



RESEARCH ARTICLE

Fine-scale spatial and temporal heterogeneities in insecticide resistance profiles of the malaria vector, *Anopheles arabiensis* in rural south-eastern Tanzania [version 1; referees: 2 approved]

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Abstract

Background: Programmatic monitoring of insecticide resistance in disease vectors is mostly done on a large scale, often focusing on differences between districts, regions or countries. However, local heterogeneities in residual malaria transmission imply the need for finer-scale data. This study reports small-scale variations of insecticide susceptibility in *Anopheles arabiensis* between three neighbouring villages across two seasons in Tanzania, where insecticidal bed nets are extensively used, but malaria transmission persists.

Methods: WHO insecticide susceptibility assays were conducted on female and male *An. arabiensis* from three proximal villages, Minepa, Lupiro, and Mavimba, during dry (June-December 2015) and wet (January-May 2016) seasons. Adults emerging from wild-collected larvae were exposed to 0.05% lambda-cyhalothrin, 0.05% deltamethrin, 0.75% permethrin, 4% DDT, 4% dieldrin, 0.1% bendiocarb, 0.1% propoxur, 0.25% pirimiphos-methyl and 5% malathion. A hydrolysis probe assay was used to screen for L1014F (*kdr-w*) and L1014S (*kdr-e*) mutations in specimens resistant to DDT or pyrethroids. Synergist assays using piperonyl butoxide (PBO) and triphenol phosphate (TPP) were done to assess pyrethroid and bendiocarb resistance phenotypes.

Results: There were clear seasonal and spatial fluctuations in phenotypic resistance status in *An. arabiensis* to pyrethroids, DDT and bendiocarb. Pre-exposure to PBO and TPP, resulted in lower knockdown rates and higher mortalities against pyrethroids and bendiocarb, compared to tests without the synergists. Neither L1014F nor L1014S mutations were detected.

Conclusions: This study confirmed the presence of pyrethroid resistance in *An. arabiensis* and showed small-scale differences in resistance levels between the villages, and between seasons. Substantial, though incomplete,

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reversal of pyrethroid and bendiocarb resistance following pre-exposure to PBO and TPP, and absence of *kdr* alleles suggest involvement of P450 monooxygenases and esterases in the resistant phenotypes. We recommend, for effective resistance management, further bioassays to quantify the strength of resistance, and both biochemical and molecular analysis to elucidate specific enzymes responsible in resistance.

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Introduction

In sub-Saharan Africa, malaria vector control relies predominantly on insecticide-based, methods, namely long-lasting insecticide treated bed nets (LLINs) and indoor residual spraying (IRS) of households. In Tanzania, LLINs are widely distributed and used as the primary and most affordable protective measure against disease vectors¹⁻³. The country has also recently implemented IRS, as a complementary vector control intervention in the north-western regions, with 11.6% – 14% of households currently covered by IRS^{4,5}. Globally, implementation of LLINs and IRS, coupled with improved case diagnosis and treatment, as well as urbanization, improved living standards, and overall improvements in health systems, have contributed to 37% and 60% reduction of malaria morbidity and mortality respectively, between 2000 and 2015⁶. In Tanzania, high malaria transmission remains, with an average prevalence of 14.8% in children under 5 years⁷. Nevertheless, the National Malaria Control Program currently has a strategic goal of reducing malaria prevalence to 1% by 2020⁸.

Despite the recent successes, efficacy of current malaria interventions is hampered by numerous challenges, particularly insecticide resistance in malaria vectors⁹⁻¹¹. This has necessitated continuous insecticide resistance monitoring and periodic changes of insecticides used¹²⁻¹⁵. Some countries have put in place mechanisms to monitor susceptibility of malaria vectors to insecticides using guidelines provided by the World Health Organization (WHO) Global Plan for Insecticide Resistance Monitoring (GPIRM)¹⁶. However, due to limited resources, insecticide resistance monitoring is mainly carried out only at large scale, often focusing on differences between districts or regions^{9,14}. In Tanzania, insecticide susceptibility monitoring in mosquito populations is conducted at district level, relying on designated sentinel sites in regions, considered to be representative of the whole country^{14,15}. Such a generalized approach to insecticide resistance monitoring is not very effective to capture local variations, where there might be pockets of high and low malaria transmission areas^{17,18}. The variations may be due to, among other factors, impacts of interventions or genetic differences in mosquito populations, in turn resulting in physiological differences in response to insecticidal pressures^{17,18}.

Different mosquito populations respond differently to insecticide pressure, depending on presence or absence, and type of resistance genes prevalent in the population¹⁹⁻²¹. This results in occurrence of geographically distinct populations, which might result in transmission variability over space and time. It is likely that these fine scale-variabilities are associated with the occurrence of residual mosquito biting “hotspots”, contributing to persistent residual malaria transmission in areas where LLINs and IRS are already widely used¹⁸. Despite this, most vector surveillance programs still use global approaches without taking population variability into consideration. Furthermore, insecticide resistance studies have mainly focused on adult female mosquitoes, with limited studies on male populations.

The present study aimed at evaluating insecticide susceptibility of the dominant malaria vector, *An. arabiensis*, at a fine-scale between

nearby villages in south-eastern Tanzania, where insecticides have been widely used for public health and agriculture, but where malaria transmission still persists.

Methods

Study villages

Sampling of mosquito larvae was carried out in three proximal villages of Minepa (-8.2665°S, 36.6775°E), Lupiro (- 8.3857°S, 36.6791°E), and Mavimba (- 8.3163°S, 36.6810°E), located in Ulanga district, south-eastern Tanzania (Figure 1). The minimum distance between villages was ~4km from Minepa to Mavimba, while the maximum distance was 9km from Minepa to Lupiro. All the villages lie between 120 and 350 meters above sea level, and are located in the flood plains of the Kilombero river, between the Udzungwa mountain ranges to the north, and Mahenge hills to the south¹⁻³. The main economic activity of the area is irrigated rice farming. The irrigation leaves rice paddies continuously flooded, creating permanent water bodies favourable for mosquito breeding habitats. It is also a perennially meso-endemic malaria area, where transmission is predominantly by *An. funestus s.s* and *An. arabiensis*²²⁻²⁵. Recent multiple assessments conducted in the same area have revealed that 100% of the *An. gambiae s.l* mosquitoes in this study area were *An. arabiensis* sibling species^{25,26}. As such, all field-collected *An. gambiae s.l* mosquitoes are henceforth referred to as *An. arabiensis*. The main malaria vector control intervention in the area is LLINs¹⁻³.

Mosquito sampling and rearing

Larval collections were carried out in the dry season between June and December 2015, and in the wet season between January and May 2016. For each village, between seven and nine breeding sites were identified, geo-referenced, and permanently established as larval sampling points for resistance monitoring during this study. Immediately after sampling, larvae were separated into anophelines and culicines to prevent cannibalism, and for easier adult morphological identifications. After morphological identification, larvae were pooled by village and reared into adults under standard insectary conditions (temperature of 27 ± 3°C and relative humidity 70–90%) in a semi-field screen house²⁷. During rearing, larvae were fed on mud, and algae collected from the respective breeding sites, and supplemented with Tetramin® fish food (Tetra, Melle, Germany). Each morning, pupae were transferred into a plastic cup and placed in a net-covered cage for adult emergence. After emergence, adults were separated by sex, transferred into individual small cages with provision of 10% glucose solution and maintained at 27–28°C and relative humidity of 70–90% for subsequent bioassays.

