# INDISCRIMINATE USE OF AGRO-VERINARY PESTICIDES AND SUSCEPTIBILITY STATUS OF *XENOPSYLLA CHEOPIS* (FAMILY: PULICIDAE) IN PLAGUE ENDEMIC FOCI IN TANZANIA

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**A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN ONE HEALTH MOLECULAR BIOLOGY OF SOKOINE UNIVERSITY OF AGRICULTURE. MOROGORO, TANZANIA.**

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## EXTENDED ABSTRACT

The control of rodent-borne fleas, which play major role in the transmission of plague, is the mainstay of plague control. The application of chemical insecticides forms the most powerful and widely used control measure. The reliability and continued use of this approach however is threatened by the development of resistance. The excessive use of agro-veterinary pesticides is increasingly associated with the development of resistance in different arthropods; however, such possibility has not been explored in flea vectors of plague in Tanzania. This study identified injudicious uses and/or malpractices which potentially enhance exposure of flea vectors to agro-veterinary insecticides and emergence of resistance thereof. Furthermore, the study assessed susceptibility status of major plague flea vector, *Xenopsylla cheopis*, to commonly used agro-veterinary and public health pesticides. The study was conducted in Lushoto and Mbulu districts, northern Tanzania, both of which are active plague foci. About 91% of the respondents in Lushoto and 93% respondents in Mbulu reported using agricultural pesticides during the cropping season. Excessive and injudicious use of agricultural and veterinary pesticides was common across the study districts. Most of the farmers reported applying agricultural pesticides for up to four times per a cropping season. The three out of fourteen most commonly used agricultural pesticides in Lushoto were master kinga72WP (mancozeb 640g/kg+ cymoxanil 80g/kg) (44%), suracron720EC (profenos 500g/l EC) (25.3%) and Sumo 5EC (lambda-cyhalothrin) (18.7%). The three out of seventeen most commonly used agricultural pesticides in Mbulu were Dursban 50W (Chlorpyrifos) (29%), Duduban 450EC (Cypermethrin 10g/lt+chloropyrifos 35g/lt) (18%) and Dursban+farmerzeb (Chlorpyrifos 48%, Mancozeb 80%WP). Cybadip (Cypermethrin) (≥45%) and paranex (alphacypermethrin) (≥13%) were the two most commonly used veterinary pesticides across the districts. Moreover, the susceptibility of *Xenopsylla cheopis*, originating from wild population in Mbulu was assessed against nine different agro-veterinary and public health pesticides via contact bioassays. The percentage mortality after exposure to recommended doses of eight insecticides tested was strongly suggestive of resistance (100% 24 h mortality, 93 - 96%). The fleas confirmed resistance to lambdacyhalothrin and carbaryl, with 90% mortality. The reference ‘susceptible’ colony *Xenopsylla Cheopis* was fully susceptible (100% 24 h mortality) to all tested insecticides. Similarly, the field *Xenopsylla Cheopis* was fully susceptible (100% 24 h mortality) to 5× and 10× the recommended doses of all insecticides indicating low resistance intensity. Conclusively, this study identified a suite of injudicious uses and/or malpractices; excessive use of agricultural chemicals, maluse of agro-veterinary chemicals as well as poor adherence to the application and safety procedures, all of which potentiate contamination of environments/surfaces and exposure of the chemicals to fleas thereof. Furthermore, the study indicates resistance in the wild population of *Xenopsylla cheopis* from Mbulu district. As such, flea vector populations across Lushoto and Mbulu districts are putatively under intense risk of resistance development, thus warranting further studies across plague endemic areas in country to understand distribution of the resistance, involved resistance mechanisms; and confirm the contribution of agro-veterinary insecticides.

**Keywords:** Agro-veterinary pesticides, misuse, malpractices, resistance, fleas, *Xenopsylla cheopis*, plague

# DECLARATION

I, **GRACE PAUL RUGALEMA,** do hereby declare to the Senate of the Sokoine University of Agriculture that this dissertation is my own original work and it has neither been nor is it being concurrently submitted for a higher degree award in any other institution.

________________________ ____________________

Grace Paul Rugalema Date

(MSc. Candidate)

The above declaration confirmed by;

________________________ ____________________

Dr. Ladslaus L. Mnyone Date

(Supervisor)

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# DEDICATION

This research work is dedicated to my beloved mother Stella P. Rugalema and all plague endemic communities in Africa and beyond.

