DETECTION AND MONITORING OF INSECTICIDE RESISTANCE IN MALARIA VECTORS IN TANZANIA MAINLAND

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i. ABBREVIATIONS/ACRONYMS

B&MGF	Bill and Melinda Gates Foundation
BCC	Behaviour Change Communication
DDT	Dichlorodiphenyltrichloroethane
DED	District Executive Director
DMO	District Medical Officer
DNA	Deoxyribonucleic acid
GFATM	Global Fund for AIDS, Tuberculosis, and Malaria
GPS	Global Positioning Systems
IEC	Information Education and Communication
IPTp	Intermittent Preventive Treatment in Pregnancy
IRS	Indoor Residual Spraying
ITN	Insecticide Treated Net
KCMC	Kilimanjaro Christian Medical College
Kdr	Knockdown resistance
KDT	Knock Down Time
LLIN	Long-Lasting Insecticidal Net
MRCC	Medical Research Coordinating Committee
NIMR	National Institute for Medical Research
NMCP	National Malaria Control Programme
PCR	Polymerase Chain Reaction
PMI	President's Malaria Initiatives
RTI	Research Triangle Institute
s.l.	sensu lato
s.s	Sensu stricto
SE	Standard Error
TPRI	Tropical Pesticides Research Institute
USAID	United States Agency for International Development
VBC	Vector Biology and Control
WHO	World Health Organization

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iv. DISCLAIMER

The authors' views expressed in this report must not be construed as necessarily reflecting the views of the National Institute for Medical Research (NIMR) whatsoever. This report is the result of the authors' investigations, except where otherwise stated. All other sources are acknowledged and a bibliography is appended. Care has been taken to ensure the accuracy of the information presented, and the authors alone are responsible for the views and any omissions contained herein. Any use of trade names is for identification purposes only and does not imply their endorsement by the authors.

v. EXECUTIVE SUMMARY

Introduction: Vector control is a major component of the global strategy for malaria control which aims to prevent parasite transmission mainly through interventions targeting adult Anopheline vectors. Insecticide treated nets (ITNs) and indoor residual spraying (IRS) are the cornerstone of malaria vector control programmes. These major interventions in most cases use pyrethroid insecticides which are also used for agricultural purposes. With widespread development of resistance to pyrethroid insecticides in malaria vectors raises concern over the sustainability of insecticide-based interventions for malaria control. Therefore, close monitoring of performance of the insecticides against malaria vectors is essential for early detection and management of resistance.

Objective: To measure pyrethroid susceptibility in populations of malaria vectors in Tanzania and to test the efficacy of LLINs/ITNs and insecticide residues on sprayed wall substrates in the IRS operation areas.

Methodology: In 2011 the National Institute for Medical Research (NIMR) in collaboration with National Malaria Control Programme (NMCP) conducted large scale surveillance to determine the countrywide susceptibility levels of malaria vectors to insecticides used for both public health and agricultural purposes. *Anopheles gambiae* Giles s.l. were collected during national surveys and samples of LLINs/ITNs in the 14 sentinel sites and houses from the IRS areas were randomly selected for bioassays to test the efficacy and insecticide residual effects on sprayed wall substrates respectively. Wild adult mosquitoes for susceptibility testing were collected by resting catches indoors. Net traps (outdoors and indoors) were set up to enhance catches. WHO Susceptibility kits were used to test for resistance status using test papers: Lambdacyhalothrin 0.05%, Deltamethrin 0.05%, Permethrin 0.75%, DDT 4%, Propoxur 0.1% and Fenitrothion 1%. The quality of the test paper was checked against a laboratory susceptible *An. gambiae* Kisumu strain. Knockdown effect and mortality were measured in standard WHO susceptibility tests and cone bio-efficacy tests. Whereas, con bioassays on treated walls and ITNs were conducted using the laboratory susceptible *An. gambiae* Kisumu strain.

Results: The results from the surveillance recorded continued susceptibility of malaria vectors to commonly used insecticides. However, there were some isolated cases of resistance and/or reduced susceptibility to pyrethroid insecticides which may not compromise the current vector control interventions in the country. *Anopheles gambiae s.l.* showed resistance (15-28%) to each of the pyrethroids and to DDT but not to Organophosphates (Propoxur 0.1%), and Carbamates (Fenitrothion 1%). The information obtained from this surveillance is expected to be used to guide the National Malaria Control Programme on the rational selection of insecticides for malaria vector control and for the national mitigation plans for management and containment of malaria vector resistance in the country.

Conclusion: The current observation warrants more vigilant monitoring of the susceptibility of malaria mosquitoes to commonly used insecticides in areas found with resistance and/or reduced levels of susceptibility of malaria vectors to insecticides, particularly in areas with heavy agricultural and/or public health use of insecticides where resistance is likely to develop. The current survey covered malaria vectors only and not the non malaria vectors (nuisance) mosquitoes such as Culex. Similar monitoring of insecticide susceptibility of this non malaria vectors may be needed to ensure public motivation for sustained use of ITNs/ LLINs in the country.

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1.0 INTRODUCTION AND BACKGROUND INFORMATION

Malaria remains a serious global health problem with half of the world's population at risk (WHO, 2009). The biggest burden of the disease is borne in sub-Saharan Africa where over 800,000 lives are lost to this disease each year. In the past five years, the level of resources and the degree of national and international commitment to malaria control has been scaled up dramatically and its impact is now being felt in many malaria endemic regions.

Vector control has been a central component of malaria control strategies ever since malaria transmission was linked to mosquitoes. With the discovery of DDT and the launch of the Global Malaria Eradication Programme (GMEP) in the 1950s, indoor residual spraying (IRS) with DDT became very widely used (WHO, 2011).

For the first time in a generation, malaria is on the decline in some parts of Africa, principally due to the wide scale application of vector control interventions homes, in the form of LLINs and IRS, coupled with effective treatment with antimalarial drugs.

In Tanzania malaria is the leading cause of morbidity and mortality, especially in children under five years (Rowe, *et al.,* 2006). The socioeconomic impact of malaria is so high that it contributes highly to poverty and underdevelopment (Mboera *et al.,* 2007). Malaria is the single most significant disease in Tanzania affecting the health and welfare of its inhabitants. The climatic conditions are favorable for vector breeding almost throughout the country. The transmission is stable-perennial to stable-seasonal in over 80% of the country and about 20% of the population live in unstable malaria transmission areas prone to frequent malaria epidemics. The geographic distribution of malaria endemicity is mainly attributed to the ecological suitability for vector propagation. Mosquitoes from the *Anopheles gambiae* complex and the *Anopheles funestus* group are the main malaria vectors responsible for nearly all malaria transmission in the country. These transmit *Plasmodium falciparum*, the parasite responsible for 96% of malaria infections in Mainland Tanzania. The other minor malaria parasite infections are *P. malariae*, *P. ovale* and (very rarely) *P. vivax*.

Vector control is an important element of strategies used to control major vector-borne diseases including malaria globally, and chemical control remains the most widely used approach. In recent years, interventions using insecticides have been scaled up in many countries. Wide scale implementation of tools such as indoor residual spraying (IRS) and long lasting insecticide impregnated bednets (LLINs) have led to spectacular decreases in malaria transmission in some regions and these interventions are the cornerstone of malaria control programmes in most African countries. In the past decade the control of malaria vectors in Tanzania as in most parts of Africa relying on vector control interventions has increased dramatically; the major interventions being the use of insecticide treated nets (ITNs/LLINs) and Indoor Residual Spraying (IRS).

However, the current front line interventions for malaria vector control are dependent on a very limited number of available insecticides. The under investment in the development of new insecticides for the public health

market since the commercialization of the pyrethroid insecticides in the 1970s and 1980s, and the emergence of resistance to the majority of existing insecticides, has severely depleted the tool box and that we are now facing the real possibility that interventions such as LLINs and IRS will fail due to lack of alternative insecticides with known long term effectiveness and safety compared to pyrethroids. Hence one of the key challenges for ensuring the continued success of malaria vector control is to maintain a pipeline of effective insecticides that are safe for use in the indoor environment

There are currently only four classes of insecticide approved by WHO for use in IRS (organochlorines, carbamates, organophosphates and pyrethroids) and only one class, the pyrethroids is suitable for use on LLINs. On a negative note is the fact that it is the same insecticide classes that are also widely used to control agricultural and veterinary pests in Africa, causing widespread contamination of ground water hence, exposing mosquitoes to sub-optimal lethal concentrations when insecticide permeate their larval habitats. The significant increase in exposure to insecticide-based malaria vector control in the past decade has been associated with increased resistance among malaria vectors. Although data are still limited, over 70% (35) of countries reporting data on insecticide susceptibility since 2009 have found resistance to one or more insecticides. More than 40 malaria endemic countries are currently reporting insecticide resistance, with the vast majority reporting at least resistance to pyrethroids. Thus, intensive exposure to insecticides has inevitably resulted in the evolution of insecticide resistance in the *Anopheles* mosquitoes, the malaria vectors.

Insecticide resistance (IR) refers to scenario where an insecticide is no longer having the desired effect: vectors are no longer being killed by the standard dose of the insecticide and are no longer susceptible to the insecticide. The emergence of insecticide resistance in a vector population is an evolutionary phenomenon with the selection of a heritable trait in an insect population. There are two major resistance mechanisms in vector population, namely molecular genotype and phenotype resistance. Molecular genotype resistance refers to the fundamental phenomenon of resistance, which naturally occurs through genetic mutations. The identification of a resistance gene provides evidence of the underlying evolutionary process. Based on the type of resistance mechanism, this allows an understanding of both the frequency and potential severity of resistance. Phenotype resistance is the development of an ability in a strain of insects to tolerate doses of toxicants, which would prove lethal to the majority of individuals in a normal population of the same species. Phenotypic resistance is the phenomenon most commonly spoken about in public health. The measurement of this phenotypic resistance is through a bioassay susceptibility test, which assesses vector mortality when subjected to a standard dose of insecticide

Resistance to the organochlorines DDT and the now obsolete dieldrin was first reported in African malaria vectors in the 1950s and 1960s (Brown *et al,* 1958, Hamon, *et al,* 1968). Pyrethroid resistance was detected in African malaria vectors in 1993 (Elissa *et al* 1993). Since then there have been enormous published reports of pyrethroid resistant populations of *Anopheles gambiae s.l*. in countries from West, Central, East and Southern Africa (Ndjemai et al 2009, Munhenga *et al* 2008) and *Anopheles funestus* in Ghana, Mozambique and South Africa (Hargreaves et all 2000; Okoye *et al* 2008). Recently, carbamate and organophosphate resistant populations of *An. gambiae* have been reported in West Africa (Corbel, 2007).

Today we are at a turning point in malaria vector control, with a small, but increasing, number of reports of insecticide resistance leading to control failure (Sharp *et al.,* 2007, WHO, 2009). Recent experimental hut trials

in Benin have also demonstrated a highly significant reduction in the efficacy of insecticide treated bednets in an area of pyrethroid resistance (N'Guessan *et al,* 2007). With the limited arsenal of chemicals for malaria vector control, it is evident that insecticide resistance will have an increasingly negative impact on the current insecticide based interventions and that reports of control failure will escalate in the coming years. What is already very clear is that levels of insecticide resistance in malaria vectors are increasing at a very rapid rate throughout sub Saharan Africa. We also know that the mutations responsible for insecticide resistance have arisen multiple times in different parts of the continent (Pinto *et al.,* 2007), highlighting the immense selection pressure for the development of resistance that is currently being exerted on some vector populations. The widespread development of resistance to pyrethroid insecticides in malaria vectors, now recorded from West Africa (Awolola *et al.* 2002, Chandre *et al.* 1999, Elissa *et al.* 1993, Etang *et al.* 2003), East Africa (Ranson *et al.* 2000, Stump *et al.* 2004, Vulule *et al.* 1994) and South Africa (Hargreaves *et al.* 2000), raises concern over the sustainability of ITNs for malaria control.

By implementing and acting upon robust resistance monitoring programmes, the country will be able to sustain malaria control with currently available insecticides. The onchocerciasis control programme in West Africa has demonstrated how judicious use of insecticides, involving rotations of chemicals with different modes of action can prolong their efficacy in control programmes and such strategies should form part of the action plan for all malaria control programmes.

Given the importance of effective vector control interventions in controlling malaria, preserving the susceptibility of malaria vectors to pyrethroids and to the other classes of insecticides used, is therefore of critical importance to maintain effective vector control in malaria. Therefore, the need to develop effective systems for pesticide management has been emphasized to ensure rational use of insecticides, manage insecticide resistance, and reduce risks to human health and the environment, within the context of an integrated vector management (IVM) approach (WHO 2010a; Matthews et al. 2011; van den Berg *et al.,* 2011; WHO 2011a).