Insecticide susceptibility tests

Phenotypic resistance tests on adults were conducted following WHO guidelines²⁸. Prior to susceptibility tests, efficacy of insecticide impregnated papers was verified against a known laboratory-reared susceptible *An. gambiae s.s.* strain (Ifakara strain)^{29,30}. A group of 20 – 25 non-blood fed wild female and male mosquitoes aged three to five days were exposed for an hour to the diagnostic concentrations of 0.05% lambda-cyhalothrin, 0.05% deltamethrin, 0.75% permethrin, 4% DDT, 4% dieldrin, 0.1% bendiocarb, 0.1% propoxur, 0.25% pirimiphos-methyl,

Insecticide resistance profile in *Anopheles arabiensis* in rural South-Eastern Tanzania

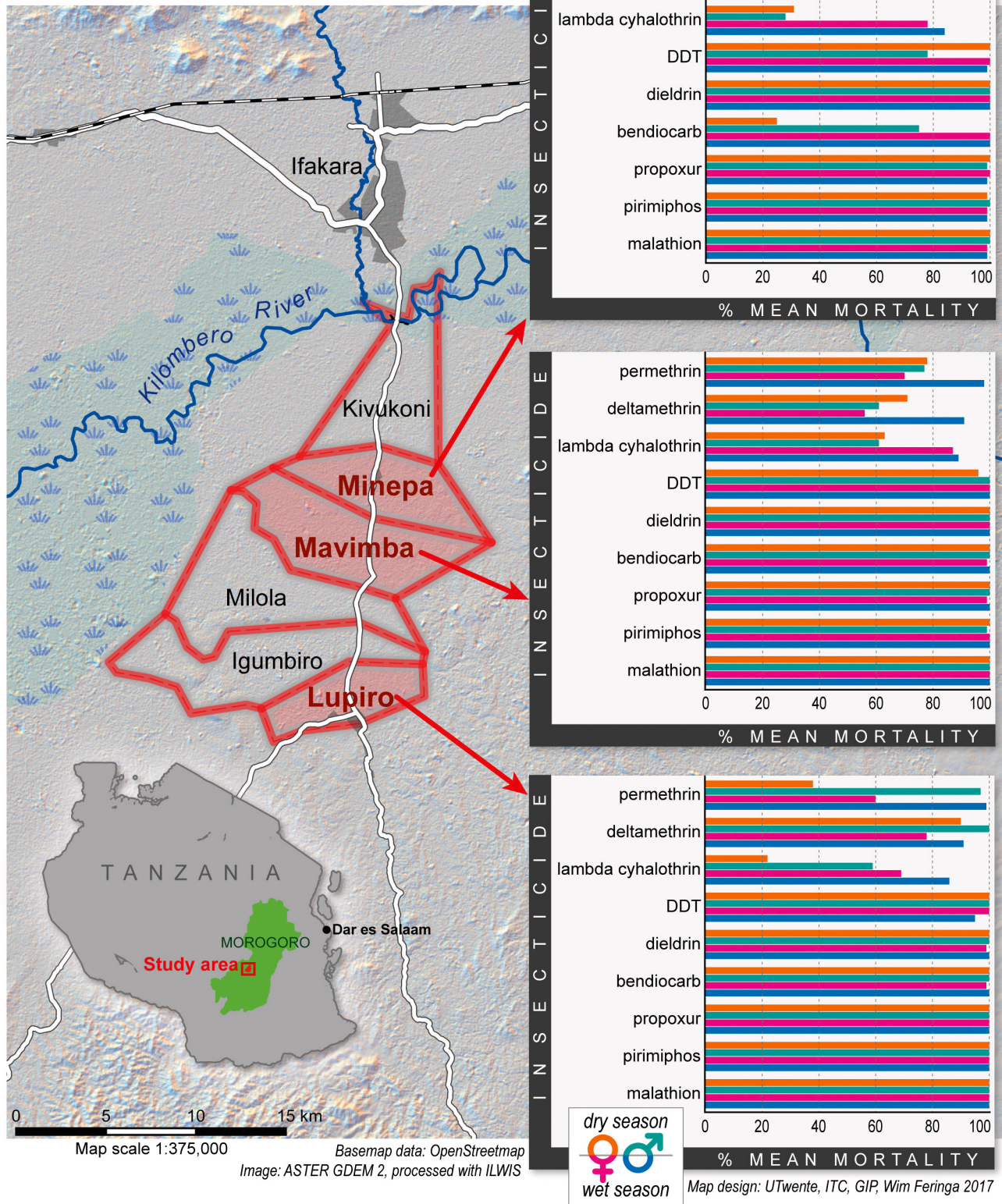


Figure 1. Geographic positions of the three study villages in south-eastern Tanzania. Embedded charts represent the fine-scale spatial and temporal variations of insecticide resistance profiles in both male and female malaria mosquitoes between the study villages.

and 5% malathion. Controls consisted of mosquitoes exposed to oil-impregnated papers. During the one hour exposure to insecticides, knockdown rates were recorded at 10, 15, 20, 30, 40, 50, and 60 minute intervals. After the exposure period, mosquitoes were transferred to holding tubes and maintained on 10% glucose solution. The final mortalities were recorded 24 hours post-exposure. Dead and surviving mosquitoes were kept separately, under preservation using silica in 1.5 ml Eppendorf tubes, for further molecular examination of resistance genes.

Synergist bioassays

Synergist bioassays using piperonyl butoxide (PBO), an inhibitor of monooxygenase, and triphenyl phosphate (TPP), an inhibitor of esterases, were performed on the adult mosquitoes, to assess whether the pyrethroid resistance phenotypes observed during WHO susceptibility assays could be reversed by synergistic activity of these insecticides, which would indicate a biochemical basis for the resistance^{28,31}. Prior to the synergist assays, the bio-efficacy and quality of PBO and TPP synergist papers was validated against a reference laboratory colony, whose pyrethroid resistance and DDT resistance is mediated by high monooxygenases (FUMOZ-R)³² and elevation of esterases (MBN-DDT)³³, respectively.

Due to limited number of mosquito sample, the PBO and TPP assays were performed only on female *An. arabiensis* collected from Minepa village, and PBO test only in female *An. arabiensis* sampled from Mavimba village between the months of September and December 2016. Non-blood fed, 2–3 day old wild female *An. arabiensis* mosquitoes were used, each test consisting of 20 to 25 mosquitoes per tube with two controls. Five replicates were performed for each exposure set. Mosquitoes were pre-exposed to (either 4% PBO or 20% TPP) for 60 minutes, followed by exposure to WHO test papers impregnated with discriminatory doses of candidate insecticides (0.75% permethrin, 0.05% deltamethrin, 0.05% lambda-cyhalothrin, or 4% DDT) for another 60 minutes. To assess the effect of insecticides alone, another group of mosquitoes without pre-exposure to the synergists were concurrently exposed to each candidate insecticide only. At the same time, the same number of mosquitoes was exposed to either 4% PBO or 20% TPP only. Another group of mosquitoes was also exposed to control filter papers treated with a mixture of olive oil and acetone, and to plain filter papers with no chemicals that were used as environmental controls. During the one hour exposure to synergist and to insecticides, the knockdown rates were recorded at 5,10,15,20,25,30,40,50 and 60 minute intervals. Mosquitoes were fed on 10% glucose solution, and mortalities from assays conducted with and without exposure to synergist were scored 24 hours post-exposure³¹.

Knockdown resistance (kdr) detection using hydrolysis probe analysis

A hydrolysis probe assay was used to screen for L1014F (*kdr-w*) and L1014S (*kdr-e*) mutations in 220 randomly selected dead and alive female specimens, which had shown resistance to both DDT or pyrethroids, using procedures previously described³³. DNA was extracted from the legs of each specimen using the ZyGEM prepGEM insect DNA extraction kit (Cat: PIN141106, ZyGEM NZ Ltd, Ruakura, New Zealand), following the manufacturer's

guidelines, except that the reaction volume was quartered. DNA extracted (10–50ng) from each individual mosquito was then used to detect the presence of *kdr-w* and *kdr-e* in two PCR master mixtures in a CFX 96 real-time PCR machine (Biorad, Hercules, CA, USA). In each instance, positive controls comprised of a DNA template from mosquitoes with known West African (*kdr-w*) genotype sampled from Sudan (SENN-DDT, homozygous for the L1014F mutation)³⁴, and DNA from Burundi mosquitoes, which had been previously genotyped as homozygous for the East African (*kdr-e*) mutation, L1014S (unpublished study, Vector Control Reference Laboratory, Johannesburg, South Africa). Other positive controls were DNA templates from a homozygous susceptible colony originating from Kanyemba, Zimbabwe (KGB). The heterozygous controls were made up by mixing equal aliquots of susceptible and resistant DNA templates. A final control consisted of a master mix containing of PCR components, except the DNA template that was set up to monitor any contamination during reaction preparation.

