# Investigation on The Level of Insecticide Resistance to Malaria Vectors in Ruangwa District Lindi Region Tanzania

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***ABSTRACT***
*High malaria prevalence remains a major problem in Ruangwa District, despite the high coverage rates of malaria control interventions. The objective of this study was to assess the level of insecticide resistance in malaria vectors in southern, Tanzania. The study was conducted in three villages of Likangara, Nandagara and Chienjere. Resistance level to insecticides in An. gambiae s.l was evaluated using a standard WHO Susceptibility Test Kit. These mosquitoes were reared from larvae collected in varoius breeding sites in a major rice, vegetable and leguminous plant cultivation area in which pesticides use is intensive. Each test was run in four replicates of 25 adult non-blood fed female Anopheles gambiae s.l mosquitoes per tube. In total, 100 specimens were exposed for standard concentration of 1% fenitrothion, 0.1% bendiocarb, 0.75% permethrin and 0.05% deltamethrin using WHO susceptibility test kit. The number of knocked down mosquitoes were recorded at 10,15,20,30,50 and 60 min and mortality rates were determined after 24 hours and results were classified according to WHO guidelines, as susceptible (97-100%), possible resistance (90%-98%) and resistance (<90%). The results indicate suggestive levels of mosquitoes resistance to Bendiocarb at (75%), possible resitance levels of Deltamethrin at (95%), Permethrin at (97%) and Fenitrothion at (96%) ($p < 0.05$). This implies that there is reduced effectiveness of insecticides used in vector control interventions in the district. Superior insecticides should made available and introduced in order to promote the sustainable management of malaria vectors and elimination malaria transmission in the district.*

**Keywords:** *Malaria vectors, Insecticide Resistance, Susceptibility, Ruangwa district, Tanzania*

## INTRODUCTION

In spite of intense national and international efforts, malaria remains one of the major tropical challenges in the world today (WHO, 2019). Malaria is one of the main public health problems in Africa, causing more than one million deaths per year and placing a strong burden on developing African countries (WHO, 2018). Vector control remains an important component of malaria prevention. In sub-sahara Africa, the primary tools for malaria vector control are long-lasting insecticidal nets (LLIN) and indoor residual spraying (IRS). However, insecticide resistance development in vector populations could impede the success of malaria control programmes in endemic areas (Riveron, *et al.*, 2018).

Currently, major classes of insecticides such as organochlorines, carbamates, organophosphates and pyrethroids which are being used in malaria control, are increasingly less effective at killing malaria mosquitoes ( WHO, 2010; Kabula, 2018; Hancock e*t al.*, 2020).The intensive use of this class of insecticides at large scale both in agriculture and public health in malaria vector control activities has led to its reduced efficacy in sub Saharan Africa (Reid, 2016; Elenora, 2018; Philbert *et al*., 2019). For example in Tanzania, some regions especially, in the southern and north western parts of the country, malaria has remained persistently high 11.7% to 24.7% despite wide coverage with LLINs, IRS and case management using Artmether-Lumephantrine (TMIS, 2017). In Lindi region the average regional malaria prevalence remains high at 11.7% (TMIS, 2017), but with great village variations. For example, in the Ruangwa District, Malaria prevalence rate of 17.7%, 18.3% and 85% were recorded from the villages of Likangara, Nandagara and Chienjere respectively (DHIS, 2017).

Tanzania's national health strategies aspire reducing malaria to 1% by the year 2020 by using malaria vector control tools such as Long-lasting Insecticidal Nets (LLINs), Artemisinin-based Combination therapy (ACT) and indoor residual spraying (IRS) (MoHSW, 2015). Despite wide LLINs distribution by 95% to most vulnerable groups such as pregnant women, school going pupils and infants from 2014 to 2017 malaria prevalence is still high at 17.7% in Ruangwa district (DHIS, 2017).