With technical and financial assistance from local and international organizations [private sector partners, RBM and Global Fund to fight AIDS, TB and malaria (GFATM)], Tanzania has been progressively scaling up use of ITN and LLIN countrywide since mid 2000. The President's Malaria Initiative (PMI) has also been funding recent introduction of indoor residual spraying since 2007 in three regions covering Lake Victoria basin. Such massive use of pyrethroids intervention in Tanzania poses the inherent risk of exerting high selection pressure to malaria vector populations. Indeed this calls for continuous monitoring of vector susceptibility status to public health insecticides for designing early proper management strategies should resistance evolve.

Given such potential selection pressure for insecticide resistance (from exposure of mosquitoes to ITNs or agricultural insecticides), detecting and monitoring of insecticide resistance in malaria vectors is paramount to (1) establish susceptibility status of local mosquito populations in Tanzania, and (2) test the efficacy of LLINs/ITNs as well as the decaying rates (residual life) of insecticides on walls in the IRS operation areas.

In view of the above, the Bill and Melinda Gates Foundation (BMGF) through WHO funded the project on "Malaria Vector Control: *filling the gap between product development and effective delivery"* in Tanzania. This project started in 2008 with the overall goal of mapping the resistance of malaria vector species in the country

while building the capacity of the malaria programme officers in basic skills related to detecting and monitoring the susceptibility of malaria vector species. Through this project, 14 sentinel districts/sites were established to coincide and include all the 11 surveillance sites established in 2004 where two surveys were conducted by the Tanzanian National Malaria Control Programme (NMCP). The surveys conducted in 1999 and 2004 provided data on mosquito insecticide susceptibility and net bio-efficacy from areas of Tanzania with increasing ITN coverage (Kulkarni *et al.,* 2007). The 2008/9 survey constituted a logical follow up from a similar survey conducted in 1999 and 2004 and provided a strong baseline against which ongoing trends can be assessed as the universal coverage campaign for LLINs was underway.

Here we report results of a large scale survey being implemented throughout Tanzania in 2011 with the aim of determining countrywide susceptibility levels of mosquito vectors to insecticides from the major four chemical classes i.e. Organochlorine, organophosphates, carbamates and pyrethroids. Information obtained from the study is expected to be used to guide the National Malaria Control Programme (NMCP) on rational selection of insecticides for malaria vector control so as to achieve sound malaria vector resistance management in Tanzania.

2.0 STUDY OBJECTIVES

2.1 Main Objective

The main objective of the study was to detect and monitor malaria vectors resistance to insecticides of public health relevance in Tanzania Mainland.

2.2 Specific Objectives:

2.2.1 The specific objectives of the study were:

2.2.2 To determine the susceptibility status of local *Anopheles gambiae* s.l. and *An. funestus* to pyrethroids (permethrin, deltamethrin and lambdacyhalothrin); carbamate (propoxur) organophosphate (fenitrothion) and organochlorine (DDT) used either for indoor residual spraying (IRS) or insecticide treated nets (ITNs/ LLINs),

2.2.3 To determine insecticidal efficacy, longevity and integrity of LLINs/ITNs under field conditions,

2.2.4 To evaluate the residual effect of the insecticide on different indoor/wall substrates by conducting cone wall bioassays in order to guide future interventions

3.0 METHODOLOGY

3.1 Study design

This was a cross-sectional countrywide survey conducted between February and September 2011 using the established 14 sentinel districts for detecting and monitoring of malaria vectors resistance to insecticides of public health relevance in Tanzania Mainland.

3.2 Study sites and selection criteria

The study was conducted in 14 sentinel districts selected from 11 out of 26 regions of Tanzania Mainland as shown in figure 1. These sites included Muheza, Handeni and Lushoto (Tanga region), Moshi (Kilimanjaro Region), Dodoma rural (Dodoma region), Arumeru (Arusha region), Babati (Manyara region), Uyui (Tabora region), Kyela (Mbeya region), Magu (Mwanza region), Ilala (Dar es Salaam region), Muleba (Kagera region), Kilombero and Mvomero (Morogoro region).

Selection of sentinel districts for the insecticide resistance surveillance was based on the WHO recommended selection criteria namely:

1) History of insecticides use by communities in the areas (both for agricultural and public health use)
2) Malaria endemicity in the country (priority was given to the districts with high malaria prevalence)
3) High coverage with ITN/LLINs
4) Demographic settings (Urban/Rural)
5) Accessibility to the sites

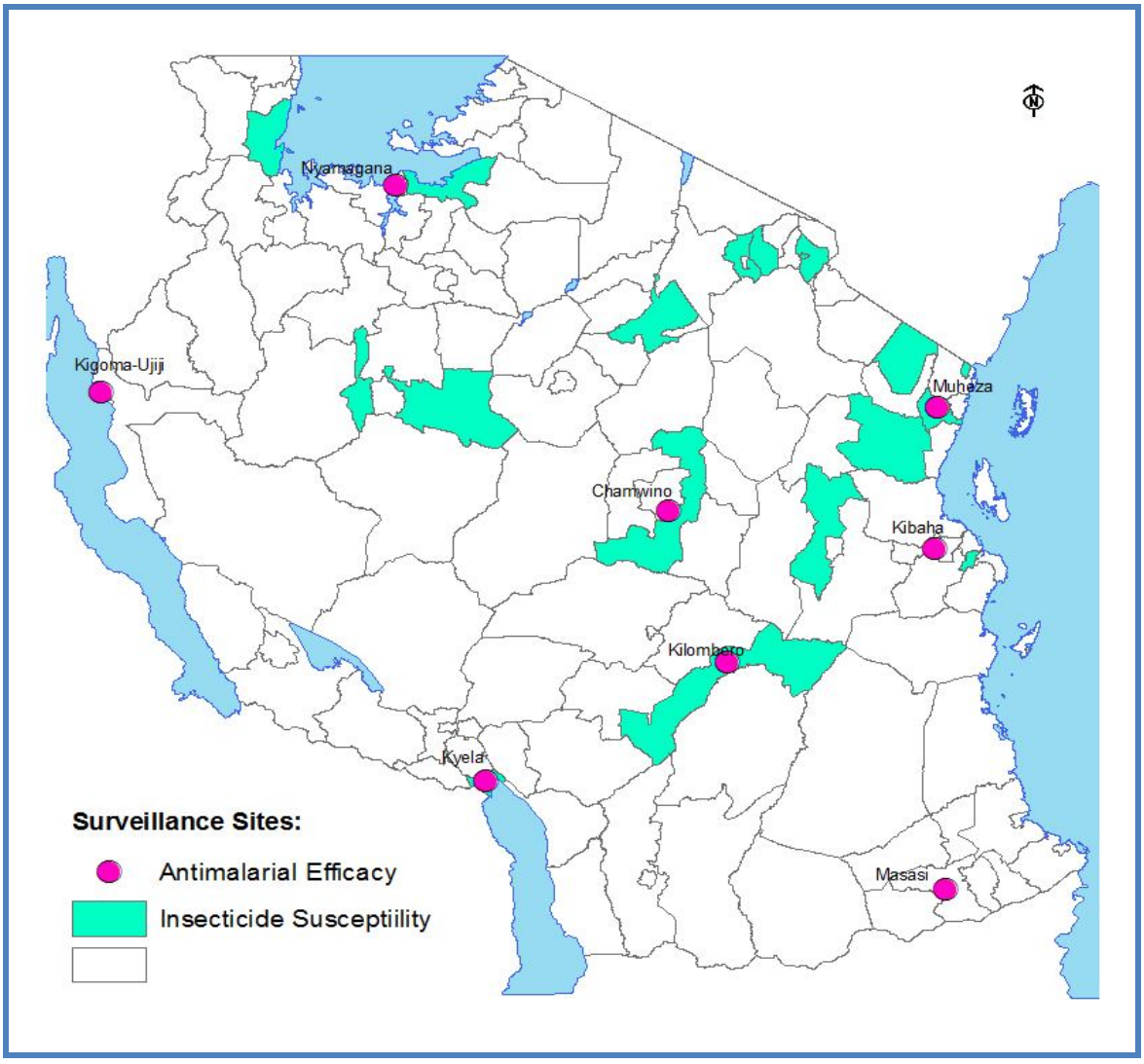

Figure 1: Distribution of sentinel districts for insecticide susceptibility surveillance in Tanzania

3.3 Description of the study sites

3.3.1 Three districts were included in Tanga region: **Muheza** (Latitude: 5°10' ^{0}S, Longitude: 38°46' ^{0}E), **Handeni** (Latitude: 5°43' ^{0}S, Longitude: 38°01' ^{0}E) and **Lushoto** (Latitude: 4°78' ^{0}S, Longitude: 38°28' ^{0}E). Muheza district is located at the foothills of East Usambara Mountains about 30km offshore Indian Ocean. Most of its inhabitants subsist on maize, cassava and few on rice while some are working on sisal plantations. The district is also famous for small scale orange plantations and animal husbandry. The district has long history of ITNs use in some areas. Handeni district is situated in the south-western part of Tanga Region and it contains both coastal and mountain forests (Nguu Mountains). The population engages in agricultural production of crops (maize, beans, cassava, millet, cotton, sunflower, pigeon peas, oranges, mangoes, coconuts, bananas and vegetables) and livestock keeping (cattle, goats, sheep and chicken). Lushoto district has an average elevation of 1,612 meter above sea level (ranging from 800 to 2300 meters above sea level) on the West Usambara Mountains. The area is mildly densely populated with 129 people per km^2. Inhabitants are extensively engaged in vegetable growing. In this district pesticides are intensively used for crop protection.

All these sites experience a bimodal pattern of rainfall; short rains from October to December and long rains from March to June. The rainfall ranges from 600 to more than 1200mm. July-September is the cool dry period with January-March the hot and drier months.

3.3.2 **Lower Moshi (37°20'E 3°21'S):** The study was carried out from Lower Moshi, an intensive rice-irrigation area, south of Mount Kilimanjaro in north-eastern Tanzania. The population in the area is engaged in agriculture. Two rivers, the Njoro and the Rau provide water for irrigation. There are two growing seasons, the main one from June to October and the second one involving sporadic cultivation of rice from September to February.

3.3.3 **Kilombero** (Latitude: 8°31' ^{0}S, Longitude: 37°22' ^{0}E) is the name of a river and a district in Morogoro Region, south-western Tanzania. The district is situated in a vast floodplain, between the Kilombero River in the south-east and the Udzungwa Mountains in the north-west. On the other side of the Kilombero River, in the south-east, the floodplain is part of Ulanga district. The majority of the villagers are subsistence farmers of maize and rice. Villages visited were Michenga, Mahutanga, Idete and Ihanga. The average net coverage is 44%.

3.3.4 **Dodoma Rural District (6^o, 30' to 8^{o}0'S, 35o, 30' to 37^{o}0'E)** in central Tanzania is located in the central plateaus at an elevation of about 800-1200m above sea level. The district has a dry Savannah type of climate characterized by a long dry season lasting between April and November. The average annual rainfall is 500-800mm, which is normally a short single wet season lasting between December and March. Temperature in the district varies according to altitude but generally the average maximum and minimum is 31^{o}C and 18^{o}C respectively. In June – August, temperatures are at times very high with hot afternoons up to 35^{o}C and chilly nights on hilly areas down to 10^{o}C

3.3.5 **Muleba District (10, 45'S, 310, 40'E),** in Kagera region of north-west Tanzania, covers an area of 10,739 km^2, of which Lake Victoria is 62%. Most of the district lies at 1200-1500m above sea level. The district covers an area of 10,739 km^2, of which Lake Victoria is 62%. Most of the district lies at 1200-1500m above sea level. The district has two rainy seasons March – June and September-December, during which malaria transmission peaks. The district was chosen as a launch site for PMI-funded spray operations in 2007. In 2009 PMI supported three rounds of IRS in Muleba district, achieving over 90% coverage, and continued support for IRS in this district will continue until there is universal use of LLINs and epidemiological data supporting the withdrawal of IRS (PMI MOP 2009).

3.3.6 **Arumeru (03°08'S 36°52'E)** is one of the five districts in Arusha Region of Tanzania. It is bordered to the north and west by the Monduli District, to the east by the Kilimanjaro Region and to the south by the Arusha District and the Monduli District. Agriculture is mainly practiced in small scale in the district where the main types of agriculture products produced are Coffee, Bananas, Vegetables Avocado, Paddy, Millet, Cassava, Irish potatoes, sweet potatoes and French beans. Farmers practice traditional farming system. Livestock production, floriculture and seed production are the main activities. High quality agriculture seeds and flowers are produced for export supported by fertile volcanic soil and available water sources draining from mountains.

3.3.7 **Babati district (4°13'S 35°45'E)** is a district of the Manyara Region. The district is the major producer of crops especially cereals, (about 60% of cereals production in the region). The rainfall in the district is usually reliable, ranging from 800-1000 mm.

3.1 Hands-on training of field implementers for vector surveillance

Insecticide resistance surveillance activities were preceeded by training of research team on a uniform research methodology. The Amani Medical Research Centre in collaboration with the National Malaria Control Programme (NMCP) conducted a one week hands-on training workshop of field implementers on the standardized protocol for vector surveillance. The major aim of this training was to impart basic entomological skills, with a particular emphasis on resistance management. Training on basic entomological skills included mapping and characterization of breeding sites (in areas where larvicides can potentially be used), adult mosquito collection and morphological identification, estimation of vector density, conducting susceptibility tests, malaria vector control and resistance management techniques.