Data analysis

Data analysis was done using R version 3.0³⁵. Susceptibility bioassay data was first summarised as mean percentage (%) mortality per insecticide per village and per season. Population susceptibility was classified according to the WHO criteria²⁸. Data for the synergist tests were summarized as mean % mortality of the four replicates, and the 95% confidence intervals were calculated to estimate probability that population means lie within the given ranges. Following an average of four replicates of each synergist test, final mortality observed 24 hours post-exposure was compared between samples with and without pre-exposure to synergists, using paired sample t-test. The time at which 50% of the experimental populations were knocked down (KDT_{50}) was determined using log-probit analysis³⁶. Resistance reduction was obtained by dividing the KDT_{50} obtained from insecticide exposure with no synergist by the KDT_{50} obtained from insecticide plus the synergist ($KDT_{50}^{\text{Insecticide alone}} / KDT_{50}^{\text{Insecticide plus Synergist}}$). The differences in mortality was considered statistically significant when $P < 0.05$. For *kdr* detection assays, the fluorescent signals detected in the experimental reactions were compared to those of the controls, and genotyping of each mosquito was done using the CFX manager software version 2.1 (Bio-Rad, Hercules, CA, USA).

Ethical statement

Permission to conduct larva sampling was obtained from the owners of the farms, after the researchers provided a description of the study aims and procedures. A brief description of the study was delivered in local language, Kiswahili. Upon agreement, participants were asked to sign written informed forms. The proposed study went through an ethical review and obtained approval from the institutional review board of Ifakara Health Institute (Ref: IHI/IRB/NO: 34-2014) and the Medical Research Coordinating Committee at the National Institute for Medical Research in Tanzania (Ref: NIMR/HQ/R.8a/Vol.IX/1903). Permission to publish this manuscript was obtained from the National Institute for Medical Research in Tanzania (NIMR; Ref: NIMR/HQ/P.12 VOL. XXII/27). Printed copies and online links to the manuscript will be provided to NIMR upon publication.

Results

Spatial and seasonal variability in phenotypic resistance in male and female *An. arabiensis* mosquitoes

The reference insectary-reared *An. gambiae* ss were fully susceptible (100% mortality) to all the insecticides tested, confirming the quality and bio-efficacy of the insecticide-impregnated papers used. The observed mortality in control groups was consistently below 5%, so no statistical correction was required. The WHO susceptibility test findings are summarized in [Figure 1](#) and [Table 1](#). There was marked seasonal and spatial variations in phenotypic resistance in both female and male *An. arabiensis* to three pyrethroids, permethrin, deltamethrin, lambda-cyhalothrin, but also to bendiocarb and DDT in the study villages.

For example, in Minepa village, the female mosquitoes were fully susceptible to bendiocarb in the wet season (mean mortality of 100%), yet highly resistant in the dry season (24.6%). Bendiocarb resistance also varied across different locations. While females collected from Minepa village in the dry season were resistant to bendiocarb, samples of the same species collected from the nearby villages of Mavimba and Lupiro during the same season were fully susceptible to the same chemical (100%). It was also observed that female *An. arabiensis* mosquitoes collected from Minepa village were fully susceptible to DDT (100%) in both seasons, while those collected from the nearby Mavimba village during dry season showed reduced susceptibility to DDT (96.5%), and resistance to the same insecticide in Lupiro village in the wet season (83.5%). Wild female mosquito populations from Minepa, Mavimba and Lupiro villages displayed variable levels of deltamethrin resistance across both seasons, but reduced susceptibility to this insecticide (90.3%) in dry season in Lupiro. Throughout the study, female *An. arabiensis* were resistant to permethrin and lambda-cyhalothrin (mortality rates between 21.6% and 87.4%) in both seasons across the study villages.

As shown in [Table 1](#), insecticide resistance variation in the male *An. arabiensis* was greater both by season and by locality, and was observed for pyrethroids, DDT and bendiocarb. Males collected from Minepa were fully susceptible to permethrin in the dry season (100% mortality), but resistant to the same chemical in the wet season (80.5% mortality); those collected from Mavimba village on the other hand were susceptible to permethrin in the wet season (97.5%), but resistant in dry season (77.4%). In Lupiro village, the males were fully susceptible to permethrin in wet seasons (98.8%), though there were also signs of weakening susceptibility among mosquitoes collected in dry season (97.2%). Deltamethrin resistance in male *An. arabiensis* was observed in wet season in Minepa (87.5%) and in dry season in Mavimba (60.8%). There was also reduced susceptibility to deltamethrin in the male mosquito population sampled from Mavimba (91.3%) and Lupiro (90.6%) in wet season, but complete susceptibility was observed in Minepa (98.5%) and Lupiro in dry season (100%).

During the study, male mosquito samples from the three villages across both seasons displayed various levels of resistance to lambda-cyhalothrin (mortality rates between 28.1% and 89.4%). For both

DDT and bendiocarb, male mosquitoes from Minepa were resistant in dry season 78.4% and 75.3% respectively, but susceptible in wet season (mortalities between 99.1% and 100%), while the males from both Mavimba and Lupiro were consistently susceptible to these two insecticides in both seasons (100%). A minor exception was specimens collected in wet season from Lupiro, where reduced susceptibility was observed against DDT (95.3%).

As illustrated in [Figure 1](#), both male and female mosquito populations across the study villages and during both seasons remained fully susceptible to propoxur, dieldrin and all organophosphates tested (mortality rates between 98.8% and 100%).

Results of the synergist bioassays conducted with samples from Minepa village

Tests with PBO. There was a reduction in time to 50% knockdown (KDT_{50}) in mosquito cohorts pre-exposed to PBO followed by deltamethrin, permethrin, lambda cyhalothrin and bendiocarb, compared to cohorts directly exposed to each of the candidate insecticides without PBO pre-exposure ([Table 2](#)). Resistance reduction levels of 1.4, 3.1, 1.9 and 1.5 fold were recorded in tests of deltamethrin, permethrin, lambda-cyhalothrin and bendiocarb, respectively. The resistance reduction ratios for all tested insecticides are shown in [Table 2](#).

There was also a significant difference in 24-hr post-exposure mortality between mosquito cohorts ([Table 3](#)). Our tests revealed significant increases in mortalities when the mosquito populations were pre-exposed to PBO followed by deltamethrin compared to when the same populations were exposed to deltamethrin alone (paired t-test, $df = 3$, $t = 18.4$, and $P < 0.001$). Pre-exposure to PBO followed by permethrin also resulted in a significant increase in mortality relative to exposure to permethrin alone (paired t-test, $df = 3$, $t = 9.80$, and $P = 0.002$). Similarly, pre-exposure to PBO followed by lambda cyhalothrin yield a significant increase in mortality compared to cohorts exposed to lambda cyhalothrin alone (paired t-test, $df = 3$, $t = 10.3$, and $P = 0.002$). In tests for bendiocarb resistance, it was observed that pre-exposure to PBO created substantial synergism, resulting in higher mortality compared to exposure to bendiocarb with no synergist (paired t-test, $df = 3$, $t = 22.46$, and $P < 0.001$).

Tests with TPP. There was a slight decrease in KDT_{50} when mosquitoes were pre-exposed to TPP followed by either deltamethrin, permethrin or bendiocarb, compared to when the same population of mosquitoes was exposed to the candidate insecticides alone ([Table 2](#)). Resistance to deltamethrin, permethrin, and bendiocarb were reduced by 0.9, 1.4, and 1.3 fold, respectively, with TPP ([Table 2](#)). However, there was no difference in mortalities in mosquitoes exposed to deltamethrin with or without pre-exposure to TPP (paired t-test, $df = 3$, $t = 0.73$, and $P = 0.520$). Also, there was no statistical difference in mean mortalities of mosquitoes exposed to TPP plus permethrin compared to when they were exposed to permethrin alone (paired t-test, $df = 3$, $t = 0.88$, and $P = 0.444$). On the other hand, there were differences in the mean mortality between bendiocarb and TPP + bendiocarb (paired t-test, $df = 3$, $t = 19.12$, and $P = 0.006$).

