

# High level of resistance in *Anopheles arabiensis* mosquito to pyrethroid insecticides from low malaria transmission zone of Moroto district, Karamoja region, Uganda: Implication for malaria vector control

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## Research

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# Abstract

**Background:** Karamoja region of Uganda previously classified as low malaria transmission zone is currently experiencing significant upsurge of malaria incidences. Long lasting insecticidal nets (LLINs) impregnated with pyrethroids constitute a major tool for malaria control in this region. Efficacy of this tool can be hampered by resistance to the pyrethroids in the *Anopheles* mosquito vectors. Resistance status of these mosquitoes in this region is poorly understood, effectively hampering better understanding of the impact of LLINs in the malaria control initiative. Here, we assessed susceptibility of the *Anopheles arabiensis* from the region to deltamethrin, permethrin (pyrethroids) and pirimiphos-methyl (organophosphate) insecticides.

**Method:** We collected anopheline mosquito larvae from their natural habitats and reared them to adult emergence *in situ* field insectary in Karamoja region. We then identified them morphological to species level and exposed 513 emerge adult female *An gambiae s.l.*, mosquitoes to diagnostic dosages of deltamethrin (0.05%), permethrin (0.75%) and pirimiphos-methyl (0.25%) pyrethroids exposure using the standard WHO insecticide susceptibility test assay. Synergic assays using piperonyl butoxide (PBO) were done to check for the involvement of detoxification enzymes in pyrethroid resistant populations. We then screened for knockdown resistance (KDR) and mosquito species diversity using Polymerase Chain Reaction (PCR).

**Results:** Majority (96%) of the mosquitoes we sampled were identified as *An. arabiensis* and 4% as *An. gambiae sensu stricto*. We observed cross-resistance to both deltamethrin (11.9%) and permethrin (47%) but susceptibility (100% mortality) to pirimiphos-methyl in *An. arabiensis*. The pre-exposure to PBO ameliorated the resistance to both pyrethroids. We detected homozygous KDR -eastern variant in 1.8 and 50% of the *An. arabiensis* and *An. gambiae s.s.* respectively.

**Conclusion:** *Anopheles arabiensis* and *An. gambiae s.s.* are the malaria vector in Karamoja region with *An. arabiensis* predominating. Both species are susceptible to pirimiphos-methyl but resistant to both deltamethrin and permethrin, through a metabolic process (phenotype). Mosquitoes with genetic (kdr) mutations for resistance were minimal and hence have minimal contribution to the pyrethroid resistance profile. *An. arabiensis* can thus be controlled in Karamoja region using deltamethrin and/or permethrin impregnated mosquito nets integrated with PBO and/or through indoor residual spraying of sprayable human dwellings with pirimiphos-methyl.

## Background

Malaria is a leading cause of morbidity and mortality in the tropics despite reduction in global malaria burden [1,2] The decline has been attributed to use of long-lasting insecticidal nets (LLINs), indoor residual spraying (IRS), introduction of artemisin-based combination therapy (ACT) for malaria treatment and intermittent preventive treatment (IPTp) during pregnancy [2]. Despite these significant gains, long-term effectiveness of malaria vector controls using LLINs and IRS is currently being challenged by widespread insecticide resistance in mosquito populations [3].

World Health Organization (WHO) recommends expansion of insecticide resistance monitoring and surveillance within national and regional control programs [4]. This initiative can 1) provide insights into

mechanisms that can drive resistance, 2) characterize effectiveness of control efforts and 3) identify causes of any failures within the controlled areas for effective implementation and sustainable vector control [5,6]. WHO recommend organochlorines, organophosphates, pyrethroids and carbamates for control of malaria vectors, among which pyrethroids are the only WHO prequalified insecticides for LLINs, and are also extensively used for IRS due to their low cost, human safety and long duration of residual action [4]. However, there are other novel pyrolle and neonicotinoid insecticide compounds like pyrolle chlorfenapyr and neonicotinoid clothianidin that are used in LLINs and IRS in combination with pyrethroids and all have different mode of actions [7–9]. The LLINs and IRS are widely promoted and implemented as public health intervention tool for malaria control in most malaria-endemic countries including Uganda [10]. In most of Uganda, LLINs is selectively implemented in the different regions, with an average operational coverage of over 95% [11]. The control programmes are concentrated in high/medium transmission zones while low malaria transmission zones, including Karamoja region have largely been neglected [12,13]. Despite extensive LLINs implementation, over the recent years Karamoja experienced significant (> 60%) malaria incidences between 2015 to 2017 [11,14] with Moroto district registering 334.5 cases per 1000 children under 5 years [15]. This translates into about 33 % disease prevalence and may be linked to factors such as pyrethroid resistance that might be impeding efficacy of the LLINs [16–18].

Pyrethroids exert their insecticidal effect on the voltage-gated sodium channel (VGSC) located on the membrane of neurons [19]. When pyrethroids bind an open channel, they block its closure, thus extending the action potential and resulting in the insect's rapid paralysis, a phenomenon known as "knockdown resistance" (kdr) [20]. However, mosquito resistance to pyrethroids is an emerging challenge to efficacy of otherwise successful insecticide-treated nets (ITN) based malaria control intervention against adult vector [21]. This is particularly of great concern to sub-Saharan Africa, with high malaria transmission levels and major vectors (*An. funestus*, *An. gambiae* s.s. and *An. arabiensis*) resistant to pyrethroids [22]. The resistance is mediated through knockdown resistance (kdr) mutations, enhanced detoxification of pyrethroids by the mosquito enzymes (esterases, monooxygenases and glutathione S-transferases [23,24] and probably other unknown mechanisms such as behavioral and penetration [25,26] known to occur in other vectors [27,28].