3.2 Mosquito collections in the field

Adult mosquitoes for vector susceptibility testing were collected by the indoor resting catch technique. Net traps (outdoors and indoors) were set up to enhance catches. Early morning (between 6.00 to 9.00 am) indoor-resting catches were carried out in all locations. Freshly blood-fed female Anopheles mosquitoes were aspirated by pooters from their resting sites on the walls and other surfaces inside houses. Captured mosquitoes were collected in paper cups, and then transported to a field laboratory or other suitable test location for morphological identification and susceptibility tests. The wild caught mosquitoes were fed with 6-10% sugar solution embedded in a cotton wool pad while being transported from the field. Caught mosquitoes

were morphologically identified using the identification key by Gillies and Coetzee, (1987) and Gillies and De Meillon, (1968).

Where adult mosquitoes were not enough for the tests, larvae searches was done and Anopheles larvae identified from their horizontal position on the surface of water were carefully collected with a 350 ml dipper and transferred into plastic containers which were then loosely capped to allow aeration. These were transported in cool boxes to the laboratory where they were reared at 27 - 30^{o}C and 76±5% relative humidity with a 12h: 12h light and dark cycle. The larvae were fed with ground Tetramin® fish food. The development of the larvae was monitored regularly and all those that pupated were transferred into shallow plastic cups /small beakers using Pasteur pipettes, and then placed in appropriately labeled cages for adult emergence. Using the Global Positioning System (GPS; Trimble Geoexplorer II, Trimble Navigation Limited, Sunnyvale, CA, USA) the geographical coordinates of each sampling site was determined.

3.3 WHO insecticide susceptibility tests

The susceptibility tests were carried out using the World Health Organization (WHO) test kits for adult mosquitoes (WHO, 1998). The kit is basically comprised of insecticide impregnated test papers and non-impregnated papers for control and plastic tubes that are marked with a red dot for exposure and a green dot for the holding tubes. Test papers impregnated with the WHO-recommended discriminating dosages of 0.75% Permethrin, 0.05% Deltamethrin, 0.05% Lambdacyhalothrin, 4% D.D.T, 0.1% Propoxur and 1% Fenitrothion were used. The quality of the test paper was checked against a laboratory susceptible *An. gambiae* Kisumu strain. Knockdown effect and mortality were measured in standard WHO susceptibility tests and cone bio-efficacy tests.

The standard methods were used for insecticide susceptibility tests (WHO, 1998). For each test, batches of 15-25 wild female mosquitoes were aspirated from paper cups and transferred into the holding tubes where they were held for 1 hour. They were then transferred into exposure tubes (through the open space between the exposure and the holding tubes). Exposure tubes were lined with the insecticide impregnated papers to which mosquitoes were exposed for 1 hour. During the exposure period, the number of mosquitoes knocked down was recorded after 10, 15, 20, 30, 40, 50 and 60 minutes for pyrethroid and organochlorine insecticides only. A mosquito was considered knocked down if it lay on its side on the floor of the exposure tube and unable to fly

At the end of exposure period mosquitoes were then transferred into holding tubes (lined with untreated papers) by gently blowing them through the open space between the exposure and the holding tubes. A cotton pad soaked in 10% sugar was placed on top of the holding tube. This is to avoid death by starvation. The mortality was scored 24 hours post-exposure and each test at each site was replicated at least four times. The resistance or susceptibility status were evaluated based on the WHO criteria i.e. 98-100% mortality indicate susceptibility; 80-97% mortality required confirmation and less than 80% mortality indicate possible resistance (WHO, 1998). When the control mortality was scored between 5% and 20%, the mean observed mortality was corrected using Abbott's formula (Abbott, 1925). Tested mosquitoes were preserved with *silica gel* in 1.5 ml eppendorf tubes and transported to Amani Medical Research Centre for further laboratory analysis (molecular species identification and detection of biochemical/molecular mechanisms of insecticide resistance).

3.4 Laboratory Analysis

3.4.1 Molecular identification of members of the Anopheles gambiae species complex

Anopheles gambiae sibling species identification was carried out according to the standard polymerase chain reaction (PCR) method (Scott *et al.,* 1993). Five oligonucleotide primers, GA, ME, AR, QD and UN designed from the DNA sequences of the intergenic spacer region of complex ribosomal DNA (rDNA) were used to amplify species-specific DNA sequences. The UN-primer is universal and anneals to the same position on the rDNA sequences of all five species, the GA anneals specifically to *An. gambiae s.s*., the ME anneals to either *An. merus* or *An. melas*, AR to *An. arabiensis* and the QD to *An. quadriannulatus.*

The PCR reaction mix of 25 μl contained 1 X PCR buffer (constituents), 200 μM of each of the deoxyribonucleotide triphosphates (dNTPs), 20 μM of oligonucleotide primers, 0.125 units of Taq Polymerase enzyme (Sigma, USA) and 0.5 μl of the extracted genomic DNA. Sterile double distilled water was added to make up the volume to 25 μl. The reaction mix was spun down briefly at 14,000 rpm and overlaid with mineral oil to avoid evaporation and refluxing during thermo-cycling.

The amplification reactions were carried out using PTC 100 thermal cycler (MJ Research Inc., USA) and the cycling parameters were as follows: 3 minutes at 94ºC (initial denaturation), followed by 35 cycles with denaturation at 94ºC for 30 seconds, annealing at 50ºC for 30 seconds and extension at 72ºC for 60 seconds and ended with a final cycle at 94ºC for 30 seconds, annealing at 50ºC for 30s and extension at 72ºC for 10 minutes. For each reaction, a positive control containing 0.5 μl of PCR products of *An. gambiae s.s.* as template DNA and a negative control that contained no DNA template were included.

The amplified products were analyzed by agarose gel electrophoresis. Ten micro-liters of each PCR product were added to 1μl of 10x Orange-G loading dye and electrophoresed in 2% agarose gel stained with 0.5 μg/ml of ethidium bromide. The electrophoresis was run in 1X Tris acetate-EDTA (TAE) buffer at 100V for one hour and were visualized and photographed over a UVP dual intensity trans-illuminator at short wavelength using a digital camera fitted with an orange filter and a hood. The amplified PCR product was identified to the sibling species on the basis of the diagnostic band size determined by comparison with the mobility of a standard 100bp DNA ladder (Sigma, USA).

3.4.2 Detection of knock down resistance (kdr) alleles in Anopheles gambiae complex

The PCR-based standard method (Martinez-Torres *et al.,* 1998) was used to detect *kdr* genes in the mosquitoes. DNA extraction was performed as described above. The primers used were Agd1 and Agd2 (Oligos Etc. Inc., USA) and Agd3 and Agd4 (Oswel, UK). Survivors and susceptibles from the bioassay were chosen at random for the *kdr* analysis. *Kdr* genotyping of susceptible and resistant individuals was possible after amplifying the DNA template from mosquitoes following the PCR conditions of 94ºC for 3 minutes (initial denaturation), followed by 45 cycles of 94ºC for 30 seconds, 50ºC for 30 seconds and 72ºC for one minute. There was a final extension cycle of 94ºC for 30 seconds, 50ºC for 30 seconds and 72ºC for 10 minutes followed by 4ºC for cooling. The products were electrophoresed through ethidium bromide-stained 2% agarose gel and visualized

under UV light. Knock down resistant (*kdr*) genotypes of both the susceptible and resistant individuals were then recorded. Expected sizes for susceptible, resistant and control were 137bp, 195bp and 293bp respectively.

3.5 Local insecticides usage

A questionnaire focusing on the history of insecticide use i.e. types of the insecticides used, mode and frequency of application and on the kinds of crops and animals on which insecticides were used was administered to agriculture and public health officers in the districts. In addition a survey of shops and agrovet stores was conducted to take an inventory of insecticides available on the market during the time of the study.

3.6 Residual efficacy of LLINs with Cone bioassays

Apart from % net coverage, Insecticidal efficacy, integrity and user acceptability are the key factors for the success of the ITNs as malaria vector prevention tool. We conducted a surveillance to monitor the insecticidal efficacy, integrity and user acceptability in 14 sentinel sites in Tanzania.

Five nets were collected from five randomly selected households in every sentinel sites for further testing of their efficacy at the laboratory of Amani Medical Research Centre. Each household was given a new LLIN. Standard WHO cone bioassay method was followed to determine efficacy of individual net by exposing 2-5 days old 50 unfed *Anopheles gambiae* Kisumu strain for three minutes then knockdown scored 1 hour post exposure and held for 24 four hour in the insectary under the condition of 27±2^{o}C temperature and 80% humidity (WHO, 1975). Mortality was scored at the end of 24h holding period.

3.7 Residual efficacy of Lambdacyhalothrin based IRS with Cone bioassays

Determination of residual activity of insecticides is essential information for the selection of appropriate indoor spraying operation. The present study was undertaken to evaluate the residual effect of three candidate insecticide formulations on different indoor surfaces in order to guide future interventions, in the context of Tanzania. The study was conducted in Kagera region where indoor residual spraying started in 2007. Lambda-cyhalothrin CS has been sprayed on the indoor wall surfaces of local cement, wood and mud houses. Their effects on the knockdown and mortality of the Kisumu susceptible strain of *Anopheles gambiae* s.s were assessed each month from July to September 2011, using WHO plastic cones test.

The tests were done three months post IRS in three villages in Kagera region which had been sprayed with Lambdacyhalothrin CS. The names of the village with respective districts were Nyamilembe village, Chato district; Mulela village, Muleba district and Kitwenchekula village, Karagwe district. In each village 4 houses were randomly selected to represent all types of wall surfaces (mud, cement, white wash and wooden). 2-3 days old 20 laboratory pyrethroid susceptible *An. gambiae s.s.* Kisumu were exposed on treated surface by cone bioassay for 30min as stipulated by standard procedure at room temperature in the field makeshift laboratory (WHO 1981). Mosquitoes were transferred to paper cups provided with cotton wool moistened with 10% glucose solution and then knockdowns scored at 1h after exposure after which time all mosquitoes were kept at room temperature. The mortality was scored at 24h after exposure. Knockdown and mortality rates were compared between different surfaces using trend Chi-square tests.

3.8 Statistical analysis

Percent mortality was corrected by Abbott.s formula when mortality in control replicates was >5% (Abbott 1925). Tests where control mortality exceeded 20% were excluded from analysis. Time taken for 50% knockdown of mosquitoes (KT_{50}) and 95% confidence intervals were determined by probit analysis using the computer program PoloPlus (Version 1.0, LeOra Software) (Finney 1971).

4.0 RESULTS

4.1 Susceptibility levels of *An. gambiae* s.l to insecticides

The results of the insecticide bioassays are shown in Figures 2, 3 and 4. For the case of 0.05% Deltamethrin the highest percentage of mosquitoes surviving the WHO diagnostic doses were seen in Muheza district (~36%) followed by Moshi (28.2%), Arumeru district (~10%) then Handeni (~7%) and the least survival rates was observed in Dar es Salaam, Kilombero and Babati (~4% each). None of mosquitoes from Muleba, Magu, Lushoto, Dodoma, Tabora, Mvomero and Kyela districts survived exposures to Deltamethrin.

In addition the highest percentage of mosquitoes surviving 0.05% Lambdacyhalothrin was recorded with mosquitoes from Moshi (45%) followed by Arumeru district (30%), Muheza (>18%), Muleba (15%) and the least surviving population from Handeni district (~2%).

The highest surviving (range: 25-26%) mosquitoes to 0.75% Permethrin exposures were observed in Muheza and Moshi followed with (~16%) mosquitoes from Arumeru and Kilombero. The least surviving population to permethrin were from Babati, Magu and Handeni districts (2-5%). No mosquito survived permethrin exposures (100% mortality) in the other six districts of Lushoto, Muleba, Dodoma, Tabora, Mvomero and Kyela.

Only 1% of tested mosquitoes from Kyela and Mvomero districts survived exposures to 0.1% Propoxur. No surviving mosquitoes were observed from other sentinel sites after exposure to propoxur. Likewise, about 1% of tested mosquitoes survived exposures to 1% Fenitrothion in Kyela district. The rest of mosquitoes from other sentinel sites did not survive to Fenitrothion exposures.

Conversely, mosquito's response to 4% DDT exposures did not show any surviving individuals across all sentinel districts except Magu where survival accounted for 5% out of the total mosquitoes tested.

