurgence of mosquito larvae after initial control with insecticides due to slow rebounding of predator populations. An increase in larval abundance has also been reported after application of organic and inorganic fertilizers in rice fields. In Madurai, India, the populations of *Cx. vishnui, Cx. tritaeniorhynchus* and *Cx. pseudovishnui* (Diptera: Culicidae) increased in a dose-related manner after application of inorganic nitrogen fertilizers[16]. In Mwea, Kenya, the populations of *Anopheles arabiensis* (Diptera: Culicidae) and culicine larvae were significantly higher in ponds treated with ammonium sulfate (AM) than in the control[7]. Mogi[10] observed a strong relationship between fertilizer application and increased pupation rate.

Despite the important role of fertilizers in supporting high populations of vector mosquitoes, little is known about the mechanisms under which they act to regulate mosquito populations. Sunish and Reuben[17] suggested that fertilizer application results in rapid multiplication of microorganisms upon which mosquito larvae feed on. Alternatively, ammonium sulfate has been suggested to act as an oviposition attractant by reducing water turbidity[7]. The objective of the present study was to determine the effect of ammonium sulfate (AM) and muriate of potash (MOP) fertilizers on survival and development of immature stages of *Cx. quinquefasciatus*.

Material & Methods

The experiment was conducted using laboratory strain of *Cx. quinquefasciatus* originally from Mwea Irrigation Scheme, Kenya maintained at Medical Entomology Laboratory, University of Illinois at Urbana-Champaign, USA. Within 6–12 h of egg hatch, 20 *Cx. quinquefasciatus* larvae were transferred to larval pans (23 x 29 cm) containing 0.422 g, 0.845 g, 1.2675 g and 1.690 g of either muriate of potash or ammonium sulfate dissolved in one litre of deionised water. These doses represented one-half, full dose, one and a half and twice the doses used in Mwea rice fields, Kenya (50 kg/acre). A control was included in which larvae were placed in one litre of deionised water without any treatment. Each treatment was replicated five times and therefore, the experiment included 45 larval pans containing 900 larvae. Larval pans were maintained at 26°C and 70% RH throughout the study. The larvae were fed with Tetramin fish food [Tetra Holding (U.S.) Inc. Blacksburg, VA, USA] every other day up to III instar when rabbit food was introduced. The pans were examined every 24 h and the number of larvae of each instar present in each pan was recorded. To do this, an observer, put his head close to the water surface and counted every individual in the larval pan. Since the number of larvae in each pan were only 20, it was possible to count each individual larva with minimum error. It is only on very rare occasions that more larvae were found in one census than in the previous census. In such cases, it was assumed that the extra individual was present in the previous census. Although it was not possible to state for certain the instar of every individual, instar size classes were distinct enough that the majority of individuals were categorised appropriately. In addition to larval instars, individuals were also categorised as pupa and adult. The pupae were transferred into larval cups containing the same kind of treatment in which the larvae developed. Pupae from each replicate of a treatment were placed in a cage and emerging adults were counted and recorded. The experiment was terminated when all mosquito larvae had died or emerged.

Statistics: All statistical analyses were performed using SPSS version 11.5 (SPSS, Inc., Chicago, IL, USA). One way analysis of variance was used to determine the effect of treatment on larval development time, the number of individuals surviving in each life stage, and the probability of surviving from one stage to the next. Tukey's HSD test was further used to examine pairwise differences among treatments in cases where a significant effect of treatment was observed. The time taken for at least 50% of the larvae in a pan to change to the next instar was taken as development period of that particular instar and the mean for the five replicates gave the mean development time.

Results

Results of ANOVA and Tukey's HSD tests revealed a strong effect of treatment on the number of larvae, pupae and adults surviving (Table 1). The numbers of I instar larvae surviving did not differ significantly among treatments. However, the numbers of other larval stages as well as the pupae and adults surviving were significantly lower in AM treatments equal to or greater than 1.2675 g compared with other treatments. Among the remaining treatments, survival of IV instar larvae, pupae and adults was also significantly lower in treatments with 1.2675 g of MOP and 0.845 g of AM than in the remaining treatments. The probability of survival from one larval stage to the next and from IV instar to pupa was generally lower in treatments containing 1.2675 and 1.690 g of AM compared with the other treatments. The 0.845 g treatment of AM had a similar effect as the higher dosages of the same treatment on probability of sur-