This present study was initiated to establish distribution of major malaria vectors and their resistance status to deltamethrin, permethrin (pyrethroids) and pirimiphos-methyl (organophosphate) insecticides in Karamoja region of Uganda. Our findings, reported herein, will contribute to designing suitable control interventions and improve implementation of resistance management strategies across Karamoja region of Uganda.

## Methods

### Study area

Karamoja lies between 1°30' to 4° 06' N and 33° 30' to 35° covering an area of 29,430 km<sup>2</sup> forming the north-eastern part of Uganda with a human population of about 1.1 million [29]. This region encompasses Abim, Amudat, Kaabong, Karenga, Kotido, Moroto, Nabilatuk, Napak and Nakapiripit districts. The region has 53.8

to 63.5% Human Poverty Indices (HPI) compared to 37.5 % Uganda national average [30]. Most of the populations are nomadic pastoralist. The region is characterized by savannah woodlands and semi-desert vegetation with 1200 and 1500m plateaus and Kadam, Akisim and Napak Mountains and Moroto mountain (about 3083m above sea level) [31]. The region is drained by Turkwel River, part of internal drainage basin of Lake Turkana in Kenya. Karamoja experiences only one rain season which occurs from May to July with malaria transmission peak picking up immediately after the rains. The mean annual rainfall level is between 350 and 750 mm [32]. Ambient temperatures range from 16°C to 30°C. The species of *Anopheles* mosquitoes in the and *Anopheles gambiae* sensu stricto Giles 1902 [33]. Malaria transmission in the region is absolutely (100%) attributed to *Plasmodium falciparum* parasite [34]. Malaria prevalence in the region has increased by 30% with average incidence ranging from 166 in 2015 to 295 in 2018 per 1000 people [11]. The LLINs which is the only malaria vector control strategy employed in the region with 57% households owning more than one ITN with ratio of use and access of 0.95 [13,35] This indicate low ratio of people sleeping inside nets.

### **Mosquito collections**

We collected mosquito larvae stages 3, 4 and pupae from four aquatic breeding habitats in Moroto (N02°32.0' E34°40.0') in the rainy month of July 2019 using dipping technique. The sites included brick pits and puddles (N2°35'4" E34°41'50"), road side drains (N2°31'52" E34°39'22") and excavations (N2°31'13" E34°31'21"), sand pits and open gardens (N2°28'57" E34°37'7"). Larvae were collected once within the three days in the month of July 2019. Immediately after sampling, the larvae were transferred to a field insectary where they were maintained at a density of 500 larvae per three liters in their natural water that contained dissolved natural foods. We kept the emergent adult mosquitoes in standard 30×30×30 cm cages in under the ambient insectary environmental conditions. We identified *An. gambiae s. l* among emerged mosquito adults using morphological keys [36] offered them 10% glucose solution *ad libitum* and tested the females for insecticide resistance 2-5 days post emergence. We obtained ethical clearance for mosquito collection from Uganda National Council for Science and Technology (authorization No SS 4610).

### **Insecticide susceptibility bioassay**

We conducted insecticide susceptibility tests on 2-5 days old non-fed adult female *An. gambiae s.l.* mosquitoes using the standard WHO tube bioassay protocol for assessing potential insecticide resistance [37] at Gulu University mosquitoes insectary. To simulate natural conditions, we maintained an average temperature in the insectary at  $29 \pm 2^\circ\text{C}$  (day) and  $24 \pm 2^\circ\text{C}$  (night), with relative humidity (RH) ranging from 57 to 70 and a photoperiod of 12:12 h (L–D). Our overall maintenance of the colony followed standard operating procedure for rearing *Anopheles* mosquitoes [38,39] We exposed 492 female *An. arabiensis* to 0.05% deltamethrin, 0.75% permethrin or 0.25% pirimiphos-methyl concentrations on insecticide-impregnated papers or control (oil-treated) (Vector Control Research Unit, Universiti Sains Malaysia) for 60 minutes. During this exposure duration, we recorded the number of mosquitoes knocked-down at 10, 15, 20, 30, 40, 50 and 60 minutes intervals post exposure. After the exposure period, mosquitoes were transferred to holding tubes and maintained on 10% glucose solution. The final mortalities were determined after 24 h post

exposure. After the bioassays, we collected dead (susceptible) and alive (resistant) mosquitoes, and stored them individually in separate Eppendorf tubes with silica gel for subsequent molecular laboratory analysis.

### **Evaluation of potential impact metabolism on resistance to the pyrethroids in the mosquitoes**

To evaluate if metabolism of the pyrethroids by the mosquitoes was responsible for the insecticide resistance we observed, we assessed relative mortality of the mosquitoes exposed to the pyrethroids with or without piperonyl butoxide (PBO) that prevents [pyrethroid catabolism](#) by Cytochrome P450 oxidase that can detoxify the active ingredient before an insecticidal effect can occur [40]. This inhibition produces a synergistic effect [41]. Briefly, we assessed this effect using WHO protocol [37] where we pre-exposed adult female *An. gambiae s.l.* to WHO papers impregnated with 4% PBO an oxidase inhibitor for 1 hour. This was followed immediately with exposure to discriminating doses of 0.05% deltamethrin and 0.75% permethrin on impregnated papers for 60 minutes. Five replicated were performed for each exposure set. We recorded the knock-down rates at 10, 15, 20, 30, 40, 50- and 60-minutes intervals during the one-hour exposure to synergist and to insecticides (deltamethrin or permethrin). We immediately assessed the resultant mortality of the flies from the three treatments (PBO + deltamethrin or permethrin exposed or unexposed mosquitoes). The live mosquitoes were fed on 10% glucose solution. Mortality rates from assays conducted with and without exposure to PBO (synergist) were scored after 24 hours of exposure. Mortality rates were compared between PBO deltamethrin or permethrin exposed and unexposed mosquitoes. Resistance was also compared with pirimiphos-methyl to ascertain which test mosquitoes were fully susceptible. All mosquitoes tested were identified to species level by PCR as described in mosquitoes species identification subsection below.