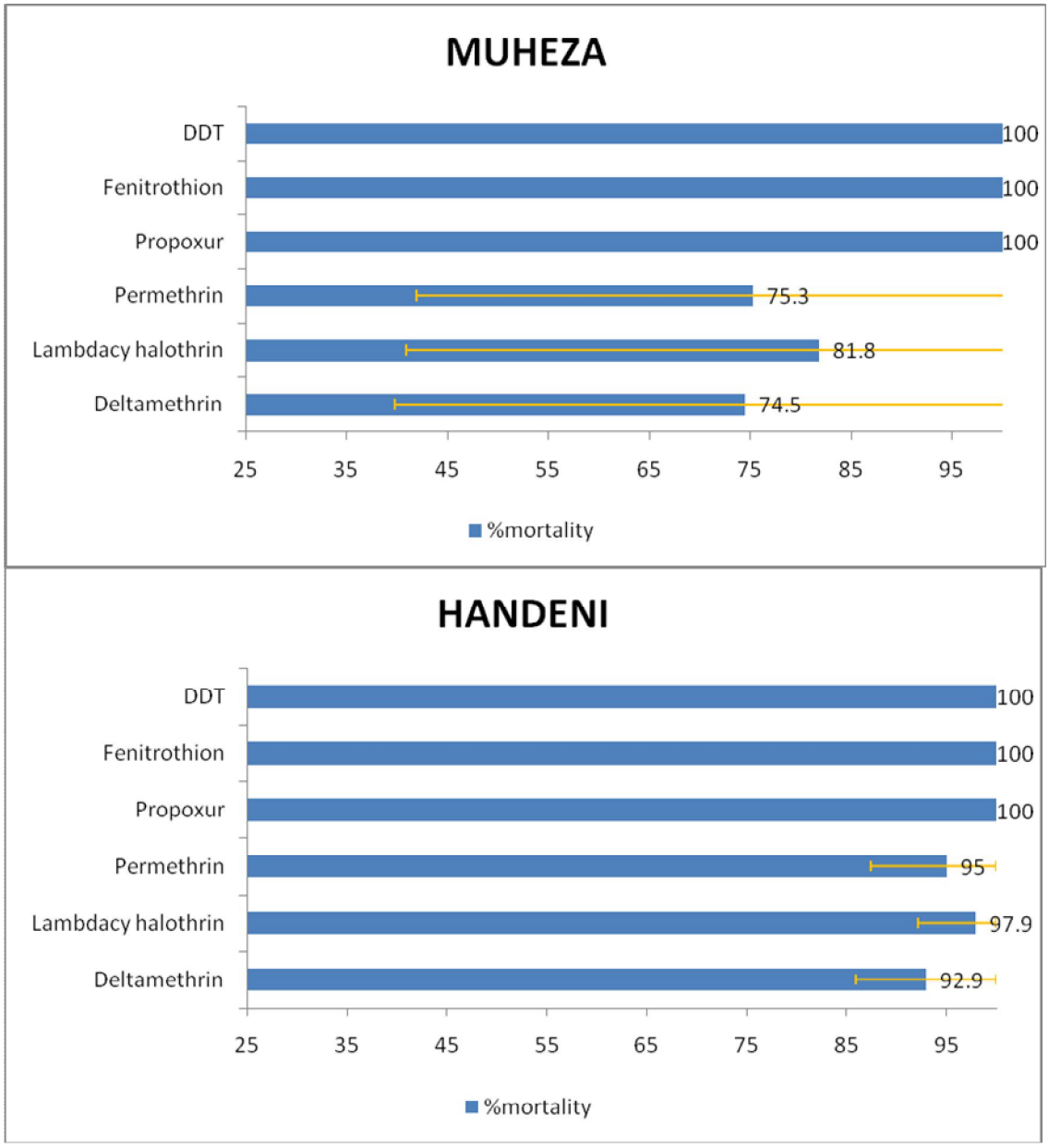

Figure 2: Vector susceptibility response to the respective Insecticide discriminatory dosage for *Anopheles gambiae s.l.* in the two sentinels Sites (Muheza and Handeni) in Tanzania. *The top graph shows percentage 24 hours mean mortality after a 1-hour exposure to the WHO diagnostic doses of insecticide in Muheza district and the bottom graph is for Handeni District. The minimum sample size for these assays was 80 and for F1 test, all individuals were non-blood fed females, 3-5 days post emergence.*

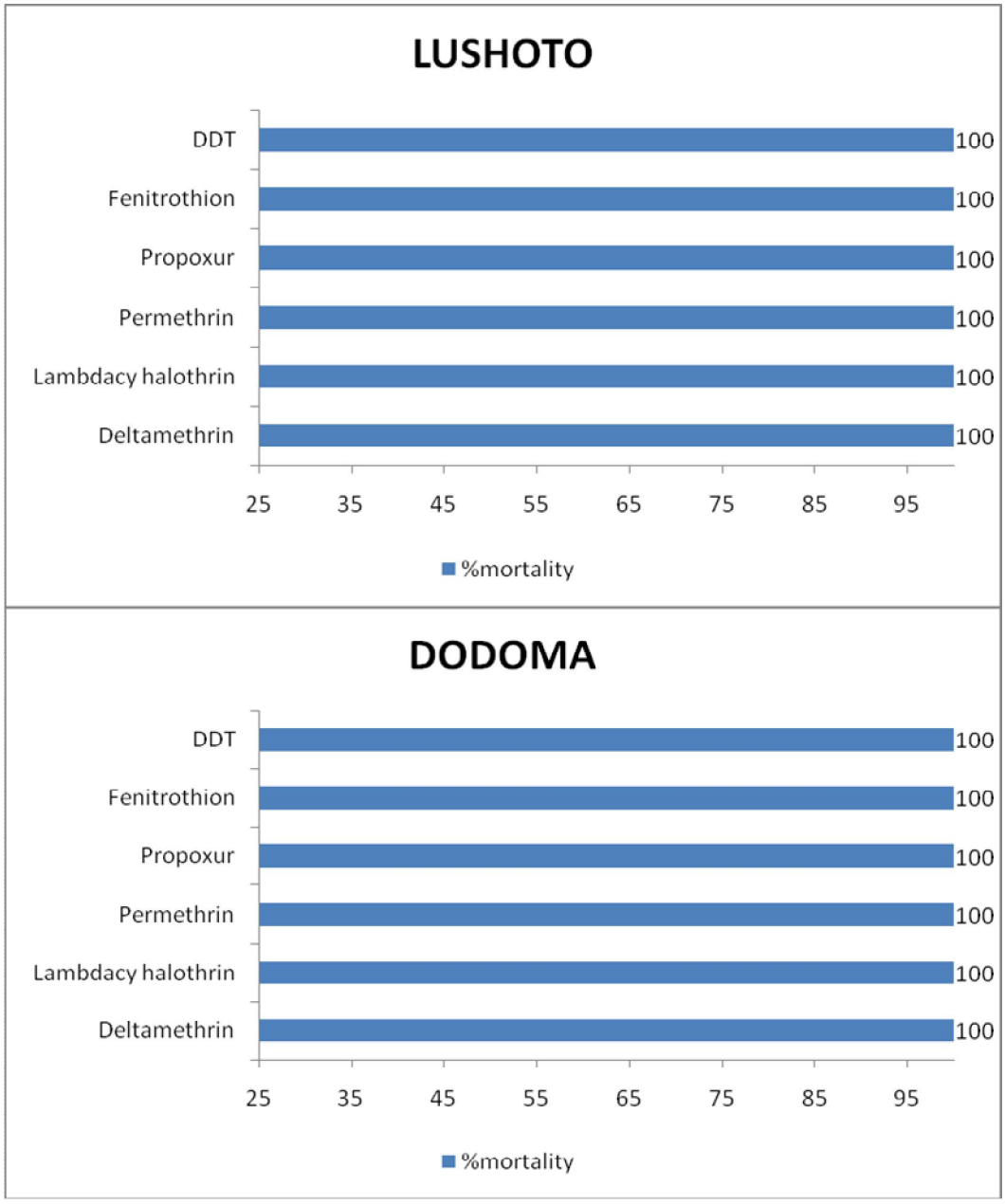

Figure 3: Vector susceptility response to the respective Insecticide discriminatory dosages for *Anopheles gambiae s.l.* in two sentinel Sites (Lushoto and Dodoma) in Tanzania. *The top graph shows percentage 24 hours mean mortality after a 1-hour exposure to the WHO diagnostic doses of insecticide. The minimum sample size for these assays was 80 and for F1 test, all individuals were non-blood fed females, 3-5 days post emergence.*

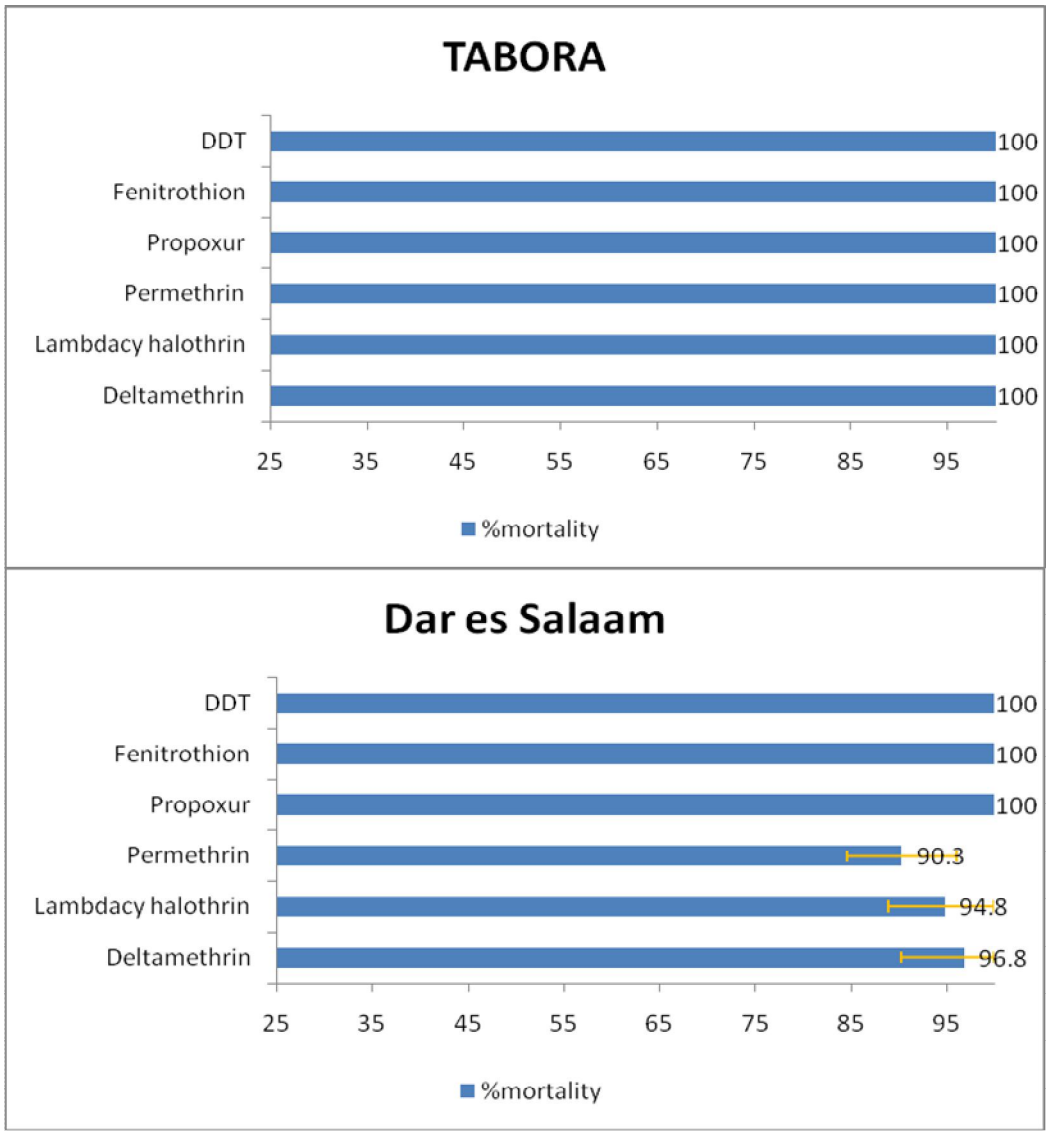

Figure 4: Vector susceptibility response to the respective Insecticide discriminatory dosages for *Anopheles gambiae s.l.* in two sentinel Sites (Tabora and Dar es Salaam) in Tanzania. *The graph shows percentage 24 hours mean mortality after a 1-hour exposure to the WHO diagnostic doses of insecticide. The minimum sample size for these assays was 80 and for F1 test, all individuals were non-blood fed females, 3-5 days post emergence.*

