THE ROLE OF AGRICULTURAL CHEMICALS ON MALARIA VECTORS' FITNESS IN A RICE AGRO-ECOSYSTEM IN KENYA

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Abstract

Control of African malaria vectors continue to depend on insecticides through Indoor residual sprays or Insecticide treated nets but insecticide-resistance is a hindrance to its excellence. An improved knowledge of mosquito ecology could inform better vector control measures. This study intended to establish any negative effects of agrochemicals on malaria vector 'fitness' in rice agro ecosystems in Mwea irrigation scheme. Four agro-chemicals in paddy and simulated field experiments were evaluated for their effects on vector 'fitness'. Pupae were sampled in agro-chemical exposed paddies and mosquito eggs incubated in simulated conditions of different agrochemical combinations. Vector 'fitness' was defined as longevity and size of emergent *Anopheles* mosquitoes. The mean longevity of agrochemical exposed *Anopheles* mosquitoes was 6.5 days in the paddy experiment and 7.1 days in the simulated experiments**.** Respective controls had a mean life span of 18 days in the paddy and 15 days for simulated experiment. Comparison for differences in these life spans on paired t- tests gave statistically significant results (P = 0.003) for the paddy trial, and for simulated experiment (P =.000). These results were taken to suggests that agrochemical exposed mosquitoes suffer reduced longevity compared to their non agrochemical exposed controls in both trials. This finding could indicate that agrochemicals can be a passive integrated vector control tool.

Key words: **Agrochemicals**, **Longevity, Size, *Anopheles* Spp**.

Introduction

Malaria is one of the most important diseases that affect humans in endemic areas in Africa. Worldwide there were 216 million cases of malaria in 2010, 81% of these in the African Region. An estimated 3.3 billion people were at risk of malaria in 2010 (WHO, 2011). An estimated 655 000 persons died of malaria in 2010. 86% of the victims were children under 5 years of age, and 91% of malaria deaths occurred in the WHO African Region (WMR, 2011). In Kenya, Malaria is still the leading cause of morbidity and mortality according to the Kenya malaria fact sheet 2012. 25 million out of a population of 39 million

Kenyans are at risk of malaria while it accounts for 30-50% of all outpatient attendance and 20% of all admissions to health facilities (MoPHS, 2011). Malaria prevention interventions play a pivotal role in the reduction of the disease burden among venerable populations. In spite of the current high level of research into the biology of the malaria parasite, control of malaria transmission in the near future will continue to depend on vector control (Clive *et al.,* 2002).

In some malaria endemic regions, adulticiding and the use of ITNs is compromised by the occurrence of insecticide resistance in the major malaria vectors (Hargreaves *et al.,* 2000; Antonio-Nkondjio *et al.,* 2011). With reports from 45 countries around the world of insecticide resistance to at least one of the four classes of insecticides used for malaria vector control and 27 of these being in sub-Saharan Africa (WHO, 2011), the war against malaria is yet to be won. The development and deployment of innovative and cost-effective malaria vector control strategies is necessary. One such method has been reduction of mosquito larvae habitats by environmental management and manipulation (Ault, 1994; Fillinger *et al.,* 2006). The current intra-domiciliary interventions, ITNs and IRS remain the most effective means for malaria vector control (WHO, 2009). However, these interventions are not sufficient to meet the goal of malaria elimination as their benefits are limited to indoor biting mosquitoes while neglecting outdoor biting populations (Pates *et al*., 2005). In Africa, indoor spraying has eliminated the endophilic *A. gambiae* Giles in some areas, only to have it replaced by the more exophilic *A. arabiensis* Parton (Hargreaves *et al.,* 2000). The vector potential of mosquitoes is inclined to their size and lifespan (longevity) (Ameneshewa and Service, 1996). Mosquito size affects the vector capacity by determining the chance of actively foraging for a host. Wing length is used as a proxy for determination of the size of a mosquito and is well correlated with weight in various studies (Ameneshewa and Service, 1996). A big sized mosquito may have advantage of covering a larger area when searching for a blood meal due to its advanced flight range. Small mosquitoes however might have increased transmission potential as they may have increased feeding cycles, which may increase their chance of infection with *Plasmodium* parasites (Tanga *et al.,* 2010). Mosquitoes must live beyond 12 days, which is required for sporogony to succeed as malaria vectors (Michel *et al.,* 2008).

The use of agro-chemicals in rice agro-ecosystem could influence the choice of mosquito ovi-position sites by gravid mosquitoes as well as subsequent development of the aquatic stages of malaria vectors (Muturi *et al.,* 2007). A clear understanding of how agrochemicals influence mosquito 'fitness' needs to be studied. Adult mosquito 'fitness' in rice agro-ecosystems may form a basis for designing integrated malaria vector control management strategies (Diuk-Wasser *et al.,* 2004).