### **PCR identification of members of *An. gambiae s.l.* and *An. funestus s.l.***

We identified our mosquitoes (post exposure) to their *An. gambiae s.l.* sibling species status using PCR methods of Scott et al. [42], and for members of the *An. funestus s.l.* group using a method of Koekemoer et al. [43]. We extracted total genomic DNA (gDNA) from whole flies using the DNeasy blood and tissue kits (Qiagen, Valencia, CA) following the manufacturer's protocols and assessed the quality of the DNA on 1.5% agarose gel as visualized on Gel Doc Imaging System (UVITEC, Cambridge). Our primers included those specific to *An. gambiae s.l.*, *An. arabiensis* as well as the universal *An. gambiae s.l.* complex primer [42]. Similarly, specific primers for *An. funestus s.l.* and universal primer for the *An. funestus* group were also used for the *An. funestus s.l.* [43]. These two Ribosomal DNA polymerase chain reaction methods for *An. gambiae s.l.* and *An. funestus* complex are based on species-specific nucleotide sequences in the ribosomal DNA internal transcribed spacer region 2 (ITS2) and are used to identify both species and interspecies hybrids in mosquitoes regardless of the life stage using extracted DNA. The primer sequences are derived from the 28S coding region and the 5' end of the intergenic spacers (IGS) of the members of the *An. gambiae* and *An. funestus* complexes. In the PCR reaction, we amplified 2.5µl gDNA with 1 unit of GoTaq Green Master Mix (Promega, Madison, MO) in the buffer in a total volume 12.5 µl. For *An. funestus* complex, conditions remained the same except that we added 0.5 mM of MgCl<sub>2</sub>. We run the reactions in touch screen thermal cycler (SimpliAmp, Applied Biosystems, Life Technologies, Singapore). The first cycle included five minutes at 95°C, 30 seconds at 50°C, and 30 seconds at 72°C. Subsequent cycles involved 1 minute at 94°C,

30 seconds at 50°C, and 1 minute at 72 °C for 30 cycles. We also run positive and no-sample negative controls. We loaded The PCR products onto 1X SYBR safe (Invitrogen, 5791 Allen Way Carlsbad CA 92008, USA) 2 % agarose gels (AppliChem GmbH Ottoweg Damstadt Germany) in a TBE (40mM Tris-HCl pH 8.3, 45 mM boric acid, and 1mM EDTA) buffer and run a 100 bp DNA ladder molecular weight marker (Life Technologies, Rockville, MD) to confirm expected molecular weights of the amplification products. We documented our PCR products using GelDoc Imaging System (UVITEC, Cambridge) and scored expected PCR product sizes for *An. gambiae s.s* at 390bp, *An. arabiensis* 315 bp and 505bp *An. funestus* [42,43].

### **Detection of East African kdr resistance mutations in *An. gambiae s.l.***

We also used the methods described by Ranson et al. [24] to detect single base pair specific single nucleotide polymorphisms (SNPs) leucine to serine substitution TTA/TCA mutation in the voltage-gated sodium channel known as knockdown resistance to DDT and pyrethroids in East Africa. Briefly, we amplified 5 µl gDNA, 0.2µM of the specific primers (AgD1, AgD2, AgD4 and AgD5) with 1 unit of GoTaq Green Master Mix (Promega, Madison, MO) in the buffer in a total volume 25µl. We run the reactions in touch screen thermal cycler (SimpliAmp, Applied Biosystems, Life Technologies, Singapore). We used touch down PCR conditions with the initial denaturation at 95°C for 5 minutes followed by 10 cycles of denaturation at 94°C for 1 minute, annealing at 54°C for 30 seconds and extension of 72°C for 30 seconds. This was followed by 30 cycles of denaturation at 94°C for 1 minute, annealing at 47°C for 30 seconds and extension at 72°C for 30 seconds with the final extension of 72°C for 10 minutes and holding at 4°C until when collected. We also run positive and no-sample negative controls. PCR products analysis was carried out as described in mosquito species identification subsection above.

### **Data analysis**

We corrected the knockdown rates for testing the toxicity of each insecticide, where the knockdown in our control treatment was greater than 5% but less than 20% using Abbott's formula [44] and then transformed them to Probits [44] for linear regression analysis and the determination of 50 % knockdown ( $KD_{t50}$ ). For Probit analysis, we used GraphPad Prism version 7.00 for Mac (GraphPad Software, La Jolla California USA). We used weighted mean summarize knockdown due to different insecticides and adopted WHO criteria of interpretation of our results. Consequently, we considered mosquito population to be 1) resistant (confirmed) to a particular insecticide if mortality rate was 0-79%, 2) resistant suspected if mortality was 80-97% and more investigations are required, and 3) susceptible when the mortality was 98-100%. We summarized data for the metabolic tests as mean percent mortality of the five replicates, and calculated 95% confidence intervals around those means.