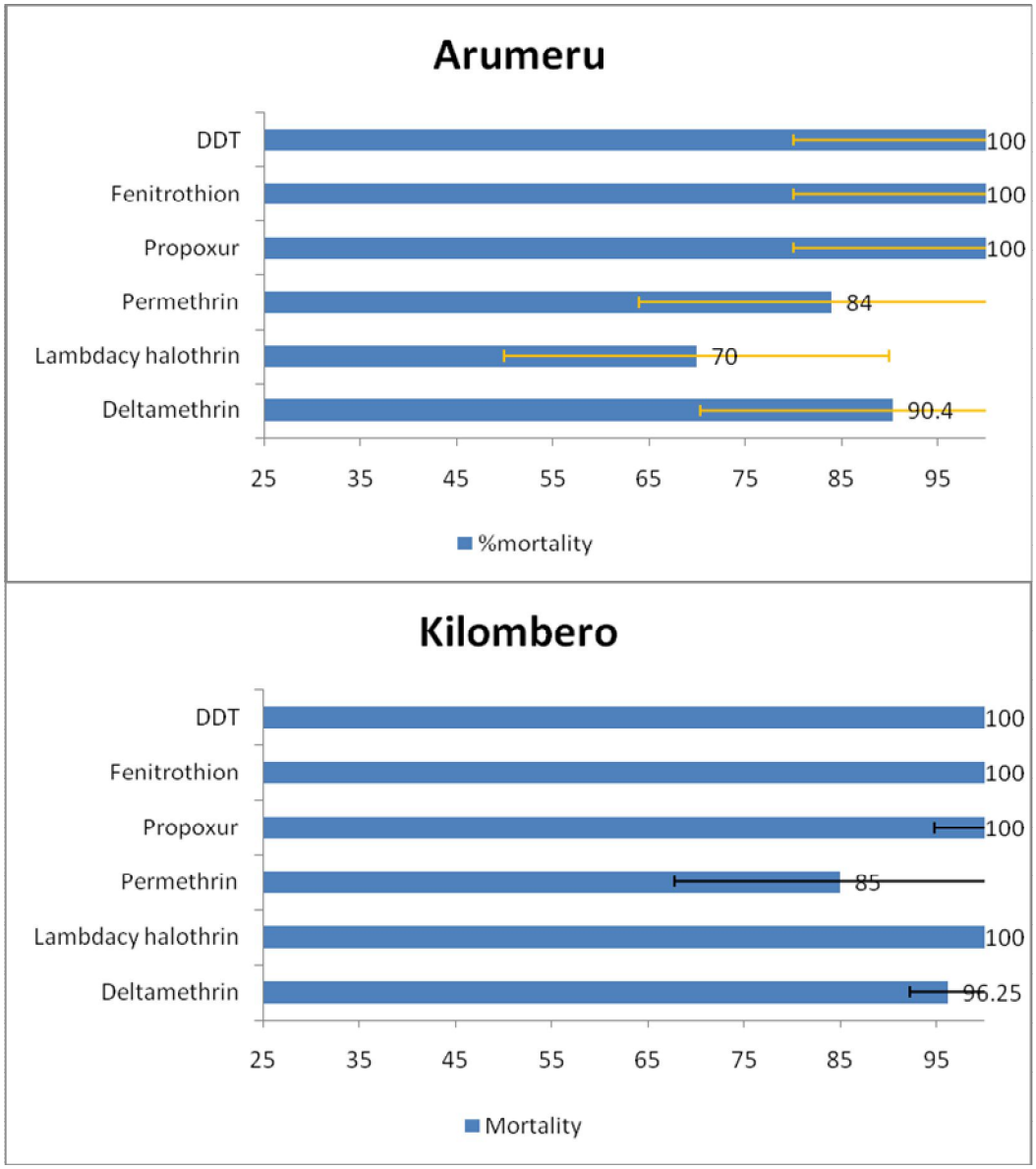

Figure 5: Vector susceptibility response to the respective Insecticide discriminatory dosages for *Anopheles gambiae s.l.* in two sentinel Sites (Arumeru and Kilombero) in Tanzania. *The graph shows percentage 24 hours mean mortality after a 1-hour exposure to the WHO diagnostic doses of insecticide. The minimum sample size for these assays was 80 and for F1 test, all individuals were non-blood fed females, 3-5 days post emergence.*

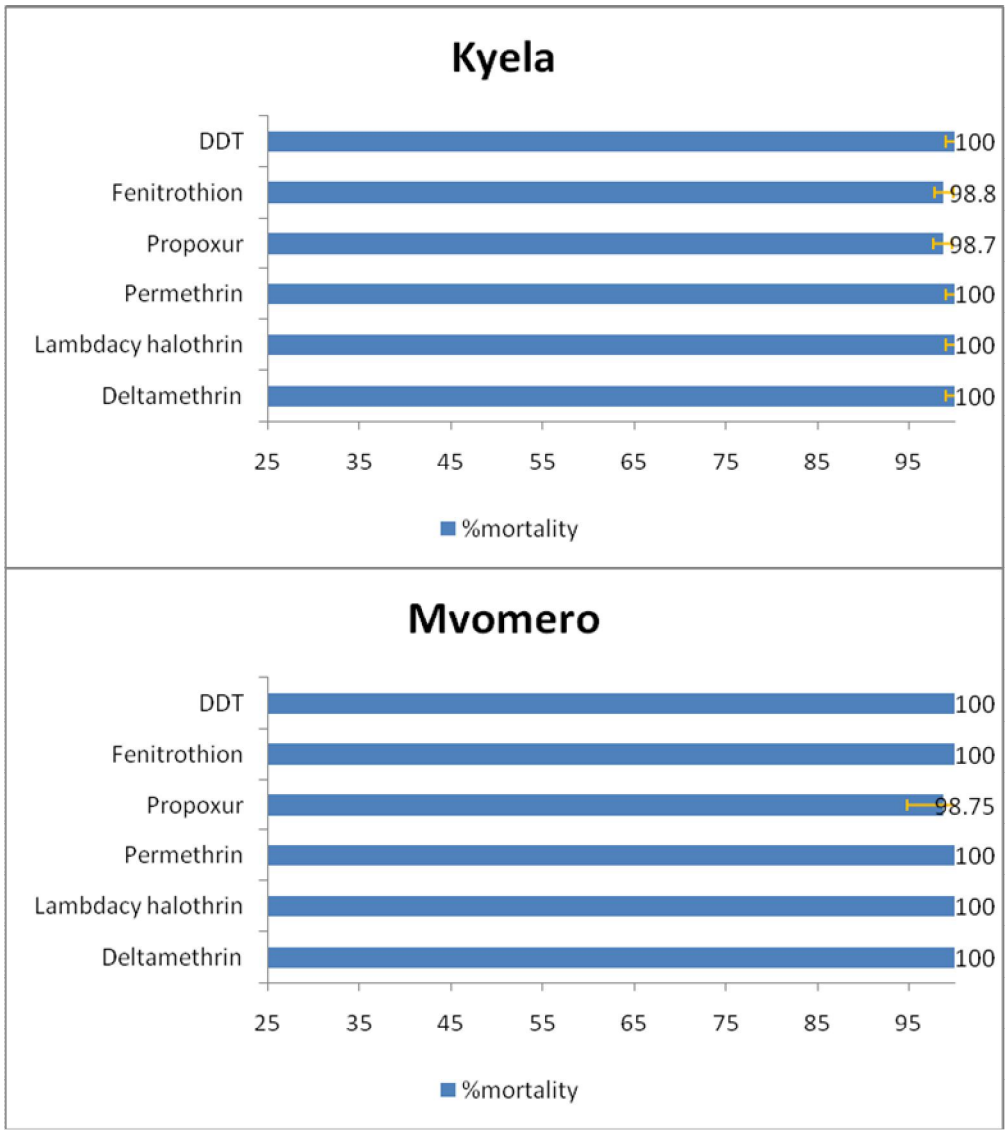

Figure 6: Vector susceptibility responses to the respective Insecticide discriminatory dosages for *Anopheles gambiae s.l.* in two sentinel Sites (Kyela and Mvomero) in Tanzania. *The graph shows percentage 24 hours mean mortality after a 1-hour exposure to the WHO diagnostic doses of insecticide. The minimum sample size for these assays was 80 and for F1 test, all individuals were non-blood fed females, 3-5 days post emergence.*

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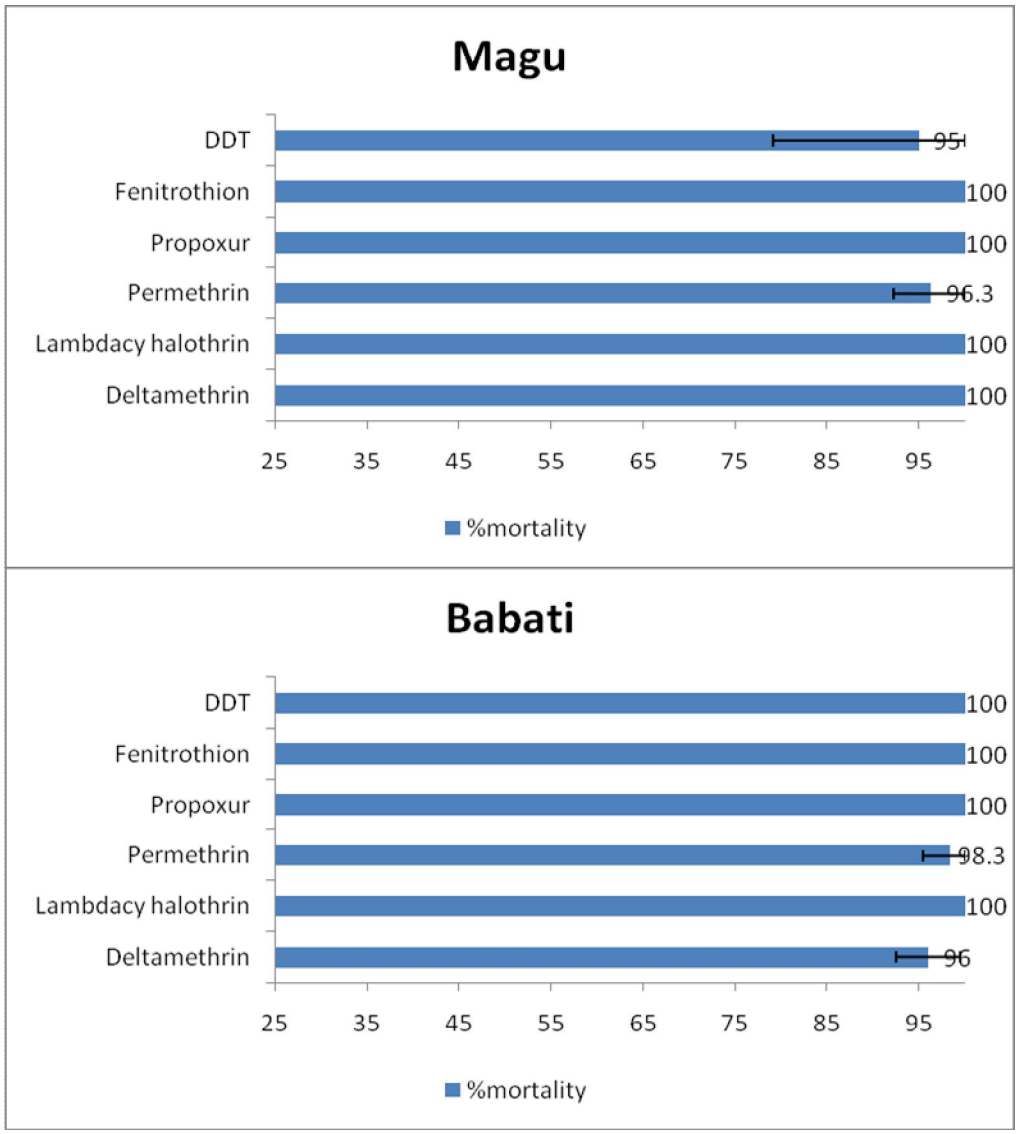

Figure 7: Vector susceptibility response to the respective Insecticide discriminatory dosages for *Anopheles gambiae s.l.* in two sentinel Sites (Magu and Babati) in Tanzania. *The graph shows percentage 24 hours mean mortality after a 1-hour exposure to the WHO diagnostic doses of insecticide. The minimum sample size for these assays was 80 and for F1 test, all individuals were non-blood fed females, 3-5 days post emergence.*