However, the main contributing factors for this persitence in malaria transmission despite widespread use of the current core vector control intervention measures are not well known. In addition. Factors such as; lack of studies to epidemiological consequences of resistance on malaria vector control (Philbert,2014) and rapid expansion of insecticide resistance to malaria vectors could be playing an important role in malaria transmission(Nkya, *et al.*, 2013; Toe, *et al.*, 2014; Kisinza, *et al.*, 2017). Furthermore, the emergence of antimalaria drug-resistant to strains of malaria parasites (WHO, 2015), resistance mechanisms to different insecticides have been documented ( Kisinza *et al.*, 2017). However, substantial collective research evidences on the dynamics of insecticide resistance in mailand Tanzania specifically in Lind Region is still limited (Govella *et al.*, 2013; Protopopoff, 2013; Killeen, 2014).

Therefore, this study aimed to monitor the status of insecticedes resistance in sourthern Tanzania were malaria transmision and is still high. The information obtained could be used as a benchmark for comprehensive approach of new tool and strategies to control insecticedes resistance in Tanzania.

## METHODOLOGY

### The study area

The study was conducted in Ruangwa district, populated with 131,080 people. It's a flat land with hilly landascape, the altitude is 313 - 549 meters above sea level. It has 707 hectares of the total irrigated area planted with annual and permenent crops. Pesticides such as insecticides, fungicides and herbcides are chemicals used for controlling insects, diseases and weeds, but insecticides were the most common pesticide used in planted area, followed by fungicides and the least was herbicides which could be threatened the present achivement of residual malaria transmission control.There's one rainy season (November – May), averaging 800 mm of rainfall per year. The daily temperature ranges from 24 to 35 degrees Celsius with very high humid air; the source of malaria prevalence rate. The three study villages (Likangara, Nandagara and Chienjere) have rural- urban and para-rural settings with low malaria prevalence of 17.7%), moderate 54.3% and high 85.7% respectively. (DMIS, 2016). The villages are located in low savannah area covered with grasses, bushes and scattered trees, narrow slow running streams with marginal vegetation and pad fields, shallow wells, bored wells and ponds which are seasonal breeding habitats. Many villagers are agriculturalists, few are petty traders.

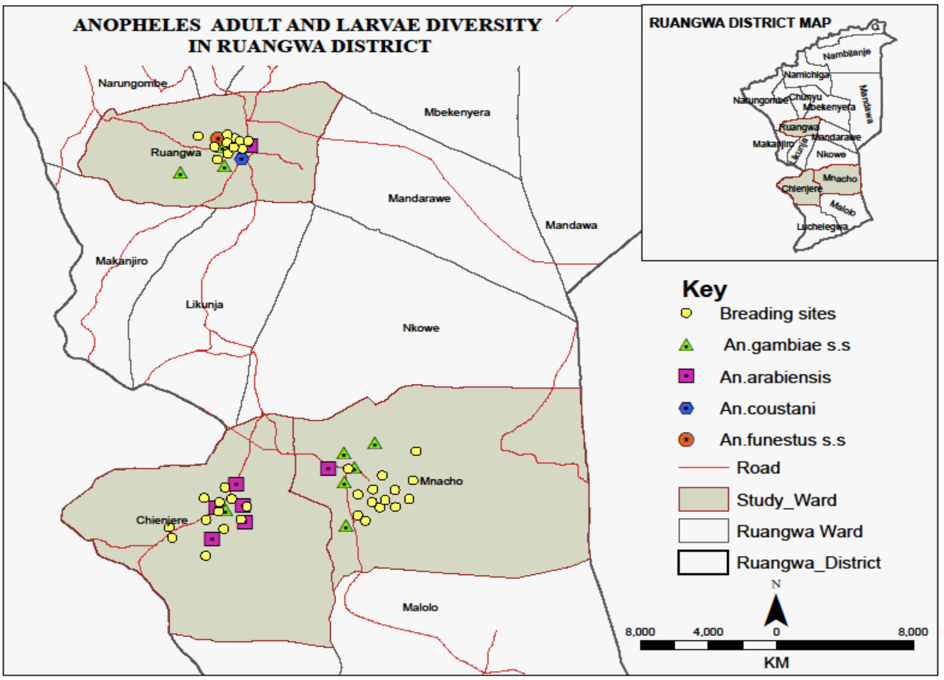


**Figure 1: Maps showing the region, district, three wards/ villages and breeding sites where malaria vectors collected in agricultural areas with intensive use of insecticides**