Materials and methods.

Study area

This study was carried out in Mwea rice irrigation scheme located 120 km North East of Nairobi. Mwea occupies the lower altitude zone of Kirinyaga District in an expansive low-lying area. Geographically,

Mwea lies between latitude 1° S of the equator, longitude 37.5° E and an altitude of 1900M above sea level. The average maximum temperatures are in the range of 16 - 29.5°C. Relative humidity varies from 52–67%. This is an area of stable, endemic malaria where *An. gambiae* Giles, *An. arabiensis* , and *An. funestus* Giles all contribute to transmission (Ijumba *et al.,* 1990).

The longevity and size experiments of *Anopheles arabiensis* were conducted during the months of October to December 2010, at the commencement of rice transplantation from nurseries to the paddies. On transplantation rice is top-dressed with fertilizers and pesticides fumigated to protect crop damage by pests and thus enhance yields. This area has a good vector borne disease laboratory, which was used for laboratory rearing of mosquitoes.

Study design

In order to get comparative data on lifespan and size of mosquitoes, two study designs were utilized. In the field studies, observational design was used. Pupa stage was sampled from paddies exposed to agrochemicals as a presumed agrochemical contaminated natural habitat. However, randomized experimental design was used in the simulated semi-field trials where the investigators exposed mosquito eggs to different agrochemical combinations and followed the emergent *Anopheline arabiensis* mosquitoes to establish their longevity and size.

Four days post transplanting, pupae were sampled in the 3 paddies on a weekly basis until the desired sample size of 192 female *Anopheles* mosquitoes was attained. This sampling method has been used earlier (Mwangangi *et al.,* 2006). Control pupa samples were collected from areas without exposure to agrochemicals in the village settlement areas simultaneously. Sampled pupae were held in holding cups with paddy-water from the sampled plot and transported to the insectary. The cups were placed inside netted emergence wire cages until emergence.

Control mosquito pupae were sampled in animal hoof spools; bicycle and vehicle ruts, tree holes and discarded cans in settled areas of the three villages where rice paddies had been selected. The collection of pupae in these areas was based on the assumption that it was highly unlikely the habitats had any agro-chemical exposure as these were found in the housing areas with no rice growing. Emerging female *Anopheles arabiensis* in individual holding cups were placed in humified holding boxes and fed on 6% glucose soaked wicks. (Clements *et al.,* 1992; Foster, 1995) and observed until their death.

In the simulated trials a suitable ground was selected within the Kimbimbi district hospital compound about 500 meters from the nearest paddies. Three replicate bowls (capacity 10 litres each) per agro-chemical combination were used to simulate field conditions at the selected site (Mboera *et al.,* 2000). Based on water loading rates of 100,000 liters of water per hectare and a 1:4 soil water ratio in rice paddies (KARI Mwea – Desk information unpublished, Muturi *et al.,* 2007) and agrochemical specific manufactures' instructions, bowl composition was arrived at as follows:-
Sulphate of ammonium– 50kgs/Hectare (0.845 g, /l)

Diammonium phosphate– 50kgs /hectare (0.845 g, /l)

Alpha-cypermethylin - 0.1mls /litre

Thiophanate methyl– 0.15mls/litre.

One liter of canal water was measured using a standard measuring jar and 8.45 g of each fertilizer dissolved. Nine more liters of water were added to make a final concentration of 0.845g/L, which is the standard concentration in paddies at 50kg/hectare. This concentration had been previously used in determining survival of *Culex quinquefasciatus* Say in inorganic fertilizers (Muturi *et al.,* 2007). 7.5 liters of the fertilizer and canal water solution were poured into the 10 liter bowl and soil used to fill up to imitate the 1:4 soil water ratio in paddies.

Anopheles arabiensis Kisumu strain eggs were collected from mouse fed mosquitoes in the Mwea DVBD laboratory. Standard rearing procedures and holding conditions for the experimental mosquitoes were used (Knols *et al.,* 2002; Mathenge *et al.,* 2002). Each bowl was inoculated with 50 eggs of *Anopheles arabiensis* Kisumu strain. Egg inoculation was done ten days after introduction of agrochemicals into the bowls this being the period observed for increased larvae densities after pesticide sprays in paddy fields (Mutero *et al.,* 2008). Netting material was used over the bowls to avoid undesired oviposition from natural wild populations. Observations were made daily and any pupa developing from the bowls was transferred to individual emergence cups. Emerging female *Anopheles arabiensis* in individual holding cups were placed in humified holding boxes and fed on 6% glucose soaked cotton wicks daily (Clements *et al.,* 1992; Foster, 1995) and observed until their death like in the field collected samples. Replicate bowls without agrochemicals were incorporated in the experiments as controls. The longevity in days was recorded as the time from emergence to time of death. Upon death, one wing was removed and measured to the nearest 0.01 mm using an ocular micrometer under a dissecting microscope.