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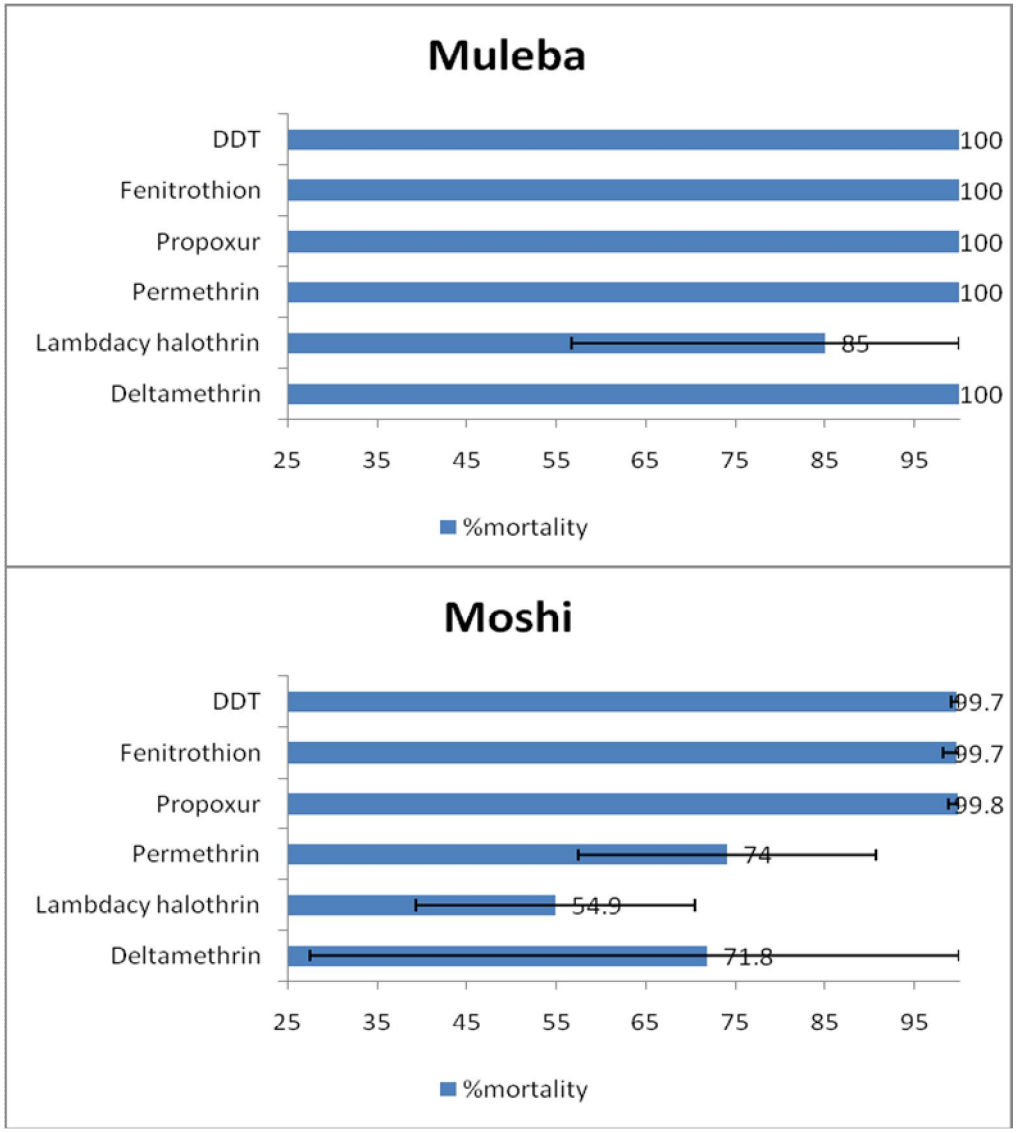

Figure 8: Vector susceptibility response to the respective Insecticide discriminatory dosages for *Anopheles gambiae s.l.* in two sentinel Sites (Muleba and Moshi) in Tanzania. *The graph shows percentage 24 hours mean mortality after a 1-hour exposure to the WHO diagnostic doses of insecticide. The minimum sample size for these assays was 80 and for F1 test, all individuals were non-blood fed females, 3-5 days post emergence.*

Table 1. Response of wild caught *Anopheles gambiae sl* local populations to discriminatory dosages of WHO insecticide treated papers from 14 sentinel districts of Tanzania[1].

Insecticide	Location (Sentinel District)	Number (N)	Mean KnockDown times KDT 50 in min 95% CI	Mean % mortality after 24h	KDT50 Ratio
Permethrin 0.75%	Kisumu	100	12.5	100	1.0
	Kirombero(Morogoro)	80	31.3 (28.8-33.9)	85	2.5
	Kyela(Mbeya)	86	15.7 (14.5-16.8)	100	1.3
	Mvomero(Morogoro)	82	17.9 (16.4-19.5)	100	1.4
	Muheza	95	28.3 (25.8-30.8)	75.3	2.3
	Lushoto	100	15.8 (13.2-18.2)	100	1.3
	Handeni	100	33.4 (27.3-42.2)	95	2.7
	Arumeru	125	84.4 (67.2-151)	73.6	6.8
	Dodoma	80	17.3 (16.3-18.3)	100	1.4
	Tabora	80	23.5 (20-27.1)	100	1.9
	Dar es Salaam	75	37.9 (36.1-39.7)	90.3	3.0
	Magu(Mwanza)	100	20.9 (19.3-22.5)	100	1.7
	Muleba	80	14.1 (12.95-15.2)	100	1.1
	Babati	123	15.5 (13.4-17.7)	99	1.2
	Moshi	542	47.8 (46.0-49.8)	74	3.8
Deltamethrin 0.05%	Kisumu	100	13.8	100	1.0
	Kirombero(Morogoro)	80	22.3(19.3-25.3)	96	1.6
	Kyela(Mbeya)	78	13.3(11.6-14.9)	100	1.0
	Mvomero(Morogoro)	80	15.3(12.9-17.4)	100	1.1
	Muheza	95	25.2 (23.7-26.9)	74.5	1.8
	Lushoto	100	23.8 (21.4-26.4)	100	1.7
	Handeni	99	26.7 (25.1-28.5)	92.9	1.9
	Arumeru	125		90.4	
	Dodoma	80	22.2 (19.6-24.9)	100	1.6
	Tabora	80	20.9 (16.6-25.4)	100	1.5
	Dar es Salaam	85	32.5 (28.6-36.5)	96.8	2.4
	Magu(Mwanza)	20	29.5 (24.9-35.4)	100	2.1
	Muleba	80	24.7 (21.1-28.6)	85	1.8
	Babati	125	34.4(31.6-37.3)	96	2.5
	Moshi	533		71.8	

[1] Based on WHO criteria for insecticide susceptibility levels [i.e., Mortality-rate based criteria) was used to determine the levels of mosquito susceptibilities: Susceptible (≤ 98 %;) Tolerant and resistance to be confirmed by molecular methods or rearing and testing of offspring of the individual survivors (97 – 80%) and resistant (≥ 8%). The results in pink and light blue colours indicate that the mosquitoes are likely to be resistant and tolerant respectively to the tested insecticide discriminatory dosages.

Insecticide	Location (Sentinel District)	Number (N)	Mean KnockDown times KDT 50 in min 95% CI	Mean % mortality after 24h	KDT50 Ratio
Lambda- cyhalothrin 0.05%	Kisumu	100	15.2	100	1.0
	Kirombero(Morogoro)	80	27.7 (23.7-32.4)	100	1.8
	Kyela(Mbeya)	83	11.9 (10.9-12.9)	100	0.8
	Mvomero(Morogoro)	83	20.6 (18.5-22.8)	100	1.4
	Muheza	95	29.6 (25.9-33.5)	81.8	1.9
	Lushoto	10	28.9 (22.7-35.1)	100	1.9
	Handeni	92	33.6 (31.8-35.4)	97.9	2.2
	Arumeru	125	32.5 (161.9-2257.8)	70.4	2.1
	Dodoma	80	21.8 (19.4-24.2)	100	1.4
	Tabora	80	26.7 (24.2-29.4)	100	1.8
	Dar es Salaam	79	41.5 (37.7- 45.9)	94.8	2.7
	Magu(Mwanza)	25	37.9 (34.04-42.8)	100	2.5
	Muleba	80	39.0 (34.9- 44.4)	85	2.6
	Babati	125	152.7 (101.9-494.9)	100	10.0
	Moshi	531	59.3 (50.2- 82.0)	54.98	3.9
DDT 4%	Kisumu	100	21.5 (18.7-24.4)	100	1.0
	Kirombero(Morogoro)	100	19.2 (15.4-23.3)	99	0.9
	Kyela(Mbeya)	85	27.9 (21.4-36.1)	100	1.3
	Mvomero(Morogoro)	83	27.3 (22.7-32.7)	100	1.3
	Muheza	100	32.7 (29.9-35.4)	100	1.5
	Lushoto	100	36.1 (34.7-37.4)	100	1.7
	Handeni	100	22.1 (12.9- 31.2)	100	1.0
	Arumeru	125	29.0 (27.9-30.1)	100	1.3
	Dodoma	80	26.4 (24.1- 28.8)	100	1.2
	Tabora	80	30.5 (24.1-38.8)	100	1.4
	Dar es Salaam	74	31.2(28.6-33.8)	100	1.5
	Magu(Mwanza)	20	29.7 (24.8-35.2)	80	1.4
	Muleba	60	-	100	0.0
	Babati	100	13.3 (11.6-14.9)	100	0.6
	Moshi	648	36.2 (34.3- 38.1)	99.7	1.7

Insecticide	Location (Sentinel District)	Number (N)	Mean KnockDown times KDT 50 in min 95% CI	Mean % mortality after 24h	KDT50 Ratio
Propoxur 0.1%	Kirombero(Morogoro)	80	-	98.8	-
	Kyela(Mbeya)	79	-	98.8	-
	Mvomero(Morogoro)	80	-	98.8	-
	Muheza	100	-	100	-
	Lushoto	100	-	100	-
	Handeni	100	-	100	-
	Arumeru	125	-	100	-
	Dodoma	80	-	100	-
	Tabora	80	-	100	-
	Dar es Salaam	68	-	100	-
	Magu(Mwanza)	90	-	100	-
	Muleba	80	-	100	-
	Babati	125	-	100	-
	Moshi	338	-	99.8	-
Fenitrothion 1%	Kirombero(Morogoro)	123	-	100	-
	Kyela(Mbeya)	80	-	98.8	-
	Mvomero(Morogoro)	81	-	100	-
	Muheza	100	-	100	-
	Lushoto	100	-	100	-
	Handeni	100	-	100	-
	Arumeru	125	-	100	-
	Dodoma	100	-	100	-
	Tabora	100	-	100	-
	Dar es Salaam	60	-	100	-
	Magu(Mwanza)	80	-	100	-
	Muleba	60	-	100	-
	Babati	100	-	100	-
	Moshi	249	-	99.9	-
Total Tested		**10,114**			
Total field mosquito tested		**9,714**			
Total Kisumu (susceptible) tested		**400**			

4.2 Mosquitoes Identification

A total of 9,672 collected mosquitoes were morphologically identified as *An. gambiae* s.l. Of these, 423 were subjected for PCR analysis. Out of 423 mosquitoes, 308 and 115 were identified as *An. arabiensis* and *An. gambiae s.s* respectively. Presence of *Anopheles gambiae s.s* and *An. arabiensis* were indicated by the diagnostic size of amplified DNA fragments which are 390 and 315 bp respectively. The distribution of these two species in different geographical zones is shown in figure 9.

Both species occurred in sympatry in Dar es Salaam where 37.1% were *An. gambiae s.s* and 62.9% *An. arabiensis*. Muheza and Lushoto were dominated by *An. gambiae* s.s at 92.3% and 94.7% respectively. Handeni however was dominated by *An. arabiensis* at 94.4%.

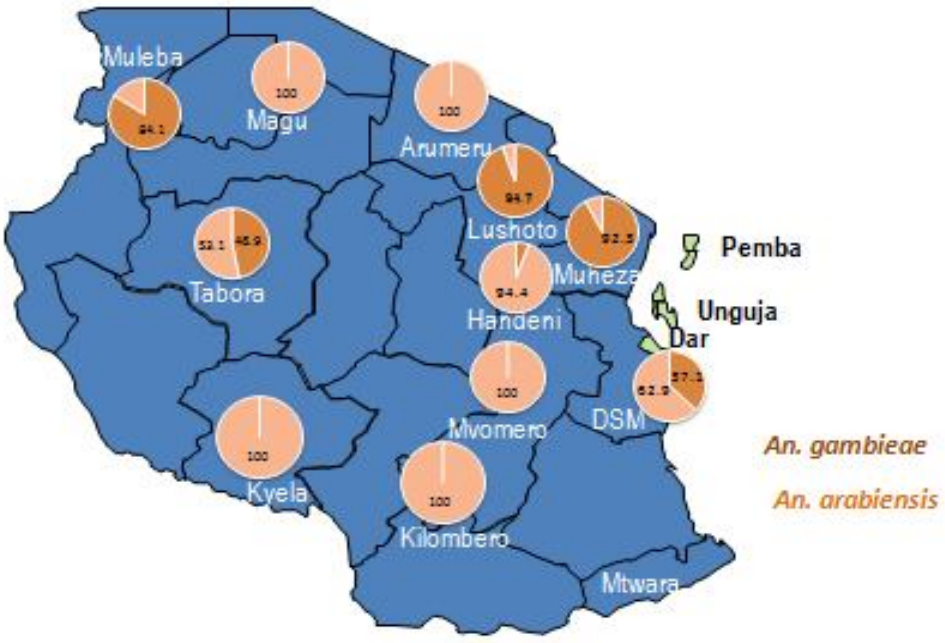

Figure 9: Distribution of the *An gambiae* sibling species per site following identification to species level by PCR technique

4.3 Kdr genes detection in the *An. gambiae s.l* population

A total of 423 An*opheles gambiae s.l were* analyzed for presence of *kdr* mutation. Of these, 115 were *An. gambiae s.s.* and 308 were *An. Arabiensis*, randomly selected from all sentinel sites There were no *kdr* mutations detected in these analyzed samples.