## Larval sampling (Immature stages of mosquitoes)

Weekly cross-sectional larval surveys in a variety of naturnal breeding sites were carried out between March and September, 2017. All samples were collected at least one km around agricultural area where most of breeding sites were found. The collected larvae were transferred into small bowl labelled with date, site of collection and type of habitat. The larvae specimen was packed in cool-box and transported to the insectary reared to adult under the following conditions: temperature 27±2°C and relative humidity at 70-90%. A 10% glucose solution was supplied in the cages for the emergent adults and maintained photoperiod 12: 12 light/dark period from 0600 hours to 1800 hours –"light period" and 1800hrs to 0600 " dark period". They were fed on King Fish food 2-3 times per day until pupation to reduce variation in larval growth rate and mosquito size at ermegence. The pupae were immediately transferred to a bowl that contained water and placed in a cage until the adults emerged. Upon emergence from pupa; the adults were fed on glucose 10% sugar solution soaked in cotton wool and maintained in the cages.

**Testing for adult susceptibility mosquitoes**

Insecticide susceptibility tests were carried out using susceptibility test kits and WHO standard procedures (WHO, 2012). Each test was run in four with replicates of 25 adult non-bloods fed female *Anopheles gambiae* s.l mosquitoes per tube, totally 100 specimens for each insecticide. Tests for mosquitoes were exposed to papers impregnated with WHO recommended discriminating concentration dose of 1% Fenitrothion, 0.1% Bendiocarb, 0.75% Permethrin and 0.05% Deltamethrin (WHO, 2013). The controls were exposed to papers without insecticides coated with specified oil, two replicates per test, totally 50 specimen for similar period. During exposure period, the number of knocked down mosquitoes were recorded at 10,15,20,30,50 and 60 min. After 60 min, tested mosquitoes were then transferred into holding tube and supplied with glucose 10% sugar solution and the mortality rates were also determined after 24 hours. Mortality was compared with the percentage of dead mosquitoes. The mosquito's population resistance results was classified according to WHO guidelines, as susceptible (98-100%), acquiring possible resistance (90%-98%) and resistance (<90%).

## FINDINGS AND DISCUSSION

The results showed that *An. gambaie s.l* mosquitoes was resistant to Bendiocarb as compared to Deltamethrin, Permethrin and Fenitrothion insecticides ($\chi^2$ = 2.65, df 2, p>0.05). In addition, significant difference in the levels of resistance was observed among Bendiocarb, Permethrin, Fenitrothion and deltamethrin insecticides tested. ($\chi^2$ =25, df =3, p <0.01).

**Table 1 *An. gambaie s.l.* Resistance profile in Ruangwa District**

| SN. | Insecticides | Concentration | No. Tested | Mortality | Percentage | P Value |
|---|---|---|---|---|---|---|
| 1. | Permethrin | 0.75% | 100 | 97 | 97% | 0.01 |
| 2. | Deltamethrin | 0.05% | 100 | 95 | 95 | 0.01 |
| 3. | Fenitrothion | 1% | 100 | 96 | 96% | 0.01 |
| 4. | Bendiocarb | 0.1%) | 100 | 75 | 75% | 0.01 |

The levels of resistance are also well illustrated in Figure 2. Red line indicates cut off efficacy of insecticides tested (98%-100%-

susceptibility; 90%-97- possible resistance; < 90- resistance) and black dots illustrate replicates conducted in ratio (Figure 2 and Figure 3).