## **Results**

### **Susceptibility of *An. arabiensis* to pyrethroids**

Results of bioassay of adult female *An. gambiae s.l.* (N= 513) for susceptibility to the three insecticides are summarized in Figure 1, 2 and Table 1. Based on WHO criteria, [37], resistance (< 95% mortality) of *An. arabiensis* to deltamethrin and permethrin was observed in all the samples, with mortality varying from 12%

to 91% in Moroto (Table 2). Susceptibility of *An. arabiensis* to pirimiphos-methyl was observed in Moroto (Table 2).

### Impact of PBO on toxicity permethrin and deltamethrin to *An. arabiensis*

An hour pre-exposure of *An. arabiensis* to PBO enhanced toxicity of deltamethrin (from 11.8 to 90%) or permethrin (from 47 to 89.2%) in Moroto, compared to cohorts directly exposed to each of the two candidate insecticides without PBO pre-exposure (Figure 1 and Table 2), suggesting potential cytochrome P450 oxidase mediated resistance [45] to these insecticides in the mosquito. However, our observations that the pre-exposure to PBO partially abolished resistance to deltamethrin and permethrin in Moroto still suggests partial role of the cytochrome P450 oxidase in the resistance phenotype while other mechanisms might also play a role (Figure 1 and Table 2).

In the absence of PBO, the  $LT_{50}$  for deltamethrin and permethrin on *An. arabiensis* were similar (Table 3 and Figure 3). However, the PBO significantly reduced the  $LT_{50}$  for both deltamethrin and permethrin on *An. arabiensis* relative to their respective native formulations (without PBO). Additionally, incorporation of PBO reduced the  $LT_{50}$  in permethrin more than it did with deltamethrin (Table 3).

### *An. gambiae* s.l. species identification

Our subsequent PCR of sibling species on most of the mosquitoes (N = 342) post exposure revealed that most of the mosquitoes were *An. arabiensis* (96%) with the rest being *An. gambiae* s.s. (4%). No *An. funestus* species was detected.

**Table 1: Knockdown summary during 60 minutes exposure**

Insecticide Tested	No. of Knocked down Mosquitoes							Total Exposed	KT50 (min)	KD60 (%)
	10	15	20	30	40	50	60			
Pirimiphos-methyl	0	1	3	9	15	30	56	110	60	51
Deltamethrin	1	3	11	25	51	79	84	101	40	83
Deltamethrin PBO	23	63	76	90	96	100	100	100	15	100
Permethrin	2	12	18	45	65	89	95	100	40	95
Permethrin PBO	34	86	94	95	98	100	101	102	15	99

**\*KT50:** Time after which 50% of the *Anopheles* tested are knocked down.

**\*KD60:** Proportion of *Anopheles* knocked down after 60 minutes.

**Table 2: Mean percentage mortalities following exposure of 2-5 day old *An. arabiensis* to pirimiphos-methyl, deltamethrin and permethrin. Mortalities were recorded 24 hours post exposure (for 60-minutes)**

Insecticide Tested	EXPOSED			CONTROL		
	Mortality	Total Exposed	% Mortality in exposed	Mortality	Total Exposed	% Mortality in control
Pirimiphos-methyl	110	110	100	00	55	00
Deltamethrin	12	101	12	00	49	00
Deltamethrin PBO	90	100	90	00	50	00
Permethrin	47	100	47	00	50	00
Permethrin PBO	93	102	91	00	50	00

**Table 3: Median Lethal Time for knockdown in Adult female *An. arabiensis* mosquitoes (from Moroto district, Uganda) by various formulations of pyrethroids.**

Pyrethroid	PBO	LT <sub>50</sub> , Min	95% CI	Slope ( $\beta \pm SE$ )	$\chi^2$
Deltamethrin	-	39.13	34.03 - 70.73	4.18 $\pm$ 0.90	3.532
	+	13.44	11.95 - 15.24	3.43 $\pm$ 0.46	3.646
Permethrin	-	37.02	30.69 - 63.59	2.73 $\pm$ 0.43	2.973
	+	11.03	10.47 - 11.67	6.09 $\pm$ 0.62	2.377

PBO – Piperonyl Butoxide, (+) - with PBO, (-) - Without PBO, CI- Confidence Interval, LT - Median lethal Time.

**Prevalence of East African (L1014S) knockdown resistance (kdr) point mutations in *An. arabiensis* and *An. gambiae s.s.* in Moroto district**

We have summarized our distribution of L1014S mutations from genotyping of *An. arabiensis* (n=328) and *An. gambiae s.s.* (15) in Table 4. Of the 328 *An. arabiensis* tested for kdr east allele, 66.2% (n=217) were homozygous for wild type alleles (SS) and 1.2% (n=4) were homozygous for mutation alleles (RR). Of the 15 *An. gambiae s.s.*, 33.3% (n=5) were homozygous for the susceptible wild type (SS), 13.3% (n=2) were homozygote mutation alleles (L1014S) and only 6.7% (n=1) was heterozygous (HR).