Table 1: Agrochemicals combination treatments in simulated experiments

TREATMENT	AGROCHEMICAL COMPONENT
TREATMENT 1 (DSTA)	Di-Ammonium Phosphate, Sulphate of ammonia, Thiophanate methyl and Alpha-cypermethylin
TREATMENT 2 (D)	Di-Ammonium Phosphate only
TREATMENT 3 (DAT)	Di-Ammonium Phosphate, Alphacypermethylin, and Thiophanate methyl
TREATMENT 4 (SAT)	Sulphate of ammonium, Thiophanate methyl and Alphacypermethylin

TREATMENT 5 (DA)	Di-Ammonium Phosphate and Alphacypermethylin
TREAMENT 6 (DT)	Di-ammonium phosphate and Thiophanate methyl
TREATMENT 7	Control – No chemicals

Sample Size Determination

The *analytical Group inc.* free automatic sample calculation software was used to determine the minimum sample size as modified and developed from Cochran's (1977) sample size formula

$$n = \frac{\frac{z^2 P(1-P)}{d^2}}{1 + \frac{1}{N}\left(\frac{z^2 P(1-P)}{d^2} - 1\right)}$$

Therefore n=1.96^2 *0.53*0.47/0.053^2/2

The minimum sample size =192

Data Management and Analysis

To evaluate the effect of agro-chemicals on vector fitness of the emergent adults, the minimum sporogonic incubation period of *Plasmodium falciparum* in the semi-field environment, was estimated at 10 - 12 days (Craig *et al.,* 1999). *Anopheles arabiensis* has a wing length range of 2.8-3.4mm (Giles, 1972). Data on longevity and wing-length of mosquitoes was compared among different treatments and compared to the respective controls using SPSS version 18. Paired sample t - test analysis was used to calculate significant differences on size and longevity between experiments and control.

Ethical considerations

The National Council for Science and Technology granted research authorization and approval . Paddy owners gave informed consent for use of their paddies in this study.

Results

Table 1: Paired sample t-tests comparison of pooled means of longevity and size of emergent adult *Anopheles* in field and simulated experiments 1 versus its controls.

Paired Sample Test									
	Variables	Paired Differences			95% Confidence Interval of the Difference		t	df	Sig. (2-tailed)
		Mean	SD	SEM.	Lower	Upper			
Pair 1	Mean LSP. field experiment Mean LSP. field control	-11.0	1.1	.60	-14.2	-8.8	-180	2	.003
Pair 2	Mean WL field experiment Mean WL field control	.187	.51	.30	-1.08	1.5	.640	2	.590
Pair 3	Mean LSP simulated experiment Mean LSP simulated control	-7.60	1.3	.52	-9.00	-6.3	-14.6	5	.000
Pair 4	Mean WL simulated experiment Mean WL simulated control	.190	.17	.068	.02	.37	2.82	5	.037

LSP- life span/longevity in days WL- wing length /size in mm

Simulated experiment (Treatment) 1 - Di-Ammonium Phosphate, Sulphate of ammonia, Thiophanate methyl and Alpha-cypermethylin.

In this experiment, where all the agrochemicals were combined, the mean lifespan of was 4.7 days against a mean of 14 days in the control. Mean mosquito wing-lengths in this treatment and control were approximately 3.8 and 3.5 mm respectively (Figure 1).