Table 1. Mean percentage mortalities following exposure to insecticides for samples of 2–5 day old *Anopheles arabiensis* adults emerging from larval collections done in the dry season (June–December 2015) and the wet season (January–May 2016) from three neighbouring villages. Mortalities were recorded 24 hours post exposure. Tests showing seasonal or spatial variability in susceptibility status are marked with symbols, ++ or ^^.

	Minepa Village % mean mortality (95% CI)		Mavimba Village % mean mortality (95% CI)		Lupiro Village % mean mortality (95% CI)	
	Dry Season (n=1298)	Wet Season (n= 1200)	Dry Season (n=1345)	Wet Season (n= 1082)	Dry Season (n= 1318)	Wet Season (n=1115)
Female Mosquitoes						
0.75% Permethrin	75.4 (64.1-84.0) ^{RR}	80.6 (69.4-88.4) ^{RR}	79.2 (65.4-88.5) ^{RR}	70.1 (58.7-79.4) ^{RR}	37.7 (26.8-50.1) ^{RR}	60.0 (48.9-70.1) ^{RR}
0.05% Deltamethrin	72.4 (60.8-81.6) ^{RR}	64.4 (51.2-75.6) ^{RR}	72.1 (56.8-83.5) ^{RR}	56.3 (44.7-67.2) ^{RR}	90.3 (80.8-95.4) ^{RS}	77.5 (67.1-85.3) ^{RR}
0.05% Lambda-cyhalothrin	31.1 (21.2-43.0) ^{RR}	63.3 (50.2-74.8) ^{RR}	63.2 (47.0-76.8) ^{RR}	87.4 (78.3-89.8) ^{RR}	21.6 (13.5-32.5) ^{RR}	69.0 (57.8-77.9) ^{RR}
4% Dieldrin	100 ^{SS}	100 ^{SS}	100 ^{SS}	100 ^{SS}	100 ^{SS}	98.8 (91.7-99.8) ^{SS}
4% DDT ^{++^^}	100 ^{SS}	100 ^{SS}	96.5 (89.7-98.8) ^{RS}	98.8 (91.6-99.8) ^{SS}	100 ^{SS}	83.5 (72.6-89.4) ^{RR}
0.1% Propoxur	100 ^{SS}	100 ^{SS}	100 ^{SS}	98.8 (91.6-99.8) ^{SS}	100 ^{SS}	100 ^{SS}
0.1% Bendiocarb ^{++^^}	24.6 (16.0-35.9) ^{RR}	100 ^{SS}	100 ^{SS}	100 ^{SS}	100 ^{SS}	100 ^{SS}
0.25% Pirimiphos methyl	99.0 (93.0-99.9) ^{SS}	99.1 (93.4-99.9) ^{SS}	100 ^{SS}	100 ^{SS}	100 ^{SS}	100 ^{SS}
5% Malathion	100 ^{SS}	100 ^{SS}	100 ^{SS}	100 ^{SS}	100 ^{SS}	100 ^{SS}
Control (untreated paper)	1.6 (0.7-3.4)	3.1 (1.3-7.0)	0.6 (0.1-2.7)	1.3 (0.5-3.2)	3.9 (2.2-7.2)	1.9 (0.9 – 3.9)
Male Mosquitoes						
0.75% Permethrin ^{++^^}	100 ^{SS}	80.5 (69.4-88.3) ^{RR}	77.4 (62.4-87.6) ^{RR}	97.5 (90.6-99.4) ^{SS}	97.2 (88.8-99.3) ^{RS}	98.8 (91.8-99.8) ^{SS}
0.05% Deltamethrin ^{^^}	98.5 (92.7-99.7) ^{SS}	87.5 (78.0-93.2) ^{RR}	60.8 (43.8-75.5) ^{RR}	91.3 (82.8-95.8) ^{RS}	100 ^{SS}	90.6 (81.6-95.4) ^{RS}
0.05% Lambda-cyhalothrin ^{++^^}	28.1 (14.2-48.0) ^{RR}	83.5 (73.0-90.5) ^{RR}	60.6 ^{RR}	89.4 (18.8-98.3) ^{RR}	58.9 (32.4-81.1) ^{RR}	85.7 (75.1-92.3) ^{RR}
4% Dieldrin	100 ^{SS}	100 ^{SS}	100 ^{SS}	100 ^{SS}	100 ^{SS}	100 ^{SS}
4% DDT ^{++^^}	78.4 (60.2-89.7) ^{RR}	99.1 (93.4-99.9) ^{SS}	100 ^{SS}	100 ^{SS}	100 ^{SS}	95.3 (87.2-98.4) ^{RS}
0.1% Propoxur	99.2 (93.9-99.9) ^{SS}	99.1 (93.4-99.9) ^{SS}	100 ^{SS}	100 ^{SS}	100 ^{SS}	100 ^{SS}
0.1% Bendiocarb ^{++^^}	75.3 (56.2-87.9) ^{RR}	100 ^{SS}	100 ^{SS}	100 ^{SS}	99.3 (93.7-99.9) ^{SS}	100 ^{SS}
0.25 % Pirimiphos methyl	100 ^{SS}	99.1 (93.4-99.9) ^{SS}	99.1 (93.6-99.9) ^{SS}	100 ^{SS}	100 ^{SS}	100 ^{SS}
5% Malathion	100 ^{SS}	99.1 (93.4-99.9) ^{SS}	100 ^{SS}	100 ^{SS}	100 ^{SS}	100 ^{SS}
Control (untreated paper)	1.4 (0.5-4.0)	1.4 (0.5-4.2)	1.2 (0.5-3.2)	2.8 (1.5-5.1)	3.8 (1.1-12.4)	0.9 (0.3-2.7)

^{SS}: Mosquitoes were susceptible to the test insecticide (WHO assays mortality between 98% and 100%).

^{RS}: Mosquitoes had reduced susceptibility indicating possible resistance and need for further investigation (WHO assays mortality of 90% to 97%).

^{RR}: Mosquitoes were confirmed resistant to the test insecticide (WHO assays mortality below 90%).

⁺⁺ Insecticides for which we observed differences in susceptibility of *Anopheles arabiensis* mosquitoes between dry and wet seasons, i.e. where mosquitoes were fully susceptible in one season and fully resistant in a different season in same village.

^{^^} Insecticides for which we observed differences in susceptibility of *Anopheles arabiensis* mosquitoes between (nearby) villages, i.e. where mosquitoes were fully susceptible in one

Table 2. Knockdown rates (KDT₅₀) and degree of resistance reduction of *Anopheles arabiensis* from two study villages after being exposed to various insecticides with and without pre-exposure to synergists.

Study sites	Insecticide	KDT ₅₀ (min)	(95% CI)	Resistance reduction [‡]
Minepa village	0.05% Deltamethrin	50.24	35.71 – 64.77	-
	4% PBO + 0.05% Deltamethrin	35.90	27.56 – 44.24	1.40
	0.75% Permethrin	70.20	34.42 – 105.98	-
	4% PBO + 0.75% Permethrin	22.72	17.82 – 27.61	3.09
	0.05% Lambda cyhalothrin	54.88	34.62– 75.13	-
	4% PBO + 0.05% Lambda cyhalothrin	29.61	22.55 – 36.66	1.85
	0.05% Deltamethrin	60.87	38.84 – 82.89	-
	20% TPP + 0.05% Deltamethrin	65.23	35.46 – 94.99	0.93
	0.75% Permethrin	38.69	30.60 – 46.77	-
	20% TPP + 0.75% Permethrin	27.65	20.59 – 34.70	1.40
	0.1% Bendiocarb	53.14	37.00 – 69.28	-
	4% PBO + 0.1% Bendiocarb	35.25	27.51 – 42.99	1.51
	0.1% Bendiocarb	56.14	40.04 – 72.24	-
	20% TPP + 0.1% Bendiocarb	43.71	33.44 – 53.98	1.28
Mavimba village	0.05% Deltamethrin	46.35	32.64 – 60.06	-
	4% PBO + 0.05% Deltamethrin	23.33	17.98 – 28.68	1.99
	0.75% Permethrin	39.78	29.16 – 50.39	-
	4% PBO + 0.75% Permethrin	21.09	15.60 – 26.59	1.89
	0.05% Lambda cyhalothrin	68.65	35.10 – 102.20	-
	4% PBO + 0.05% Lambda cyhalothrin	34.87	26.82 – 42.93	1.97