Table 1. ANOVA results for number of larvae and survival for each treatment

Response variable	Sum of squares	df	Mean square	F	P-value	Tukey's HSD
No. of larvae						
II instar	22.000	8	2.750	7.500	<0.001	h,i< a,b,c,d,e,f,g
III instar	99.644	8	12.456	28.025	<0.001	h,i< a,b,c,d,e,f,g
IV instar	218.178	8	27.272	62.936	<0.001	h,i< d,g<a,b,c,e,f
Pupae	386.400	8	48.300	57.197	<0.001	h,i< d,g<a,b,c,e,f
Adults	403.600	8	50.450	40.181	<0.001	h,i< d,g<a,b,c,e,f
Probability of survival from						
I to II instar	550.000	8	68.750	7.500	<0.001	h,i< a,b,c,d,e,f,g
II to III instar	824.035	8	103.004	5.152	<0.001	h,i<g<a,b,c,d,e,f
III to IV instar	974.325	8	121.791	6.393	<0.001	g,h,i<a,b,c,d,e,f
IV instar to pupa	1489.476	8	186.184	6.316	<0.001	h,i<a,b,c,d,e,f,g
Pupa to adult	82.965	8	10.371	1.213	0.320	

a = Control; b–e = MOP 0.422 g, 0.845 g, 1.2675 g and 1.690 g, respectively; f–i = AM 0.422 g, 0.845 g, 1.2675 g and 1.690 g, respectively.

vival of III to IV instar. In addition, the probability of survival of II to III instar in this treatment was also lower than the remaining treatments. The effect of treatment on probability of survival from pupa to adult did not vary among treatments (Table 1; and Figs. 1 & 2).

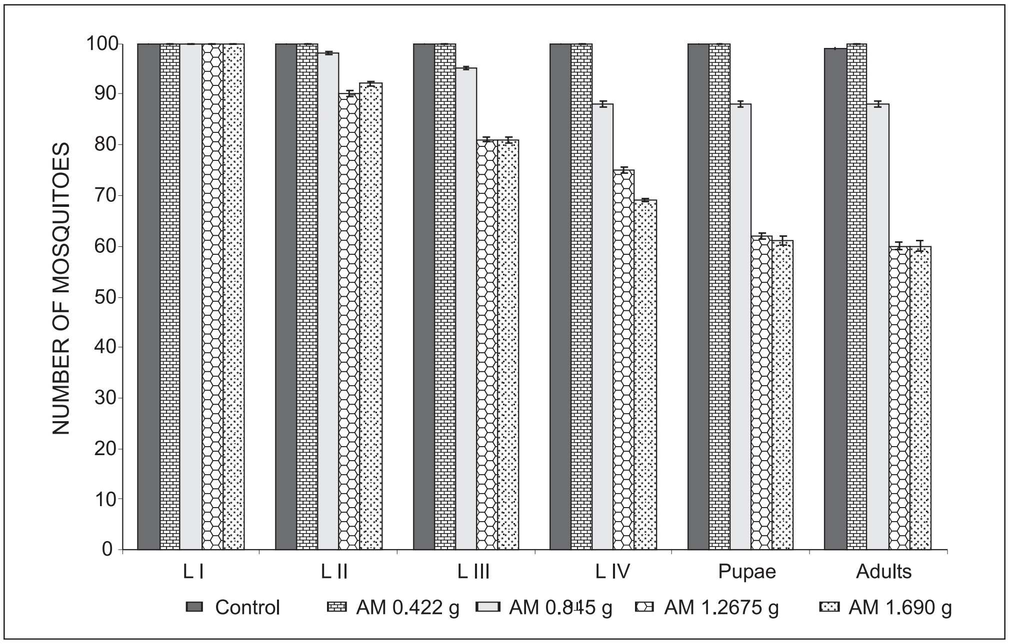

Fig. 1: Number of mosquitoes (± standard error) alive in each life stage in each treatment of ammonium sulfate (AM)