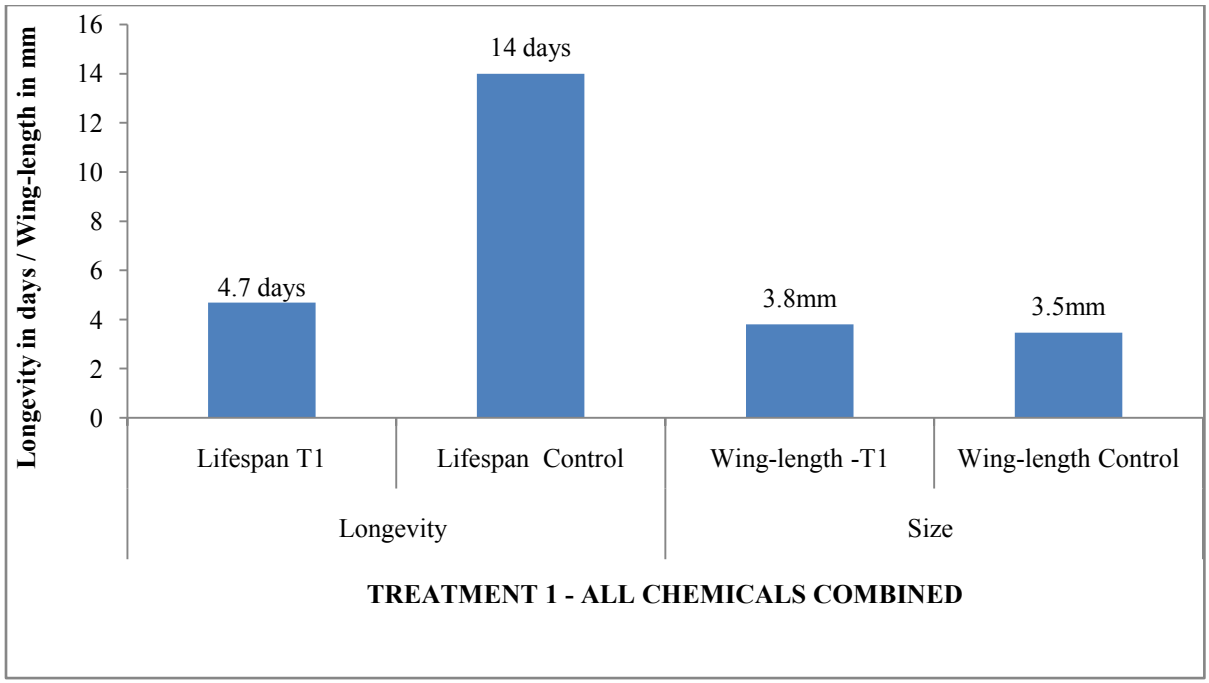

Figure 1: Comparison of longevity and size of mosquitoes in simulated experiment 1 (All chemicals) and the simulated experiment control

Paired t- test treatment (experiment) T1

In treatment one both the mosquito longevity and wing-length were significantly different at 0.05 level. Longevity was statistically different from control (P = 0.000). Wing length (P = 0.013) was likewise significantly different from that of the control (Table 2)

Table 2: Paired t - tests comparing the differences between mean mosquito longevity and mean wing length in treatment T1 (All chemicals) against the control

Variable	Paired Differences					t	df	Sig. (2-tailed)
	Mean	S. D.	S.EM	95% CI of the Difference Lower	Upper			
Pair 1								
Life span T1 Lifespan Control Wing length T1	-9.30	6.53	1.6	-12.8	-5.80	-5.71	15	.000*
Wing length Control	.338	.477	.12	.083	.592	2.83	15	.013
Pair 2								

Discussion

The findings in this study revealed reduced longevity of *Anopheles* vectors in paddies and simulated field conditions when exposed to some agrochemical combinations either singly or in combination and therefore reduced vectorial 'fitness'. This finding is collaborated with other related studies on disease vectors. For example, larval and adult nutrition critically affect not only survival, but also flight potential (Nayar and Sauerman, 1971a; 1971b;1975), biting persistence (Nasci, 1991), and thus disease transmission by mosquitoes (Nayar and Sauerman, 1975; Foster, 1995).

The mean longevity of agrochemical exposed *Anopheles* mosquitoes was 6.5 days in the paddy experiment and 7.1 days in the simulated experiments**.** The controls had a mean life span of 18 days in the field and 15 days for simulated experiment. Comparison for differences in these life spans on paired t- tests gave statistically significant results (P- 0.003) for the paddy trial, and for simulated experiment (P- .000). This suggests that agrochemical exposed mosquitoes suffered reduced longevity compared to their non agrochemical exposed controls in both trials. The field control mean mosquito lifespan of 18 days did not compare well with the expected longevity in the wild mosquito lifespan of 34 days as documented by Giles *et al*. (1968). This reduced longevity in study control samples can be explained by rearing laboratory conditions of the vectors from pupae stage to death. Laboratory rearing of mosquitoes does not provide plant sugars common in the field conditions. In similar experiments, mosquitoes lacking natural sugars had shortened lifespan than those in sugar rich fields (Gu *et al.,* 2011). The vector fitness of malaria

transmitting mosquitoes is largely dependent on the size of the mosquito and its longevity (Ameneshewa and Service, 1996). This suggests few agrochemical exposed vectors survive long enough to transmit *Plasmodium* parasites in irrigated agro-ecosystems.

Conclusion

In this study larvae exposure to agrochemicals resulted in tangible reduction of adult life span. Since mosquitoes at larvae stage in irrigated paddies cannot change or choose their habitat, these findings show that control interventions targeting larvae are still promising in malaria vector control. Larvae habitat characteristics greatly influence the fitness of emergent anopheles mosquito vector. This study has generated further ecological knowledge on relationships between aquatic and terrestrial stages of malaria vectors on size and longevity. This study confirms interplay of rice irrigation and malaria transmission and that vectors bred in irrigated-agrochemical exposed habitats may have phenotypic variations that reduce their longevity.

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