**Table 4: KDR genotypes**

Mosquitoes species	Insecticide	Survival status after exposure	# mosquitoes tested	Homozygote mutation (RR)	Heterozygote mutation (RS)	Homozygote wild type (SS)
<i>An. arabiensis</i>	Deltamethrin + PBO Exposed	Dead	55	1	0	39
<i>An. gambiae s.s.</i>	Deltamethrin + PBO Exposed	Dead	4	0	0	3
<i>An. arabiensis</i>	Deltamethrin	Live	81	1	0	76
<i>An. gambiae s.s.</i>	Deltamethrin	Live	2	1	0	0
<i>An. arabiensis</i>	Permethrine + PBO Exposed	Dead	110	0	0	80
<i>An. gambiae s.s.</i>	Permethrine + PBO Exposed	Dead	3	0	1	2
<i>An. arabiensis</i>	Permethrin	Live	40	0	0	40
<i>An. gambiae s.s.</i>	Permethrin	Live	1	0	0	1
<i>An. arabiensis</i>	Primiphos methyl	Dead	42	2	0	22
<i>An. gambiae s.s.</i>	Primiphos methyl	Dead	4	1	0	0
<i>An. arabiensis</i>	Primiphos methyl	Live	0	0	0	0
<i>An. gambiae s.s.</i>	Primiphos methyl	Live	0	0	0	0

KDR - knockdown resistance, PBO – Piperonyl Butoxide, # - number. SS represents homozygous for wild type alleles, RR homozygous mutation alleles and HR heterozygous mutation alleles.

## Discussions

We report the first results of resistance in *An. arabiensis* against pyrethroid in Karamoja region. We established *An. arabiensis* as the major malaria vector in Karamoja areas followed by *An. gambiae* s.s. As in other studies, the major malaria vectors in Uganda include *An. gambiae* s.s. *An. arabiensis*, *An. Bwambae*, and *An. funestus*, with 90% of malaria transmission in the country being attributed to *An. gambiae* s.l [46–48]. *An. arabiensis* and *An. gambiae* s.s. reported in our study have different genetics and behavior that can have important implications for the epidemiology of malaria and their control. The current use of LLIN in Karamoja region for malaria vector control might be less effective against *An. arabiensis*. This is mainly due to resistance of the mosquito to the insecticides (deltamethrin and permethrin), more exophagic, exophilic and zoophilic nature of *An. arabiensis*.

*An. arabiensis* being less killed by they two insecticides deltamethrin and permethrin commonly used for the treatment of LLIN. Similar studies have been reported in Kenya and Tanzania [49,50] This happens as a result of the more exophagic, exophilic and zoophilic tendencies of *An. arabiensis* compared to *An. gambiae* s.s. behaviors. As shown in other studies, *An. arabiensis* have peak biting activities happening during the early parts of the night when most people have not gone to bed [51,52] . What this could means is that the early evening and outdoor biting of *An. arabiensis* can compromise on the efficacy of the LLINS in Karamoja region as seen in Ethiopia [52] and Tanzania [49]. The exophagic and exophilic nature of *An. arabiensis* similarly can affect the performance of IRS since these mosquitoes tend to rest and feed outdoors [53].

The high prevalence of malaria seen in Karamoja region as describe in other studies [11,14] may be linked to population dynamics of *An. arabiensis* in the region. Exophagic and exophilic nature of *An. arabiensis*, increases the outdoor biting and resting of these mosquitoes leading to high malaria transmission in the area. Besides, *An. arabiensis*, bite early evening or morning when the population is not protected by LLIN potentially sustaining residual malaria in area with high coverage of net [54,55]. Additionally communities in Karamoja region thrive on pastoralism looking for pasture and water for their livestock which pre-expose them to outdoors activities when keeping the livestock and increasing the chances of more outdoor mosquitoes' bites and thus more malaria in the area.

In this study, we confirm existence of both mechanisms of insecticide resistances: knock down resistance (kdr) and metabolic resistance in the *An. gambiae*s.s. and *An. arabiensis* in Karamoja region. We confirm the restoration of *An. arabiensis* susceptibility following the pre-exposure to PBO indicating increased activity of detoxification of enzymes which are contributing to pyrethroid resistance in Karamoja region. However, future further studies are needed in characterizing these mechanisms of insecticide resistances. Besides, future planned roll out of LLINs enhanced with PBO might not be a magic bullet since more than one resistance mechanisms were observed in *An. gambiae* s.l. in Karamonja region.

While the two pyrethroids seems to be having similar performance, PBO significantly enhances the performance of both deltamethrin and permethrin and further enhances that of permethrin over that of deltamethrin (Table 3). This suggests that permethrin incorporated with PBO would have a much better performance in addressing the issues of pyrethroids resistance in Moroto than deltamethrin. These insecticide resistances could as well have important implications for the epidemiology of malaria as well as malaria vector control in the low malaria transmission zones of Karamoja areas. Although pyrethroids are

currently used in all LLINs as recommended by the World Health Organization, there are also used in combination with either synergists (PBO) [55] or other active ingredient like clothianidin, chlorfenapyr in Interceptor G2 [7,8]. However, pyrethroid resistance of malaria vectors is still widespread in Africa as well as other classes of insecticides [23,37,56]. Increased resistance has been attributed to selection pressure from the scale-up of LLINs and IRS [23,57] and use of similar classes of insecticides in agriculture [58], although the relative contribution of these mechanisms varies by area [59].

Given that resistance has been reported in *An. gambiae* s.s and *An. funestus* [57,60–66] that are the major malaria vector in Uganda and now *An. arabiensis* in this current study in Karamoja region, this is a major threat to LLINs and IRS use in malaria vector control programmes in the country and the whole of the neighbouring countries. This appears to reflect the need to monitor malaria vector resistance. As demonstrated in other studies on the host feeding and insecticide resistance, the fitness costs associated with insecticide resistance can have influence on malaria transmission directly by altering host seeking feeding and mating behaviours [67–69] or fecundity [70] and reducing mosquito life span [71,72] It can also indirectly influence malaria transmission by impairing parasite development inside the mosquitoes [70,73,74].