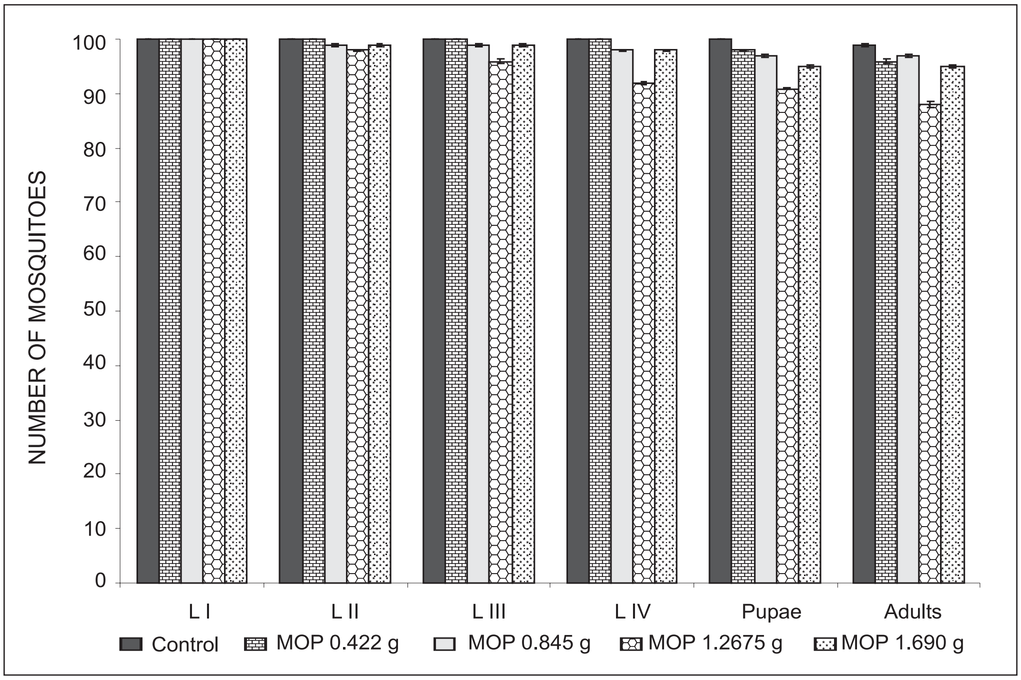

Fig. 2: Number of mosquitoes (± standard error) alive in each life stage in each treatment of muriate of potash (MOP)

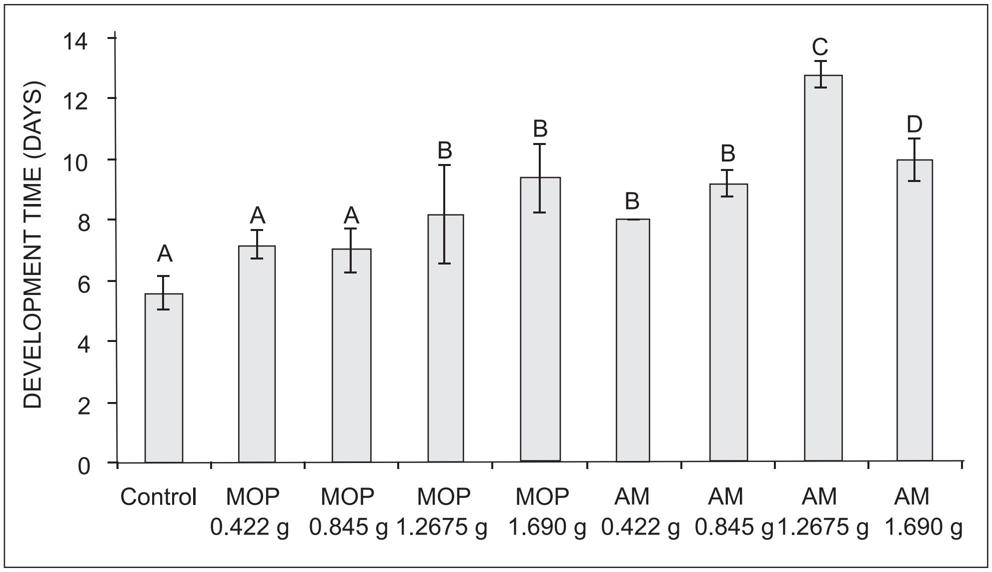

Fig. 3: The average number of days (± standard error) taken by *Cx. quinquefasciatus* larvae to develop from I instar to adult in each treatment. Columns with same letters are not significant while those bearing different letters are significant at 0.05 level of significance

The development time of *Cx. quinquefasciatus* from I instar to adult stage differed significantly among the different fertilizer treatments ($F = 33.025$, df = 36, $p < 0.001$, Fig. 3). It took approximately six days for larvae in the control to develop into adults and this was not significantly different from seven days in the 0.422 and 0.845 g treatments of muriate of potash. However, it took 2–3 days longer than the control treatment for larvae to develop into adults in 1.2675 and 1.690 g treatments of MOP and 0.422 and 0.845 g treatments of AM. In addition, it took 4 and 7 days longer than the control for the larvae in 1.690 and 1.2675 g of ammonium sulfate, respectively to complete development.