[‡] Resistance reduction = $KDT_{50 \text{ insecticide alone}} / KDT_{50 \text{ insecticide plus synergist}}$

Results of the synergist bioassays conducted with samples from Mavimba village

Prior exposure to PBO partially restored susceptibility to deltamethrin by 2.0 fold and decreased the KDT₅₀ from 46.35min for deltamethrin alone to 23.33 min for deltamethrin and PBO (Table 2). The time required for 50% of the mosquitoes to be knocked down was also reduced from 39.78min for permethrin alone to 21.09 min after being exposed for permethrin and PBO. Resistance reduction level for permethrin following PBO pre-exposure was 1.9 fold (Table 2). Similarly, the resistance to lambda-cyhalothrin was reduced by 2.0 fold with PBO, with a shift in KDT₅₀ from 68.65min to 34.87min (Table 2). There was a significant increase in mortality in mosquito populations pre-exposed to PBO followed by deltamethrin compared to when the

same populations were exposed to deltamethrin alone (paired t-test, $t = 18.4$, $df = 3$, $p < 0.001$) (Table 4). Similarly, when the mosquito populations were pre-exposed to PBO followed by lambda-cyhalothrin this resulted in a significant increase in mean mortality compared to when the same population was exposed to lambda cyhalothrin alone (paired t-test, $t = 17.9$, $df = 3$, $p < 0.001$) (Table 4).

Results of the molecular assays to detect knockdown resistance (*kdr*) alleles

A total of 74 adult female *An. arabiensis* mosquitoes from Minepa, 66 from Mavimba and 80 from Lupiro were assayed for *kdr* allele mutations L1014F (*kdr-west*) and the L1014S (*kdr-east*). All specimens were negative for both mutations.

Table 3. Mortality *Anopheles arabiensis* from Minepa village exposed to insecticides and the synergists, PBO or TPP.

Treatment	No. replicates done	Sample size *	% mean mortality (95% CI)			
			Minepa village			
			0.05% Deltamethrin	0.05% Lambda cyhalothrin	0.75% Permethrin	0.1% Bendiocarb
Environmental control	4	375	0	NA	0	0
Solvent control	4	375	0	NA	0	0
20% TPP only	4	375	0	NA	0	0
20% TPP & Test insecticide	4	375	27.0 (18.3 – 35.7) ^b	NA	29.5 (20.3 – 38.7) ^b	72.0 (62.9 – 81.0) ^a
Test insecticide only	4	374	24.0 (13.4 – 34.6) ^b	NA	26.5 (21.1 – 31.9) ^b	55.5 (46.4 – 64.6) ^a
Environmental control	4	370	0.2 (-0.2 – 0.6)	0	0	0
Solvent control	4	370	0.2 (-0.2 – 0.6)	0	0	0
4% PBO only	4	370	0	0	0	0
4% PBO & Test Insecticide	4	370	73.0 (63.5 – 82.5) ^b	97.5 (94.7 – 100) ^a	56.8 (46.9 – 66.6) ^a	76.0 (60.4 – 91.6) ^a
Test Insecticide only	4	370	45.0 (35.5 – 54.5) ^b	20.0 (5.6 – 34.4) ^a	08.8 (03.0 – 14.1) ^a	33.0 (23.5 – 42.5) ^a

NA=No assay was performed on this insecticide.

^a There are significant differences in mean mortalities between exposure to insecticides with and without synergists.

^b No significant difference in mean mortalities between exposure to insecticides with and without synergists.

Table 4. Mortality *Anopheles arabiensis* from Mavimba village exposed to insecticides and the synergists, PBO.

Treatment	No. replicates done	Sample size*	% mean mortality (95% CI)		
			Mavimba village		
			0.05% Deltamethrin	0.05% Lambda cyhalothrin	0.75% Permethrin
Environmental control	4	260	0.4 (-0.4 – 1.2)	0.4 (-0.4 – 1.2)	0
Solvent control	4	262	0.3 (-0.3 – 0.9)	0	0
4% PBO only	4	262	0	0	0
4% PBO & Test Insecticide	4	241	92.5 (86.2 – 98.8) ^a	85.2 (74.6 – 95.8) ^a	91.3 (82.9 – 99.6) ^a
Test Insecticide only	4	240	27.5 (24.7 – 30.3) ^a	20.0 (03.5 – 36.5) ^a	67.5 (54.5 – 80.5) ^a

^a There are significant differences in mean mortalities between exposure to insecticides with and without synergists.

^b No significant difference in mean mortalities between exposure to insecticides with and without synergists

Discussion

The increasing spread of insecticide resistance in malaria vectors jeopardizes control and elimination efforts^{9–14}, thus necessitating regular resistance monitoring to design setting-specific and successful resistance management programmes^{16,28,37}. Overall, this study detected widespread resistance against pyrethroids, bendiocarb, and DDT; but not against propoxur, dieldrin, and the two organophosphates, pirimiphos-methyl and malathion, for which there was full susceptibility across all the villages and

seasons. This study also found marked temporal and fine-scale fluctuations of insecticide resistance profiles in both male and female *An. arabiensis* against three insecticides in the pyrethroid class, DDT, and bendiocarb. In all the three villages, deltamethrin, permethrin, lambda-cyhalothrin, DDT and bendiocarb resistance of male *An. arabiensis* mosquitoes fluctuated between seasons and villages. Resistance of female *An. arabiensis* mosquitoes against DDT and bendiocarb also fluctuated between seasons and villages. The most resistant populations were

observed in Minepa for bendiocarb, lambda-cyhalothrin and DDT and in Lupiro for lambda-cyhalothrin and permethrin. In Minepa, bendiocarb resistance was detected in the dry season, but completely diminished in wet seasons for both male and female populations, and DDT resistance followed a similar trend in the male population. However, in Lupiro village, DDT resistance was observed during the wet season only.

The seasonal and spatial variation in insecticide resistance detected in this study is not unique. Variations in both phenotypic and genotypic insecticide resistance in both *Anopheles* and *Aedes* mosquitoes over small spaces and time have been reported previously^{22,38–40}. A recent report in Chad found a significant spatial changes in insecticide resistance in an *An. arabiensis* population³⁸. Similarly, there was significant difference in phenotypic and genotypic resistance at a fine geographical scale in *Ae. aegypti* populations to chlorpyrifos-ethyl and deltamethrin sampled from nearby study sites in Mexico³⁹. The seasonal and spatial fluctuations in insecticide resistance might be attributed to differences in the biology and genetics of the vector populations in particular ecological settings, as reported in a previous study by Verhaeghen *et al.*⁴⁰.

Perhaps the presence of chemical contaminants in a particular environment, possibly due to leached agricultural chemicals and other pollutants at a particular time might cause selection pressure in mosquitoes, and subsequent resistance to insecticides. Also, the existence of phenotypic resistance in the study areas to lambda-cyhalothrin, bendiocarb and DDT that are not used for LLINs or IRS, suggest cross-resistance between classes or alternative sources of insecticide resistance pressure, most likely from agriculture. The impact of agricultural pesticides in the selection of resistant mosquitoes has already been reported extensively^{19,41–48}. This hypothesis is also supported by our preliminary observations that the majority of farmers in the study villages reported applying more pesticides in dry seasons than in wet seasons (Matowo N, Munhenga G, Tanner M, Koekemoer L, Coetzee M and Okumu F, unpublished study, Ifakara Health Institute). The differences in insecticide resistance between adjacent study villages suggests that other than variations that have been reported between districts and regions^{10,14,15}, there might also be fine-scale differences even within the villages that require further investigations. All these variations signify an important challenge to the vector control programs that might require proper consideration in the timing/season and choosing different insecticides for application even in a particular small area.