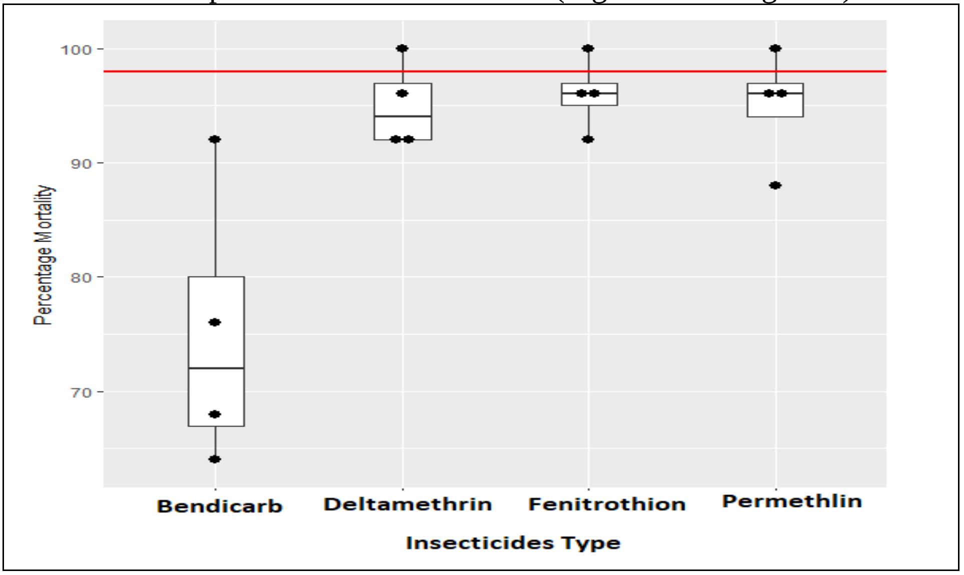


**Figure 2: Results for An. gambiae s.l susceptibility tests indicate the four replicates per insecticide tested. Black dots indicate the mortality rate of mosquitoes in percentage**

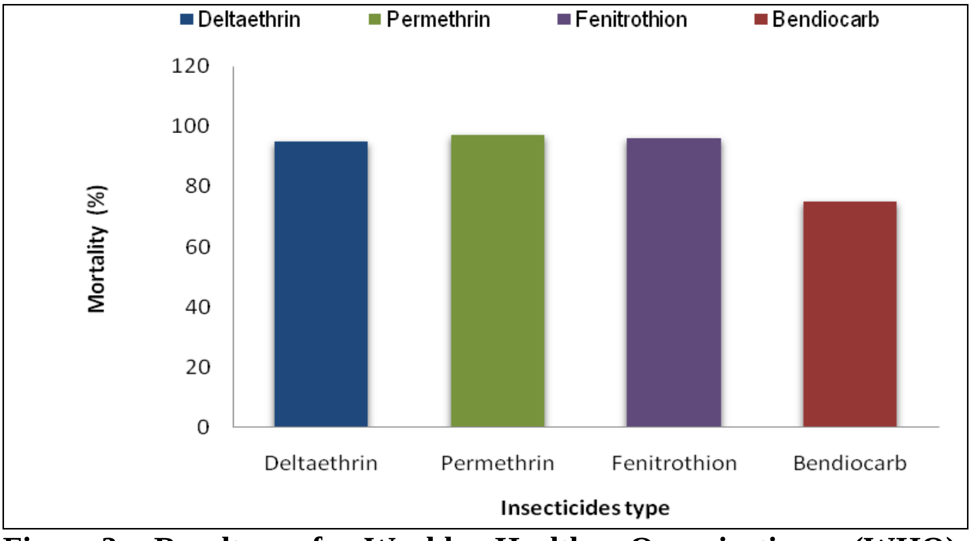


**Figure 3: Results of World Health Organization (WHO) Susceptibility tests for *An. gambiae* s.l mosquitoes collected in Ruangwa district**

The present study was designed to evaluate under field conditions the efficacy of a carbamate (bendiocarb) pyrethroids (deltamethrin, and permethrin) and organophosphate (fenitrothion) resistance level against malaria vectors in Ruangwa district, southern part of Tanzania. The results showed that *An. gambaie s.l* mosquitoes were resistant to Bendiocarb as compared to Deltamethrin, Permethrin and Fenitrothion insecticides. The resistance level of bendiocarb to malaria vectors reported in the present study concurs with the one reported by Kisinza (2017), in Ngara and Mbozi districts, Tanzania which accounted for 58% in mortality rates of *Anopheles* species tested. The results also concur with (Antonio-Nkondijio, 2016) in a study conducted in Younde, Cameroon in which he reported *An. gambiae* s.s resistance to Bendiocarb insecticides. Besides, Wanjala (2018) also confirmed Bendiocarb resistance to malaria vectors in in Iguhu and Kabula areas of Western Kenya.