Current WHO guidelines recommend combining ITNs and IRS in various transmission settings, especially in areas with holoendemic and epidemic malaria [5]. LLINs and IRS could be employed together in the same households in Karamoja region. However, due to unique bionomics of *An. arabiensis*, local anthropological and variable environmental factors in Karamoja region, the combined entomological outcomes of IRS and LLIN interventional trails can be carried out. Besides, insecticide resistance management should rely on tactical deployment of the active ingredients used for IRS and on LLINs in rotation, combinations (particularly LLINs), mosaics and mixtures as recommended in other studies [5]. What we observed would be important is, if LLINs are to be combined with IRS for malaria prevention and control, the selection of appropriate LLIN types and design should put in consideration the housing structures and cultural issues concerning in-house sleeping patterns and IRS chemicals should be done with caution to avoid further exacerbating existing resistance.

### **Implication for future malaria vector control**

Nevertheless, the increasing intensity of resistance currently seen in *An. arabiensis* mosquito populations in Karamoja region will reduce the efficacy of pyrethroid-based interventions. The right course of action for malaria vector control program is therefore to adopt a proactive approach and modify current practices so as to delay the spread of resistance and preserve the effectiveness of deltamethrin and permethrin insecticides.

The high frequencies or intensities of resistance can lead to failure of IRS and can thereby have an epidemiologically significant effect on malaria incidence [75]. There is also cross-resistance between different classes of insecticides that share the same mode of action. The existence of cross-resistance and multiple resistance restricts the choice of alternative insecticides in situations where resistance has been detected. Besides, the impact of the observed spread of resistance will have effectiveness of current vector-control programmes. Therefore, a key element of effective resistance management is the use of alternations,

rotations, or sequences of different insecticide mode of action classes. Other studies have shown that even in the presence of pyrethroid resistance, insecticide-treated mosquito nets (ITNs) perform better than untreated nets in terms of protection against blood-feeding, and ITNs can induce significant mosquito mortality [76].

We also recommend the use of Larval Source Management (LSM) in order to prevent the completion of development of the immature stages. Mosquitoes breeding sites in Karamoja region can easily be mapped out during the dry season. LSM can be done through 1) Larviciding; the regular application of biological or chemical insecticides to water bodies and 2) biological control: the introduction of natural predators into water bodies.

## Limitations

We acknowledge the limitations of the current study including:

- The time constraints of conducting this research during.
- The sites sampled were predominantly suited for *gambiae* s.l breeding, thus findings don't rule out possibility existence of *An. funestus* s.l in the area.

## Conclusions

Our findings show that *Anopheles arabiensis* and *An. gambiae* s.s. are the malaria vectors in Karamoja region with *An. arabiensis* predominating. Both species are still susceptible to pirimiphos-methyl but resistant to both deltamethrin and permethrin, through a metabolic process (phenotype). *An. arabiensis* with genetic (kdr) mutations for resistance were minimal and we think this is having minimal contribution to the pyrethroid resistance profile seen in the region. However, we confirm restoration of *An. arabiensis* susceptibility following the pre-exposure to PBO indicating elevated levels of detoxifying enzymes in these vectors in Karamoja region. Therefore guidelines for management and controlling *An. arabiensis* insecticide resistance in Karamoja region are needed. *An. arabiensis* in Karamoja region can be controlled using deltamethrin and/or permethrin impregnated mosquito nets integrated with PBO and/or through indoor residual spraying of sprayable human dwellings with pirimiphos-methyl.

## List Of Abbreviations

IRS: indoor residual spraying

LLINs: long lasting insecticide-treated nets

WHO: World Health Organization

KDR: knockdown resistance

PCR: Polymerase chain reaction assay

PBO: Piperonyl butoxide

MFOs: mixed-function oxidases

## Declarations

### Authors' contributions

RE, JI and DO conceived, contributed design of the study, field collections, performed laboratory work, analyzed the data, and drafted an initial version of the manuscript. JA, PM, GMM and TI performed laboratory work and analyzed the data. EAO conceived, designed the study, coordinated fieldwork and provided guidance. All authors read and approved the final manuscript.

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### Competing interests

The authors declare that they have no competing interests.

### Availability of data and materials

The authors declare that all the main data supporting the findings of this study are available within the article. Any additional data sets are available from the corresponding author upon reasonable request.

### Consent for publication

Not applicable.

### Ethics approval and consent to participate

This study was approved by Gulu University Ethical Review Committee. Formal approval to conduct the study was granted by the Uganda National Council for Science and Technology and the Office of the Ugandan president (SS4610). Community leaders in Moroto district provided written informed consent.