Male mosquitoes are considered to be more delicate and susceptible to insecticides as they have a shorter life expectancy than their females counterparts²⁸. In this study, males were found to be resistant to the same insecticides as the females, but at a lower level. These observations are consistent with previous studies that have reported that adults male *An. arabiensis*, with previous exposure to insecticides, could also experience resistance similar to females⁴⁹. For example, a high level of glutathione-S-transferase (GSTs) activity was found in both male and female *An. arabiensis*

selected for resistance to DDT, but only elevated esterases was found in the male-DDT selected strain⁴⁹. Resistance in male mosquitoes was reported previously to adversely affect their mating competitiveness, as shown in *Culex pipiens* and *An. gambiae*^{50–52}. This suggests the need for regular monitoring of susceptibility status of male mosquitoes, particularly in interventions targeting male mating behaviour, such as the sterile insect technique, which involves mass-rearing, sterilization, and release of sterile male mosquitoes into the wild population to prevent females from reproducing^{53,54}. Other interventions that have been proposed for mosquito-borne disease elimination includes targeting male swarming behaviour⁵⁵, sugar-seeking behaviour through the use of attractive toxic sugar baits^{56,57} and larval control⁵⁸. In summary, our findings and the current evidence suggest the need for regular monitoring of susceptibility status of both males and females, especially for end-game scenarios where LLINs and IRS have already been widely used, but malaria transmission still persists.

As revealed in the synergist assays, the reduction in knockdown rates and increase in mortalities was due to synergistic action of piperonal butoxide (PBO), as an inhibitor of P450 monooxygenases, and triphenol phosphate (TPP), as an inhibitor of the esterases activity. Synergists have an effect by augmenting the penetration of the insecticides into the mosquito body and counteracting the metabolic pathways that would otherwise metabolize the insecticides, thus restoring susceptibility to varying degrees^{31,59–61}. The observed effects in the present study suggest involvement to a significant degree of one or both of the two enzyme classes in conferring pyrethroid and bendiocarb resistance within the mosquito populations sampled from the study sites. However, esterases seem not to be involved in deltamethrin and permethrin resistance in the mosquito population sampled from Minepa village. Susceptibility to lambda-cyhalothrin was completely restored by 4% PBO in the mosquito population sampled from Minepa village, indicating that the resistance is metabolic mediated by monooxygenases. However, the inability of PBO and TPP to completely reverse the deltamethrin, permethrin and bendiocarb resistance across the study sites indicates that either other enzymes might be playing a role in the metabolic resistance, or there is presence of other mutations that require further investigation. These questions will need to be further explored through biochemical and genetic analyses. Our findings agree with previous studies that have consistently reported the combining effect of synergists and insecticides against resistant disease-transmitting mosquitoes and incomplete suppressions of pyrethroids resistance due to the synergists action^{17,31,59,62–64}.

The absence of L1014F and L1014S resistance alleles in the field-collected adult female mosquito populations suggests that the phenotypic resistance to pyrethroid and DDT was not associated with target site insensitivity of the voltage-gate sodium channel. The findings supports an earlier study by Okumu *et al.*, who also showed absence of *kdr* mutations in wild population of *An. arabiensis* from Lupiro village, five years before this current study⁶⁵. Similarly, a recent multi-region study in Tanzania by Kabula *et al.*⁶⁶ reported absence of both L1014F and L1014S

mutations in *An. arabiensis* populations from Kilombero district, which neighbours Ulanga district where our study was conducted. However, these gene mutations were detected in both *An. arabiensis* and *An. gambiae s.s.* from other sentinel districts of Tanzania where studies were carried out⁶⁶.

Conclusions

This study revealed multiple spatial and temporal fluctuations of insecticide resistance profiles in the *An. arabiensis* populations from the three neighbouring villages in south-eastern Tanzania, and confirmed the presence of pyrethroid, DDT and bendiocarb resistance in each of these three villages. The substantial, though not absolute reversal of pyrethroid and carbamate resistance when mosquitoes were pre-exposed to PBO or TPP, coupled with the absence of *kdr* resistance alleles, suggests involvement of P450 monooxygenases and esterases as key determinants conferring the resistance phenotypes. We recommend further intensity bioassays to determine the strength of phenotypic resistance, as well as biochemical and molecular analysis to elucidate various enzymes involved in the resistance. Such additional tests are essential for an effective resistance management programmes in this or similar areas. Overall, these results highlight the importance of periodic and continuous insecticide susceptibility surveillance and emphasize the need to consider fine-scale variations in insecticide resistance levels, even in small geographical locations, when implementing insecticidal-based interventions.

Data availability

Raw datasets for this study are available from the Ifakara Health Institute data repository (<http://dx.doi.org/10.17890/ihl.2017.09.9967>).

Competing interests

No competing interests were disclosed.

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References

- Renggli S, Mandike R, Kramer K, *et al.*: **Design, implementation and evaluation of a national campaign to deliver 18 million free long-lasting insecticidal nets to uncovered sleeping spaces in Tanzania.** *Malar J.* 2013; **12**: 85. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Khatib RA, Killeen GF, Abdulla SM, *et al.*: **Markets, voucher subsidies and free nets combine to achieve high bed net coverage in rural Tanzania.** *Malar J.* 2008; **7**: 98. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Schellenberg JR, Abdulla S, Minja H, *et al.*: **KINET: a social marketing programme of treated nets and net treatment for malaria control in Tanzania, with evaluation of child health and long-term survival.** *Trans R Soc Trop Med.* 1999; **93**(3): 225–231. [PubMed Abstract](#) | [Publisher Full Text](#)
- West PA, Protopopoff N, Rowland M, *et al.*: **Malaria risk factors in North West Tanzania: the effect of spraying, nets and wealth.** *PLoS One.* 2013; **8**(6): e65787. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- West PA, Protopopoff N, Wright A, *et al.*: **Indoor residual spraying in combination with insecticide-treated nets compared to insecticide-treated nets alone for protection against malaria: a cluster randomised trial in Tanzania.** *PLoS Med.* 2014; **11**(4): e1001630. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- WHO: **World malaria report 2015.** World Health Organization. 2016. [Reference Source](#)
- Ministry of Health CD, Gender, Elderly and Children (MoHCDGEC) [Tanzania, Mainland] MoHMZ, National Bureau of Statistics (NBS), *et al.*: **Tanzania Demographic and Health Survey and Malaria Indicator Survey (TDHS-MIS) 2015–16.** 2016. [Reference Source](#)
- The Tanzania National Malaria Control Programme (2014–2020), The National Malaria Control Programme Strategic Plan: **Invest in the future defeat Malaria.** [Reference Source](#)
- Ranson H, Abdallah H, Badolo A, *et al.*: **Insecticide resistance in *Anopheles gambiae*: data from the first year of a multi-country study highlight the extent of the problem.** *Malar J.* 2009; **8**: 299. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Ranson H, N'Guessan R, Lines J, *et al.*: **Pyrethroid resistance in African anopheline mosquitoes: what are the implications for malaria control?** *Trends Parasitol.* 2011; **27**(2): 91–98. [PubMed Abstract](#) | [Publisher Full Text](#)
- Ranson H, Lissenden N: **Insecticide Resistance in African Anopheles Mosquitoes: A Worsening Situation that Needs Urgent Action to Maintain Malaria Control.** *Trends Parasitol.* 2016; **32**(3): 187–196. [PubMed Abstract](#) | [Publisher Full Text](#)
- PMI: **Presidents Malaria Initiative, Malaria Operational Plan: Tanzania FY2015.** USAID, 2015. [Reference Source](#)
- Protopopoff N, Matowo J, Malima R, *et al.*: **High level of resistance in the mosquito *Anopheles gambiae* to pyrethroid insecticides and reduced susceptibility to bendiocarb in north-western Tanzania.** *Malar J.* 2013; **12**: 149. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Kabula B, Tongu P, Malima R, *et al.*: **Distribution and spread of pyrethroid and DDT resistance among the *Anopheles gambiae* complex in Tanzania.** *Med Vet Entomol.* 2014; **28**(3): 244–252. [PubMed Abstract](#) | [Publisher Full Text](#)
- Kisinja WKB, Tongu P, Sindato C, *et al.*: **Detection and Monitoring of Insecticide Resistance in Malaria Vectors in Tanzania Mainland.** Technical Report of the