4.4 Insecticide usage

Responses from interviews with agriculture and public health officers in the districts revealed a long history of insecticide use. Crops for which insecticides are applied include cotton, maize, tomatoes, coffee, beans and vegetables. Animal species sprayed with insecticide are cattle and poultry. In public health, insecticides are used for control of mosquitoes, houseflies, cockroaches, bedbugs and lice. Modes of application include spraying and fumigation in homes, guesthouses, hostels, restaurants, hospital wards and grain stores. In the shops and agrovet stores insecticides found on sale included Karate® (Lambdacyhalothrin 50g/L), Helarat® 5EC (Lambdacyhalothrin 5%W/V), Cypercald® (Cypermethrin 15g/L & Dimethoate 120/L ULV) and Fenom® (Lambdacyhalothrin 15g + profenofos 300g) all of which are used for cotton spraying. Insecticides used in vegetable production included Cypercald®, Fenom® and BANCO® 500 SC (Chlorothalonil 500g/L SC). For grain storage Actellic Super®; Permethrin 3g/Kg; and Pyrimifos-methyl 16g/Kg were commonly used. Insecticides used for livestock spraying were Stelladone® (Chlorfenvinphos 300g/L), Dominex® (Alphacypermethrin 10%), Ectomin® (Cypermethrin) and Sevin® dust (Cabaryl 75g/Kg). Others included Tactic®, Triatix®, Norotrax® and Amitix® all of which contain Amitraz 12.5% for cattle only. Cotton spraying is done 6 times a year while dipping or spraying of animals is done two to four times a month. For public health purposes insecticides used included Neocidol® (Diazinon), Kill IT® (Dichlorvos 5.0g/Kg, Tetramethrin 2.0 g/Kg, Pironyl butoxide 10g/Kg & Permethrin 2.9g/Kg), Raid IT® (Tetramethrin 0.3%, Cypermethrin 0.1% & Propoxur 0.74%), DOOM® Fast Kill (d-Phenothrin 1.0g/Kg & Imiprothrin 0.4g/Kg) and HATARI (Fenitrothrin 0.8 W/W, Tetramethrin 0.2 W/W & Piperonyl butoxide 1.0W/W).

4.5 Insecticidal Efficacy, Longevity and Integrity of LLINs/ITNs under field conditions

4.5.1 Insecticidal Efficacy of LLINs/ITNs under field conditions

After two years of field usage the mean mortality of the sampled LLIN and ITNs were scored as 36% and 26%, respectively. However, the observed difference was not statistically significant (p = 0.001) . Likewise, the difference in mean knock down between the LLIN nets (40%) and that of the conventional ITN (32.9%) was not found to be statistically significant (p = <0.001).

Daily use of LLIN was reported to be 93% during the survey. All but four of the surveyed households reported that net washing was done using cold water. It was estimated that nets were washed on an average of 2.5 times per year.

Mean number of size 1 holes was 16 for LLIN and 22.4 for ITN while mean number of size 2 holes was 10 for both LLIN and for ITN and for the size 3 holes was 5 for LLIN and 2 for ITN.

The mean mortality of LLINs nets (36%) was significantly higher (p < 0.33) than that of the conventional ITNs (22%). On the other hand 12% of the LLIN nets recorded mortality below 80%, while 58% of the ITNs recorded mortality below 80%. Therefore, ITNs had lost their insecticidal efficacy, while LLINs were still efficacious after two years of field usage. According to WHO standards LLIN suppose to remain effective for 3 to 4 years but the effectiveness recorded in this survey raise an alarm regarding longevity of the insecticidal efficacy and durability of the nets fabrics.

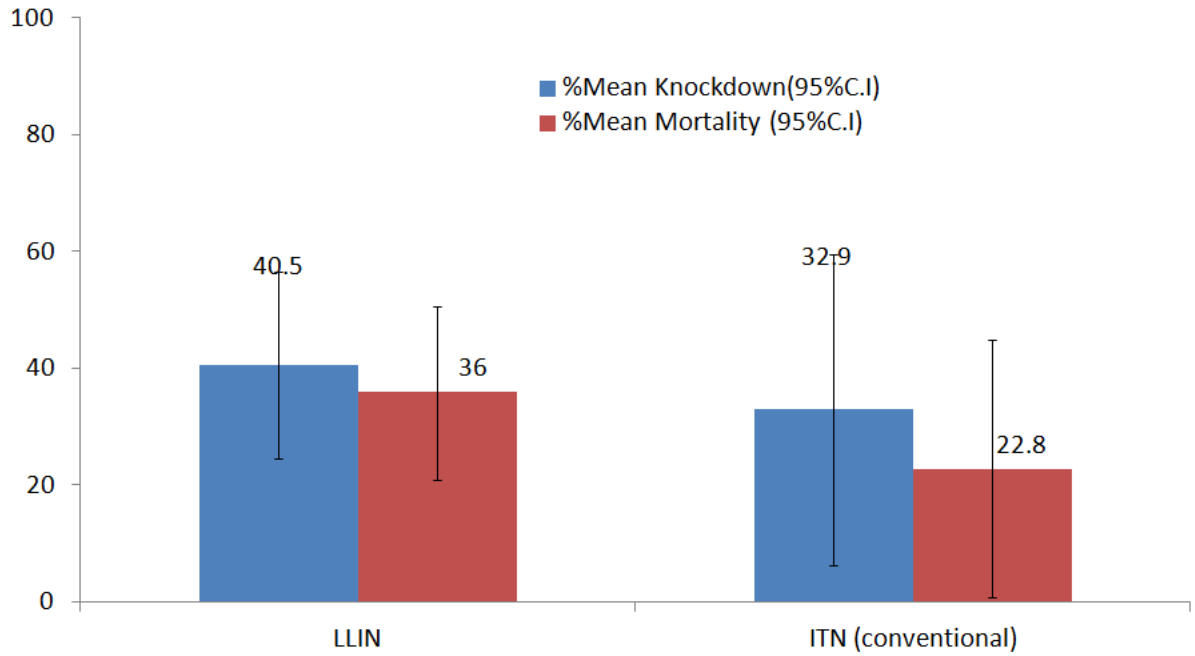

Figure 10: Bed nets insecticidal efficacy under field conditions

4.5.2 Longevity and Integrity of LLINs/ITNs under field conditions

To determine the physical integrity of LLINs and ITNs after 2 years of field use, the Wilcoxon-Mann-Whitney test was performed to compare the median number of holes between LLINs and ITNs, there was no statistically significant difference found between the total number of holes on the two net types (p=0.47).

Table 2: Physical integrity of LLIN and conventional ITNs after 2 years of field use

Net type	Percentage of holes per category sizes of holes[2]			Mean number of holes per position of the net (SE)	
	Size 1	Size 2	Size 3	Lower half of the net (SE)	Upper half of the net (SE)
LLINs	16.6	10.7	5	25(1.05)	2.6(0.3)
ITNs	22.4	10	2	30.6(1.3)	3.6(0.25)

[2] Kilian, *et al* (2010) Review of delivery strategies for insecticide treated mosquito nets – are we ready for the next phase of malaria control efforts? TropIKA.net http://journal.tropika.net

4.6 Lambdacyhalothrin CS insecticide Residual Bio-efficacy on different types of wall substrates

These are the results from a community-based trial in Karagwe, Muleba, and Chato districts in Kagera region with Lambdacyhalothrin 10 CS (Figures 11 to 13). The knockdown rates remained 100% on painted surfaces during the three months trial. However, it significantly decreased on mud, concrete and wood surfaces between 35%, 78% and 80% respectively ($P<0.05$).

In Karagwe district the 24 hours mortality rates ranged from 98.7% to 100% well above the WHO recommended threshold of ≥80% mortalities (Fig. 11). The results indicate that the Lambdacyhalothrin 10 CS insecticide residue was still effective on all treated wall substrates over three month's period post Indoor Residual Spraying.

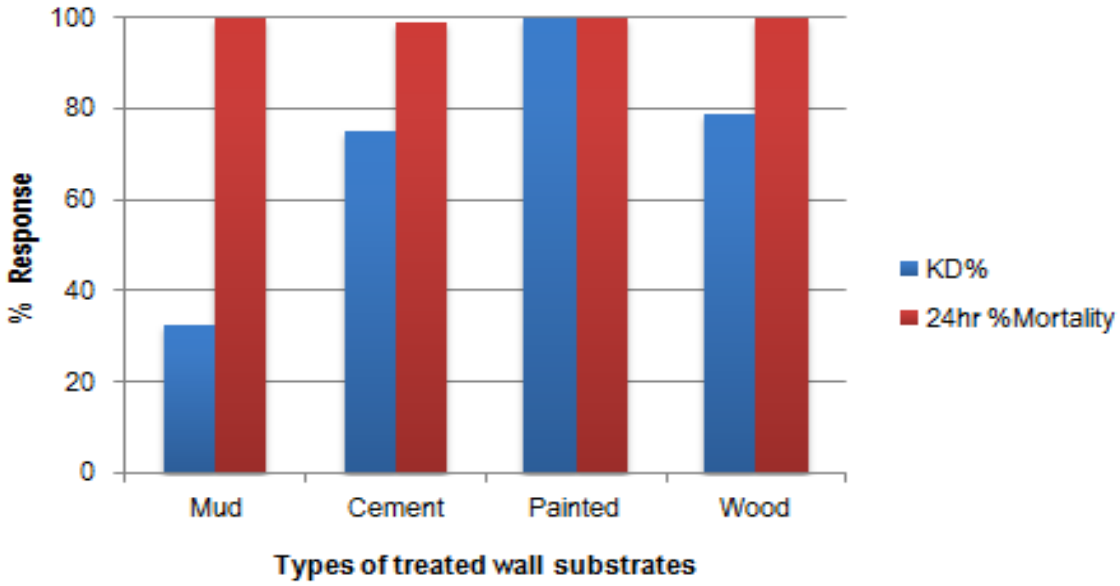

Figure 11: Response of *An. gambiae s.s* susceptible strains to insecticide treated wall surfaces in KARAGWE DISTRICT, three months after spraying (July – September 2011)

Figure 12 below shows the response of *An. gambiae s.s* to Lambdacyhalothrin CS sprayed on different types of wall substrates in Muleba district. The results showed that the 24h hours mortality rates of mosquitoes exposed to treated wall surfaces ranged from 98.7% to 100% (above the WHO recommended threshold of ≥ 80% mortalities) which indicate that Lambdacyhalothrin CS still had high residual effect over three months period post IRS. The highest knockdown rate was observed on the painted treated substrate (67%) followed by the wood walls (60%) and least to the mud substrate (45%).

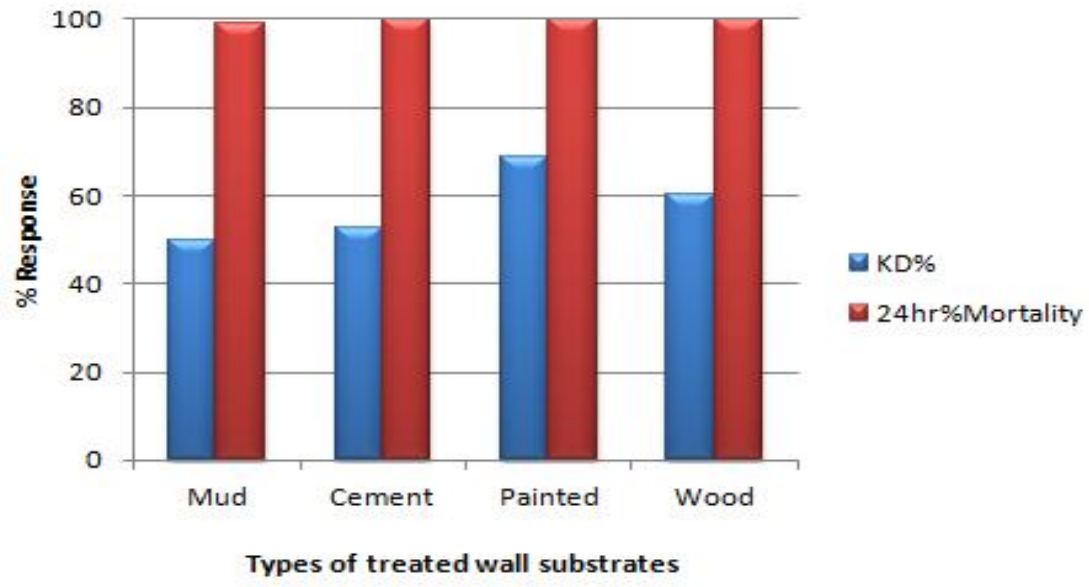

Figure 12: Response of *An. gambiae s.s* susceptible strains to insecticide treated wall surfaces in MULEBA DISTRICT, three months post-spray (July – September 2011)

The results from figure 13 below show that the 24h mortality rates of mosquitoes exposed to treated wall substrates ranged from 82.5% to 98.5% which is above the WHO threshold for insecticide to be effective post IRS (i.e. ≥80%). The mud and painted wall substrates retained the highest insecticide residues (98%) followed cement (87%) and wood (82%) over three months period post IRS in Chato district.