In the present study, Deltamethrin showed a possible resistance level at (0.05%) in all wards. Similar results have been reported by (Matowo *et al.*, 2010; PMI, 2016) in several districts in northern and western Tanzania. Similarly, Wanjala (2018) and Kweka (2018) have reported possible resistance to Deltamethrin in Kenya. Another Pyrethroid, Permethrin has been reported to have possible resistance level of 3% in Musoma rural district (PMI, 2016).

Nonetheless, (Akogbeto, 2006; Ochomo *et al.*, 2013; Massebo *et al.*, 2013; Matananga *et al.*, 2015) in Kenya and Malawi respectively, observed that, even in the presence of possible pyrethroid resistance, LLINs perform better than untreated nets in terms of protection against mosquito biting in endemic countries.

The efficacy shown by the organophosphate (fenitrothion) in the present study is supported by studies by Maharaj and Sharp (2005) in KwaZulu Natal South Africa, in which DDT was re-introduced after the failure of pyrethoids to control *Anopheles funestus*. Furthermore, a study by Abilio *et al*., (2011) in Mozambique reported that both *An. gambiae* s.s. and *An. funestus* were controlled effectively with the DDT-based IRS programme in Zambezia, an excercise that led to reduction of disease transmission and burden. However, *Anopheles gambiae* s.l . is becoming resistant to pyrethoids and DDT in several parts of Tanzania (Kabula *et al*., 2014 ; Matowo *et al.,* 2014; Matiya, *et al*., 2019). The discovery of potential resistance to carbamates and pyrethroid in in Ruangwa district may threatens the gains made here and may impair the effectiveness of these interventions in place and therefore demand close monitoring and the adoption of a resistance management strategy (Kabula *et al*., 2014).

In the present study, *Anopheles mosquitoes* were collected in lowland areas in which; rice, fruits, maize, cashew nuts and vegetable cultivation was being practiced and there is an extensive application of pesticides. The possible resistance levels could emanate from the above practices. Nkya *et al.,* (2013) observed that resistance and possible resistance of insecticides to malaria vectors were due to cross contamination from cumulative pesticide compounds used to control pests in agriculture and livestock as well as weed control. This supports the findings by Philbert (2014) in Dar Es Salaam while assessing the role of agriculture pesticide use on the development of to insecticides resistance to in malaria vectors and the potential impact on control activities. In addition, these observations, coincide with those reported in Cote d' Ivory, Kenya, Cameroon and Benin, which reported that, resistance in *Anopheles* mosquitoes was originating from rice farms, tomatoes and vegetable growing areas (Bigoga *et al*., 2007; Menze *et al.,* 2008; Djegbe *et al.,* 2011; Edi *et al.,* 2012; Antonio-Nkondijio, 2015).

The present study confirms the resistance of Bendiocarb, possible resistance to Deltamethrin and Permethrin. Moreover, the results indicate that Fenitrothion is still highly effective in malaria control in the study area. Moreover, the findings of the present study have shown that mosquito populations in the study area remain susceptible to the organophosphates which indicates that these can be used to good effect in Indoor Residual Spraying as part of a resistance management strategy in the study area.

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**Author's contributions**
Clement Godfrida was the principal investigator of the study and led collection of data, designed the sampling technique, conducted data analysis, and interpreted results. Prof. Emmanuel S. Kigadye and Dr. Nicodem J. Govella and Dr B. Kabula provided technical support guidance. All authors read and the Open University of Tanzania approved the manuscript.

**Ethical clearance**: This received an ethical approval from the Medical Research Coordination Committee of the National Institute of Medical Research in Tanzania Reference no NIMRI/HQ/R.8a/Vol. XI /3232. I collected mosquito larvae from various productive breeding sites of Likangara, Nandagara and Chienjere villages. Permission to collect mosquito larvae in the breeding sites in their area was obtained from Lindi region, Ruangwa district, ward, village officials and landlords after explaining the objective and benefit of the study.