- NIMR, Tanzania. 2011; 1–45.
[Reference Source](#)
16. WHO: **Global Plan for Insecticide Resistance Management in Malaria Vectors.** Geneva, World Health Organization. 2012.
[Reference Source](#)
 17. Djègbè I, Boussari O, Sidick A, *et al.*: **Dynamics of insecticide resistance in malaria vectors in Benin: first evidence of the presence of *L1014S* kdr mutation in *Anopheles gambiae* from West Africa.** *Malar J.* 2011; 10: 261.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 18. Opondo KO, Weetman D, Jawara M, *et al.*: **Does insecticide resistance contribute to heterogeneities in malaria transmission in The Gambia?** *Malar J.* 2016; 15: 166.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 19. Nkya TE, Akhouayri I, Kisinza W, *et al.*: **Impact of environment on mosquito response to pyrethroid insecticides: facts, evidences and prospects.** *Insect Biochem Mol Biol.* 2013; 43(4): 407–416.
[PubMed Abstract](#) | [Publisher Full Text](#)
 20. Poupardin R, Reynaud S, Strode C, *et al.*: **Cross-induction of detoxification genes by environmental xenobiotics and insecticides in the mosquito *Aedes aegypti*: impact on larval tolerance to chemical insecticides.** *Insect Biochem Mol Biol.* 2008; 38(5): 540–551.
[PubMed Abstract](#) | [Publisher Full Text](#)
 21. Saavedra-Rodriguez K, Beaty M, Lozano-Fuentes S, *et al.*: **Local evolution of pyrethroid resistance offsets gene flow among *Aedes aegypti* collections in Yucatan State, Mexico.** *Am J Trop Med Hyg.* 2015; 92(1): 201–209.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 22. Matowo NS, Moore J, Mapua S, *et al.*: **Using a new odour-baited device to explore options for luring and killing outdoor-biting malaria vectors: a report on design and field evaluation of the Mosquito Landing Box.** *Parasit Vectors.* 2013; 6: 137.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 23. Okumu FO, Moore J, Mbeyela E, *et al.*: **A modified experimental hut design for studying responses of disease-transmitting mosquitoes to indoor interventions: the Ifakara experimental huts.** *PLoS One.* 2012; 7(2): e30967.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 24. Mayagaya VS, Nkwengulila G, Lyimo IN, *et al.*: **The impact of livestock on the abundance, resting behaviour and sporozoite rate of malaria vectors in southern Tanzania.** *Malar J.* 2015; 14: 17.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 25. Kaindoa EW, Matowo NS, Ngowo HS, *et al.*: **Interventions that effectively target *Anopheles funestus* mosquitoes could significantly improve control of persistent malaria transmission in south-eastern Tanzania.** *PLoS One.* 2017; 12(5): e0177807.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 26. Kaindoa EW, Mkandawile G, Ligamba G, *et al.*: **Correlations between household occupancy and malaria vector biting risk in rural Tanzanian villages: implications for high-resolution spatial targeting of control interventions.** *Malar J.* 2016; 15: 199.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 27. Ferguson HM, N'g'habi KR, Walder T, *et al.*: **Establishment of a large semi-field system for experimental study of African malaria vector ecology and control in Tanzania.** *Malar J.* 2008; 7: 158.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 28. WHO: **Test procedures for insecticide resistance monitoring in malaria vector mosquitoes.** Geneva, World Health Organization. 2016.
[Reference Source](#)
 29. Ogoma SB, Lorenz LM, Ngonyani H, *et al.*: **An experimental hut study to quantify the effect of DDT and airborne pyrethroids on entomological parameters of malaria transmission.** *Malar J.* 2014; 13: 131.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 30. Ogoma SB, Ngonyani H, Simfukwe ET, *et al.*: **The mode of action of spatial repellents and their impact on vectorial capacity of *Anopheles gambiae* sensu stricto.** *PLoS One.* 2014; 9(12): e110433.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 31. Chouaïbou M, Zivanovic GB, Knox TB, *et al.*: **Synergist bioassays: A simple method for initial metabolic resistance investigation of field *Anopheles gambiae* s.l. populations.** *Acta Trop.* 2014; 130: 108–111.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 32. Hunt RH, Brooke BD, Pillay C, *et al.*: **Laboratory selection for and characteristics of pyrethroid resistance in the malaria vector *Anopheles funestus*.** *Med Vet Entomol.* 2005; 19(3): 271–275.
[PubMed Abstract](#) | [Publisher Full Text](#)
 33. Nardini L, Christian RN, Coetzer N, *et al.*: **Detoxification enzymes associated with insecticide resistance in laboratory strains of *Anopheles arabiensis* of different geographic origin.** *Parasit Vectors.* 2012; 5: 113.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 34. Oliver SV, Brooke BD: **The effect of larval nutritional deprivation on the life history and DDT resistance phenotype in laboratory strains of the malaria vector *Anopheles arabiensis*.** *Malar J.* 2013; 12: 44.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 35. R Core Team: **R: A Language and Environment for Statistical Computing.** R Foundation for Statistical Computing, Vienna, Austria. 2012.
 36. Vincent K: **Probit analysis.** San Francisco: San Francisco State University, 2008.
[Reference Source](#)
 37. WHO: **Management of Insecticides Resistance in Vectors of Public Health Importance.** Geneva, World Health Organization. 2014.
[Reference Source](#)
 38. Foster GM, Coleman M, Thomsen E, *et al.*: **Spatial and temporal trends in insecticide resistance among malaria vectors in Chad highlight the importance of continual monitoring.** *PLoS One.* 2016; 11(5): e0155746.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 39. Deming R, Manrique-Saide P, Medina Barreiro A, *et al.*: **Spatial variation of insecticide resistance in the dengue vector *Aedes aegypti* presents unique vector control challenges.** *Parasit Vectors.* 2016; 9: 67.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 40. Verhaeghen K, Bortel WV, Roelants P, *et al.*: **Spatio-temporal patterns in kdr frequency in permethrin and DDT resistant *Anopheles gambiae* s.s. from Uganda.** *Am J Trop Med Hyg.* 2010; 82(4): 566–573.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 41. Diabate A, Baldet T, Chandre F, *et al.*: **The role of agricultural use of insecticides in resistance to pyrethroids in *Anopheles gambiae* s.l. in Burkina Faso.** *Am J Trop Med Hyg.* 2002; 67(6): 617–622.
[PubMed Abstract](#) | [Publisher Full Text](#)
 42. Nkya TE, Poupardin R, Laporte F, *et al.*: **Impact of agriculture on the selection of insecticide resistance in the malaria vector *Anopheles gambiae*: a multigenerational study in controlled conditions.** *Parasit Vectors.* 2014; 7: 480.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 43. Nkya TE, Moshia FW, Magesa SM, *et al.*: **Increased tolerance of *Anopheles gambiae* s.s. to chemical insecticides after exposure to agrochemical mixture.** *Tanzan J Health Res.* 2014; 16(4): 329–32.
[PubMed Abstract](#) | [Publisher Full Text](#)
 44. Elissa N, Mouchet J, Riviere F, *et al.*: **Resistance of *Anopheles gambiae* s.s. to pyrethroids in Côte d'Ivoire.** *Ann Soc Belg Med Trop.* 1993; 73(4): 291–4.
[PubMed Abstract](#)
 45. Akogbeto MC, Djuaka R, Noukpo H: **[Use of agricultural insecticides in Benin].** *Bull Soc Pathol Exot.* 2005; 98(5): 400–405.
[PubMed Abstract](#)
 46. Yadouleton A, Martin T, Padonou G, *et al.*: **Cotton pest management practices and the selection of pyrethroid resistance in *Anopheles gambiae* population in Northern Benin.** *Parasit Vectors.* 2011; 4: 60.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 47. Yadouleton AW, Asidi A, Djuaka RF, *et al.*: **Development of vegetable farming: a cause of the emergence of insecticide resistance in populations of *Anopheles gambiae* in urban areas of Benin.** *Malar J.* 2009; 8: 103.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 48. Georghiou GP: **The effect of agrochemicals on vector populations.** *Pesticide Resistance in Arthropods.* Springer. 1990; 183–202.
[Publisher Full Text](#)
 49. Matambo TS, Abdalla H, Brooke BD, *et al.*: **Insecticide resistance in the malarial mosquito *Anopheles arabiensis* and association with the *kdr* mutation.** *Med Vet Entomol.* 2007; 21(1): 97–102.
[PubMed Abstract](#) | [Publisher Full Text](#)
 50. Berticat C, Boquien G, Raymond M, *et al.*: **Insecticide resistance genes induce a mating competition cost in *Culex pipiens* mosquitoes.** *Genet Res.* 2002; 79(1): 41–47.
[PubMed Abstract](#) | [Publisher Full Text](#)
 51. Platt N, Kwiatkowska RM, Irving H, *et al.*: **Target-site resistance mutations (*kdr* and *RDL*), but not metabolic resistance, negatively impact male mating competitiveness in the malaria vector *Anopheles gambiae*.** *Heredity (Edinb).* 2015; 115(3): 243–52.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 52. Rowland M: **Activity and mating competitiveness of gamma HCH/dieldrin resistant and susceptible male and virgin female *Anopheles gambiae* and *An. stephensi* mosquitoes, with assessment of an insecticide-rotation strategy.** *Med Vet Entomol.* 1991; 5(2): 207–222.
[PubMed Abstract](#) | [Publisher Full Text](#)
 53. Bellini R, Calvitti M, Medici A, *et al.*: **Use of the sterile insect technique against *Aedes albopictus* in Italy: First results of a pilot trial.** *Area-Wide Control of Insect Pests.* Springer. 2007; 505–515.
[Publisher Full Text](#)
 54. Harris AF, McKemey AR, Nimmo D, *et al.*: **Successful suppression of a field mosquito population by sustained release of engineered male mosquitoes.** *Nat Biotechnol.* 2012; 30(9): 828–830.
[PubMed Abstract](#) | [Publisher Full Text](#)
 55. Diabate A, Tripet F: **Targeting male mosquito mating behaviour for malaria control.** *Parasit Vectors.* 2015; 8: 347.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 56. Müller GC, Beier JC, Traore SF, *et al.*: **Successful field trial of attractive toxic sugar bait (ATSB) plant-spraying methods against malaria vectors in the *Anopheles gambiae* complex in Mali, West Africa.** *Malar J.* 2010; 9: 210.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 57. Stewart ZP, Oxborough RM, Tungu PK, *et al.*: **Indoor application of attractive toxic sugar bait (ATSB) in combination with mosquito nets for control of**