Discussion

In the current study, fertilizer application had a significant impact on survival and development of aquatic stages of *Cx. quinquefasciatus*. These effects were more pronounced in AM treatments than in the MOP treatments. None of the MOP dosages had significant impact on survival of immatures of *Cx. quinquefasciatus* and only the higher dosages showed significant impact on development time but in significantly lower magnitudes compared with similar dosages of AM. Ammonium sulfate accounted for up to 40% mortality rate and up to one week delay in development time. Interestingly, the effects of 1.2675 and 1.690 g of AM on survival of *Cx. quinquefasciatus* immatures were similar, contrary to the expected dose-dependent relationship. Moreover, the larvae developed much slower in 1.2675 g than in 1.690 g of AM. Because fertilizer application enhances multiplication of microorganisms[18], it is likely that the higher dosage (1.690 g) of AM promoted growth of microorganisms that were able to break down the fertilizers thereby reducing their toxic effects. Also, the larvae may have acquired the ability to compensate for the damages caused by higher dosages of AM. Alternatively, the differences in dosages between the two groups might have been too little to impact significantly on larval survival. Further studies using widely spaced dosages could reveal more information.

The mechanisms under which fertilizers act to impact larval survival and development time is least known. At physiological level, it is likely to involve ionic balance of the larvae. Considering that fertilizer treatment did not affect the probability of survival from pupae (non-feeding) to adults, it appears that consumption of treated water together with food materials may have interfered with ionic balance of the larvae resulting in reduced survival rate and increased development time. The breakdown of ammonium sulfate into NH_3^+ and SO_4^{2+} could impact negatively on mosquito larvae. At high level of internal concentration, ammonia is known to interrupt nervous transmission[19] and this could result in high mortality rate and increased development time. Exposure of two snail species to 1–1.25 g/L doses of AM for 48 h resulted in 100% mortality[20]. Sulphur-coated urea has also been shown to be a possible mosquito control agent in rice fields[21], suggesting that the sulphur component of AM may partly have contributed to the observed mortality. Higher levels of muriate of potash (KCl) may also affect mosquito osmotic balance since K^+ and Cl^- ions are the primary constituents of urine in larval malpighian tubules[22].

Fertilizer application in rice fields has been reported to enhance multiplication of microorganisms, which form the main diet of mosquito larvae and in particular, ammonia nitrogen has been found to be an oviposition attractant[18]. Therefore, in contrast to the current findings, fertilizer application in rice fields has been shown to increase larval abundance[7] in a dose-related manner[16] and also to accelerate pupation rates[10]. However, it should be noted that in the field, several factors may act interdependently to impact larval abundance and survival, and fertilizer may be one of these factors[5, 17, 23]. Fertilizer application in the rice fields is also applied mostly by broadcasting and this result in heterogeneous distribution of fertilizers and mosquitoes may have the ability to discriminate areas with toxic dosages of fertilizers and avoid them. In addition, the rate of fertilizer breakdown and utilisation may be greatly higher in the rice fields because of presence of a wide range of biotic factors including decomposition microorganisms, aquatic animals and vegetation including rice plants. In contrast, the fertilizers used in this study were scaled and dissolved in one litre of deionised water before the larvae were introduced. Secondly, the study was restricted to larval pans and only the food material was added. The rate of fertilizer decomposition and utilisation may therefore have been much slower than in the field, providing a toxic environment for mosquito larvae. Further studies on the effect of fertilizers on survival and development of rice land vector mosquitoes should be conducted with the aim of devising control strategies based on farm managed practices.

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Corresponding author: Dr. Ephantus J. Muturi, Department of Medicine, William C. Gorgas Center for Geographic Medicine, 206-C BBRB, 845 XIX Street South, Birmingham, Alabama–35294, USA.
E-mail: emuturi@uab.edu

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