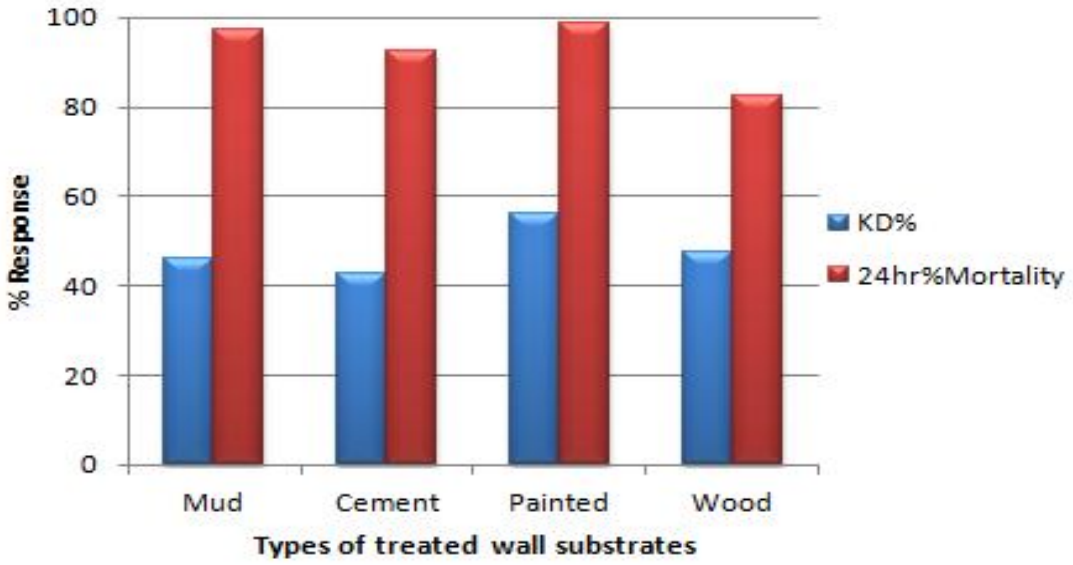

Figure 13: Response of *An. gambiae s.s* susceptible strains to insecticide treated wall surfaces in CHATO District, three months post-spray (July – September 2011)

5.0 DISCUSSION

The current study demonstrates the rapid emergence of insecticide resistance to pyrethroids used against malaria vectors, particularly in areas of Tanzania where ITNs have been used for more than 20 years. Data from this study revealed varied responses of mosquito population after exposures to WHO insecticide treated papers across all sentinel sites. Some vector population survived exposures to all synthetic pyrethroid compounds tested (Permethrin, Deltamethrin and Lambdacyhalothrin) compared to Organochlorine, carbamate and organophosphate compounds to which vectors were fully susceptible.

Anopheles gambiae s.l mosquito population from Moshi and Muheza showed resistance to Deltamethrin, while those from four sentinel districts (Arumeru, Dar es Salaam, Handeni and Kilombero) were suspected to be resistant. Furthermore, mosquito population in Arumeru, Moshi, and Muleba districts were resistant to Lambdacyhalothrin whilst those in other four sentinel districts (Muleba, Muheza, Handeni and Dar es Salaam) were suspected to be resistant to the same insecticide. Similar findings were observed on slightly reduced levels of susceptibility to Permethrin in Dodoma (90%) and Morogoro (87%) in 1999 and in Ifakara (97%), Arumeru (91%) and Moshi (96%) in 2004 (Kulkarn *et al.,* 2007). In the 2009 insecticide resistance survey, Moshi rural district registered resistance to both Lambdacyhalothrin and reduced susceptibility to Deltamethrin (WHO/GATES PROJECT ON VBC, 2009: Unpublished Technical Report). In 2009, Muheza, Handeni, Dar es Salaam and Arumeru registered low level of susceptibility (suspected resistance), while Muleba had fully susceptibility to all insecticides tested.

Likewise, mosquito population from Moshi and Muheza was resistant to Permethrin. Resistance to Permethrin was also suspected in further six sentinel districts (Babati, Magu, Handeni, Dar es Salaam, Arumeru and Kilombero) mosquito population. The resistance due to Permethrin in Moshi is consistent with the 2009 survey. The rest of the districts showed more or less the same trend as was in 2009 with the exception of Muleba where this time *An. gambiae* s.l were found to be resistant to the pyrethroids, Lambdacyhalothrin.

Such rapid changes of susceptibility status of mosquitoes to insecticides could be the result of long term cumulative effect of treated bed nets scaling up (under-five catch up, pregnant mothers and universal coverage campaigns) which have been going on in the country since 2005, coupled with the recent IRS application in Kagera region since 2007. Similar observations have also been documented in other parts of Africa including Niger, Bioko Island and in Kenya (Czeher *et al.,* 2008, Sharp *et al.,* 2007 and Stump *et al.,* 2004).

On the other hand, mosquito populations from all the sentinel sites districts were fully susceptible to Propoxur WP which belong to carbamate group of insecticides; this was the case also for organophosphates Fenitrothion WP and Organochlorine DDT insecticides. However, a suspected resistant population to DDT was observed in Magu district. Similarly, data on the killing effect of Lambdacyhalothrin deposits on various wall substrates (mud, cement, woods and painted surfaces) against susceptible mosquito population in Karagwe, Muleba, and Chato districts indicated that its insecticidal residual efficacy was still very high at the time of this survey and therefore validating the observation of reduced susceptibility in some mosquito population around Muleba district.

6.0 CONCLUSION

Resistance to one or two synthetic pyrethroids (Permethrin, Deltamethrin and Lambdacyhalothrin) is on the increase across Tanzania mainland. Pyrethroid resistance is now emerging at high rates never seen before even in areas of long history of insecticide treated nets such as Muheza Township. The rate of resistance emergence is even more aggravated particularly in Muleba district where large scale use of pyrethroid based LLINs and IRS tools constitute the main mosquito control interventions. Although it is quite early to make assumption on the root-cause of the prevailing situation in the surveyed areas, it is more likely that the scaling up of vector control activities, particularly universal coverage of with long-lasting insecticidal nets (LLINs) and possibly the use of pyrethroids for agricultural purposes in those areas has accelerated the evolution and spread of pyrethroid resistance in Tanzania. However, the full susceptibility of *An. gambiae s.l* to carbamates (Propoxur and bendiocarb), and organophosphate (Fenitrothion WP) indicates that these insecticides can be suitable alternatives for IRS in Tanzania mainland. Reliable data on resistance is the cornerstone of successful resistance management and key to this is the availability of sound regularly updated data on the susceptibility status of malaria vectors to insecticides.

7.0 RECOMMENDATIONS

1) The fact that reduced susceptibility to pyrethroids was recorded in some areas; **this should be considered to be a strong indication for increasing trends in insecticide resistance among malaria vectors in Tanzania.** Such observation calls for continued vigilant monitoring of malaria vectors for their susceptibility to pyrethroids, carbamates and organophosphates.
2) As the country is now using pyrethroids for both IRS and LLINs/ITNs; **we strongly recommend that Pyrethroids should NOT be used for IRS in the presence of high LLIN/ITN coverage**, since the combination of pyrethroids on the wall and on the net is expected to produce extremely intense selection pressure for insecticide resistance. Thus *Combination interventions involve using different insecticide classes is highly encouraged* by either using organophosphates or carbamates for IRS so as to decrease the pressure of pyrethroids to malaria vectors.
3) As part of the insecticide resistance management strategies, **Indoor Residual Spraying (IRS) should be adopted using a rotation between pyrethroids and fast killing organophosphate or carbamate insecticides targeting preferably to the insecticide resistance sites** in order to check the spreading of resistant genes among mosquito population.
4) Since monitoring and evaluation activities are too frequently marginalized in large-scale vector control programmes elsewhere and this missed opportunity has contributed to the paucity of data on the impact of vector insecticide resistance to malaria transmission. For this reason, **monitoring vector susceptibility to insecticides for purposes of resistance management must be given a higher priority in the decision-making process in vector-control programmes** than is currently the case.
5) **There is an urgent need for epidemiological studies into the impact of insecticide resistance on LLINs and IRS at the programmatic level**. Therefore, Longitudinal comparative studies should be designed to assay whether pyrethroid resistance mechanisms other than kdr mutation may make pyrethroid treated materials or house spraying less effective against malaria vectors and malaria control efforts in general.
6) **In the presence of insecticide resistance, Integrated Malaria Vector Management approach (i.e., Environmental management, biological control and larviciding) should be encouraged depending on different local settings**, particularly targeting those areas with resistance and/or reduced susceptibility to insecticides.
7) Despite of the available scientific evidence that the currently WHOPES approved LLIN products can remain effective even after 3-5 years of field usage, still **there is an urgent need of conducting biannual quality control in the field to determine nets' durability, insecticidal efficacy, and user acceptability**. This is vitally important not only for purpose of quality control but also for making sure that we achieve the intended intervention goal.

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9.0 APPENDICES

INSECTICIDE SUSCEPTIBILITY TEST FORM FOR ADULT MOSQUITOES

AREA INFORMATION:

Village:		Ward:		Division	
District:		Region:			
GPS Coordinates:	**S**				
	E				

Collection method:

Human landing indoor		Resting night indoor	
Human landing outdoor		Resting night outdoor	
Others		**OUTDOOR MOSQUITO NET TRAPS USING HUMAN BAIT**	
From larval collection, F1 progeny;			

Type of breeding site:

Rice field		River bed		Irrigation channel	
Rain water pool		Wells		Water storage containers	
others (specify)			Species Control:		
SAMPLE INFORMATION:			Age:(days)		
Species tested:		Gravid			
Sex:		Blood fed		Semigravid	
Physiological state:				Date tested(dd-mm-yy)	
Non-blood fed					
Date collected(dd-mm-yy)				Concentration:	
INSECTICIDE INFORMATION				Batch number	
Insecticide tested:				Date of expiry	
Impregnated papers prepared by:			Number of times paper is used		
Date of impregnation		Room temperature			Refrigerated
Date paper removed from pack					

Storage conditions:		**Temperature**	**Relative humidity**			
TEST CONDITIONS						
Exposure period: start						
Holding period: after 12 hours						
Holding period: end	**Replicate 1**	**Replicate 2**	**Replicate 3**	**Replicate 4**	**Total tested**	**Control**
TEST R ESULTS: Period of exposure(minutes)						
		No of mosquitoes knocked down after exposure				

No. exposed	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Total tested	Control
Time(minutes)						
START						
10						
15						
20						
30						
40						
50						
60						
**80						
Number of mosquitoes killed at the end of the holding period(after 24 hours)						
Number killed						
Observed mortality (%)						
Corrected mortality (%)						

SUMMARY OF ALL INSECTICIDES TESTED

	Deltamethrin 0,05%	Delta control	Lamdacyhalothrin 0.05%	Lambda control	Permethrin 0,75%	Permethrin control	DDT 4%	DDT control	Fenithrthion	Fenitrothion control
Number of mosquitoes exposed										
Number of mosquitoes knocked down after exposure (time in minutes)										
10'										
15'										
20'										
30'										
40'										
50'										
60'										
80'										
Number of mosquitoes killed at the end of the holding period(after 24 hours)										
Observed mortality (%)										
Corrected mortality (%)										

HISTORY OF PESTICIDE USE

Name (Group)	Purpose	Formulation	Active ingredient	Dosage	Year introduced/ terminated	Remarks

NET SURVEY FORM

TITLE OF THE PROJECT: Surveillance of Malaria Vectors Mainland Tanzania
Name of Principle Investigator: Dr William N Kisinza
Name of Organization: National Institute for Medical Research, Amani Research Centre, Tanzania

HOUSEHOLD NET SURVEY

DISTRICT: _____

Village _____

Hamlet_____

Date: ___/___/___ (*dd/mm/yy*)_____ Household code _____
Type of house ……
No. of sleeping spaces ……
No. of nets owned …… ……
Type of fabric of the nets owned …… ……
Whether the net(s) was/were treated with insecticide? ……
If yes, when was the last treatment done? ……
How did you acquire the net? ……..
Why do you use nets/ITNs? ……
Choice of colour/size of net ……..

Information on net usage provided by:
1) User of this net
2) Caretaker of those using the net
3) Head of household
4) Other (specify) ……

Information on net usage:
1) Year-round and every night.
2) Year-round but occasionally.
3) Seasonally but every night.
4) Seasonally and occasionally

How did you get the net ?
1) Paid or purchased yourself ?
2) Given free
3) Specify

How the net was last washed? ……

When was the last time you washed the net? …….. (month)

How many times a net is washed in a year time? ……. times

Name of interviewer. …… **Signature of interviewer……**