- pyrethroid-resistant mosquitoes. *PLoS One*. 2013; **8**(12): e84168.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
58. WHOPEs: WHOPEs-recommended compounds and formulations for control of mosquito larvae. Geneva, World Health Organization Pesticide Evaluation Scheme. 2013.
[Reference Source](#)
59. Nwane P, Etang J, Chouaibou M, *et al.*: Multiple insecticide resistance mechanisms in *Anopheles gambiae* s.l. populations from Cameroon, Central Africa. *Parasit Vectors*. 2013; **6**: 41.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
60. Jao LT, Casida JE: Insect pyrethroid-hydrolyzing esterases. *Pestic Biochem Physiol*. 1974; **4**(4): 465–472.
[Publisher Full Text](#)
61. Farnham AW: The mode of action of piperonyl butoxide with reference to studying pesticide resistance. *Piperonyl Butoxide*. the Insecticide Synergist, Academic, London. 1999; 199–213.
[Publisher Full Text](#)
62. Darriet F, Chandre F: Combining piperonyl butoxide and dinotefuran restores the efficacy of deltamethrin mosquito nets against resistant *Anopheles gambiae* (Diptera: Culicidae). *J Med Entomol*. 2011; **48**(4): 952–955.
[PubMed Abstract](#) | [Publisher Full Text](#)
63. Bingham G, Strode C, Tran L, *et al.*: Can piperonyl butoxide enhance the efficacy of pyrethroids against pyrethroid-resistant *Aedes aegypti*? *Trop Med Int Health*. 2011; **16**(4): 492–500.
[PubMed Abstract](#) | [Publisher Full Text](#)
64. Vijayan VA, Sathish Kumar BY, Ganesh KN, *et al.*: Efficacy of piperonyl butoxide (PBO) as a synergist with deltamethrin on five species of mosquitoes. *J Commun Dis*. 2007; **39**(3): 159–163.
[PubMed Abstract](#)
65. Okumu FO, Chipwaza B, Madumla EP, *et al.*: Implications of bio-efficacy and persistence of insecticides when indoor residual spraying and long-lasting insecticide nets are combined for malaria prevention. *Malar J*. 2012; **11**: 378.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
66. Kabula B, Kisinza W, Tungu P, *et al.*: Co-occurrence and distribution of East (L1014S) and West (L1014F) African knock-down resistance in *Anopheles gambiae sensu lato* population of Tanzania. *Trop Med Int Health*. 2014; **19**(3): 331–341.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
67. Matowo N, *et al.*: Tanzania - Fine scale spatial and temporal monitoring of insecticide resistance in malaria vector in rural south-eastern Tanzania. DDI_IHI_ENV003_MVRMV_2017. Ifakara Health Institute.
[Data Source](#)

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Delenasaw Yewhalaw

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Comments to Authors

Title “Fine-scale spatial and temporal heterogeneities in insecticide resistance profiles of the malaria vector, *Anopheles arabiensis* in rural south-eastern Tanzania”

General

This paper evaluated spatial and seasonal variations of insecticide resistance profile in the major malaria vector, *An. arabiensis*. Generally, this is a well written paper with important information showing how resistance level in malaria vectors vary periodically and among proximal localities in the same geographic area. The work further highlights the need to consider this small-scale variation in implementing insecticide resistance monitoring and management strategies which mainly relies on insecticide resistance data often coming from few selected district sentinel sites. Such an approach is important for countries which initiated malaria elimination programs where residual transmission could be sustained due to resistance by local mosquito populations.

Introduction:

OK, however, it would have been good to provide information on the type of insecticides which have been used for crop protection in the 3 villages as this may help understand the observed variation in resistance profile

Methodology:

The methodology is sound and clear. However, it is not clear why authors used 3-5 days old adult female mosquitoes for insecticide susceptibility test and 2-3 days old adult female mosquitoes for synergist assays.

Results:

This reviewer would suggest to use “KD rate reduction” instead of “Resistance reduction”. Authors should also interpret the results of PBO and TPP assays with caution as the results from these assays indicate partial involvement of P450 and esterases. Hence, those enzymes may not be key determinants conferring resistance in the mosquito populations.

Discussion:

OK

Conclusion

OK

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

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doi:[10.21956/wellcomeopenres.13662.r26599](https://doi.org/10.21956/wellcomeopenres.13662.r26599)



Eric Ochomo 

Centre for Global Health Research, Kenya Medical Research Institute (KEMRI), Kisumu, Kenya

This manuscript is very well written and adequate in form and content. It could be improved greatly if the authors did the following:

1. Methods: It is not clear whether the papers used for the WHO tube bioassays were procured from WHO sources or if these were made locally. If they were made locally, it would be nice to include a statement about QA and QC.
2. Results: The bar charts used to present resistance data should have error bars.
3. Table 2: The authors need to include some statistics of significance for the resistance reduction indices.
4. Discussion: One paper that would be useful to cite is the Ochomo *et al* 2014 paper which describes heterogeneity of insecticide resistance in Kenya¹.

References

1. Ochomo E, Bayoh NM, Kamau L, Atieli F, Vulule J, Ouma C, Ombok M, Njagi K, Soti D, Mathenge E, Muthami L, Kinyari T, Subramaniam K, Kleinschmidt I, Donnelly MJ, Mbogo C: Pyrethroid susceptibility of malaria vectors in four Districts of western Kenya. *Parasit Vectors*. 2014; **7**: 310 [PubMed Abstract](#) | [Publisher Full Text](#)

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Referee Expertise: Entomology, Insecticide Resistance, Malaria, Epidemiology, Surveillance

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.