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## Figures

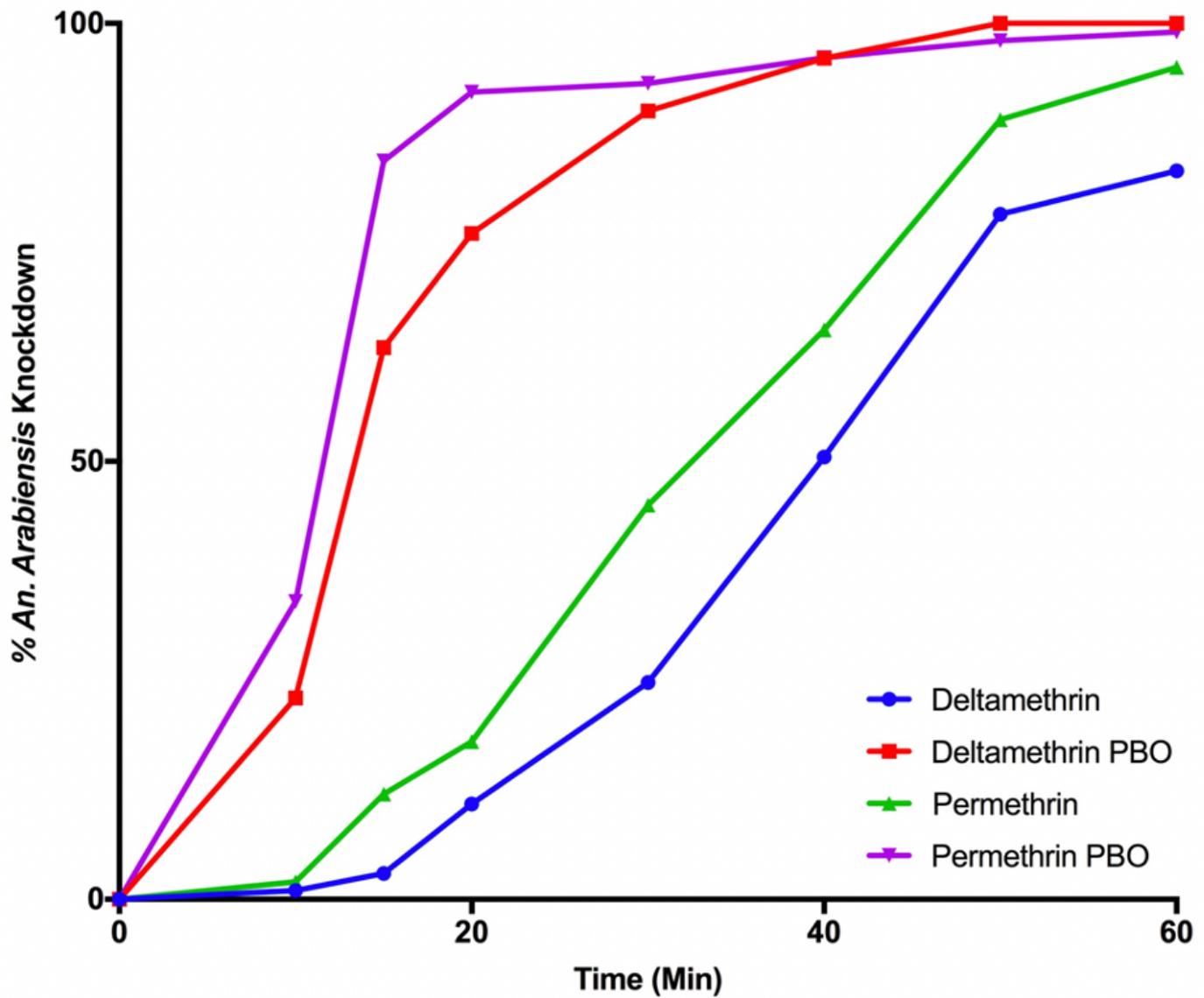
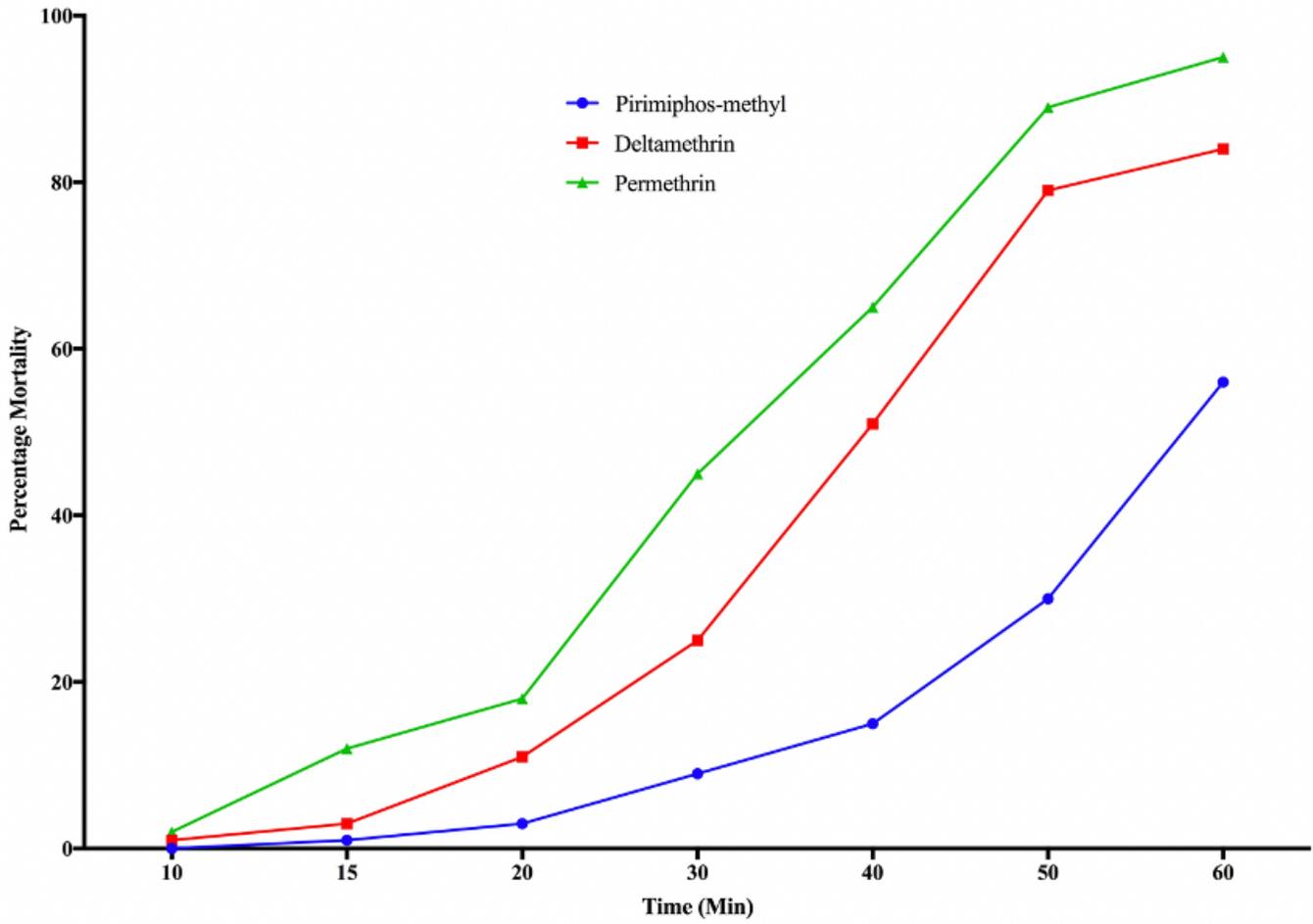


Figure 1

Percentage of *An. arabiensis* knocked down during 60-minutes exposure to deltamethrin or permethrin and PBO using WHO tube assay in Moroto District in Uganda, July 2019 (Larval collections)



**Figure 2**

Percentage of *An. arabiensis* knocked down during 60-minutes exposure to pirimiphos-methyl, deltamethrin or permethrin using WHO tube assay in Moroto District in Uganda, July 2019 (Larval collections)

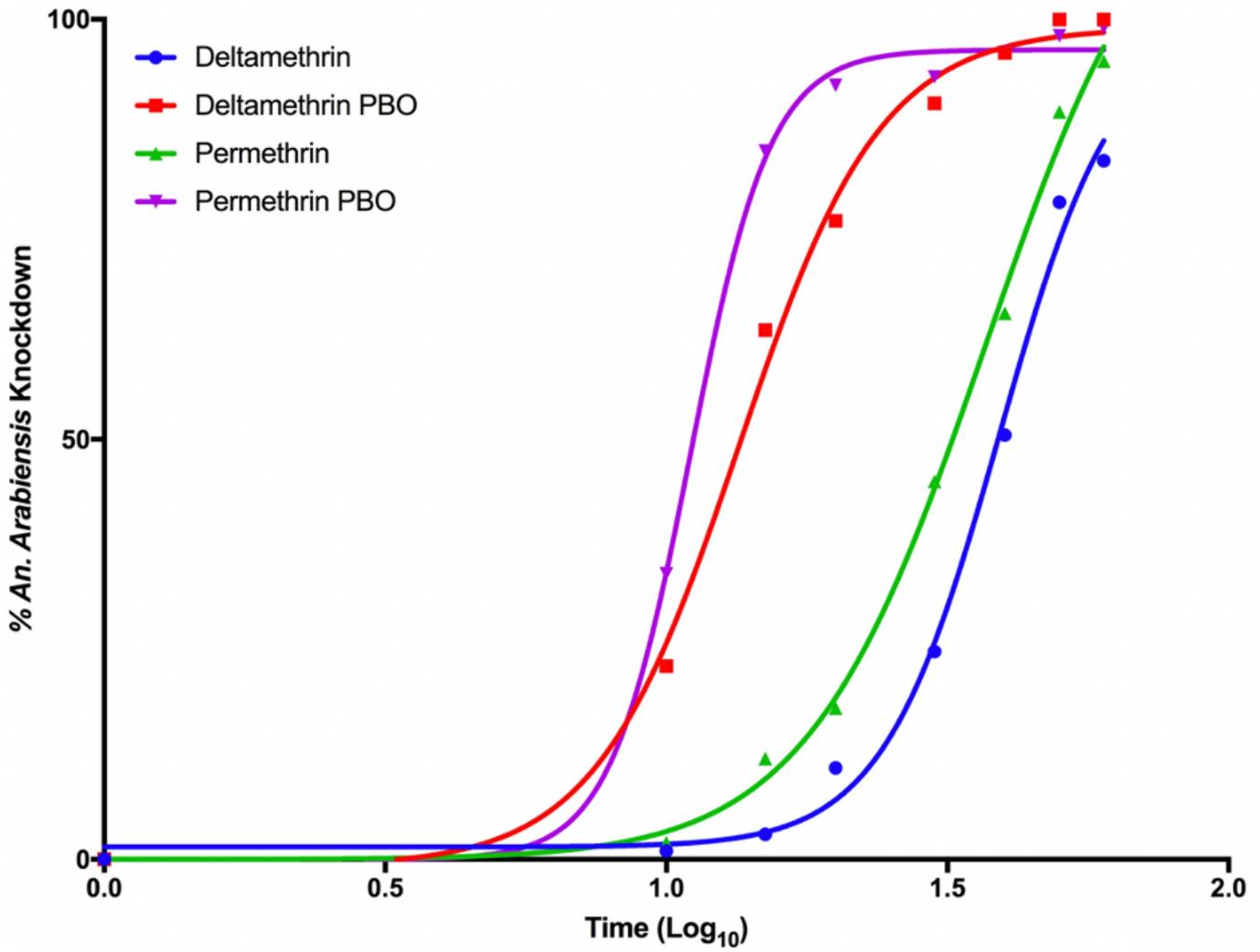


Figure 3

Temporal (log<sub>10</sub> minutes) percentage knockdown of Adult female *An. arabiensis* mosquitoes (from Moroto district, Uganda) by various formulations of pyrethroids.