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## LIST OF ABREVIATIONS AND SYMBOLS

| | |
|---|---|
| CDC | Centers for Disease Control and Prevention |
| DDT | Dichloro-Diethyl-Trichloroethane |
| DRC | Democratic Republic of Congo |
| EC | Emulsifiable Concentrate |
| IRS | Indoor Residual Spraying |
| NBS | National Bureau of Statistics |
| SC | Suspension Concetrate |
| SPMC | SUA Pest Management Center |
| SUA | Sokoine University of Agriculture |
| WHO | World Health Organization |
| WP | Wettable Powder |

# CHAPTER ONE

## 1.0 GENERAL INTRODUCTION

### 1.1 Background

Plague, which is caused by *Yesinia pestis*, is primarily a flea-borne infectious zoonotic disease often transmitted to humans from rodents. Although relatively fewer cases and deaths are experienced now than before, plague remains a serious public health threat worldwide. Plague pandemics across the mediaeval Europe caused over 50 million deaths, leave alone millions others that occurred elsewhere (Drancourt and Houhamdi, 2006; Walloe, 2008; Hufthammer *et al.,* 2013). To date, Sub-Saharan Africa contributes greater than 90% of the global plague burden (WHO, 2017). The most affected countries are Madagascar and Democratic Republic of Congo (DRC). Madagascar accounts for nearly 30% of plague cases worldwide, with an average annual incidence rate of 900 cases (WHO, 2010). Mahajanga city in Madagascar experienced plague outbreak after over 63 years of quiescence (Laventure *et al.,* 1991).

Although other endemic countries like Tanzania, Mozambique and Uganda have remained quiescent for several years, they are equally vulnerable to this devastating disease. Due to shifts in climate, land-use patterns, human population size and rodent migration, plague outbreaks can recur in inactive foci and/or establish in new geographical locations. In Mbulu district, Republic of Tanzania, two consecutive plague outbreaks were reported after nearly 30 years of quiescence (Bertharat *et al.*, 2007; Neerinck *et al.,* 2010).

Plague can be controlled by targeting flea vectors and or the disease bacterium *Y. pestis* However, flea control, particularly using chemical insecticides, remains the mainstay of control across many if not all, plague endemic countries. Indeed, the World Health

Organization recommends deployment of insecticides formulated as a dust or low volume spray as the most effective means of controlling plague flea vectors. The classes of insecticides that have been and/or are used from time to time include pyrethroids, organochlorines, organophosphates and carbamates (Rust, 2016). This control approach has substantially reduced transmission risk of plague in Tanzania and other epidemic countries. However, the long term effectiveness and sustainability of this contemporary approach is increasingly threatened by the development of resistance in flea vectors. Preliminary studies conducted in Madagascar indicate the development of resistance in *Xenopsylla cheopis* and other major plague flea vectors virtually against all existing classes of insecticides. Madagascar has recorded resistance in the major plague flea vector, *Xenopsylla cheopis*, to several classes of recommended insecticides available for use (Rust, 2016; Miarinjara *et al.,* 2017). Of the four classes of insecticides, however, pytheroids face the greatest threat. These insecticides are the most widely preferred and frequently used across plague endemic countries because of their high safety index and low cost. The insecticide resistance reported so far is largely associated with indiscriminate use public health insecticides particularly those recommended for use against plague and other vectors. Studies in other arthropods, however, are increasingly demonstrating the evolution of resistance resulting from agricultural and veterinary insecticides (Reid and McKenzie, 2016).

## 1.2 Distribution and Burden of Plague

There have been three great world plague pandemics. The first one, known as the Justinian plague, which occurred in the Byzanite Empire in the 6th century and spread through the Middle East, the Mediterranean and Europe (Rosen, 2007). This pandemic eventually claimed lives of over 25 million people (Rosen, 2007). The second pandemic, known as the Black Death, started in India, China and some regions of Russia and reached Western

Europe in 1347 where it claimed lives of approximately 60% of the population (Benedictow, 2008). The third pandemic started in 1855 CE in Yunnan Province, China where it claimed lives of nearly 10 million people (Khan, 2004; Perry and Fetherston, 1997; Dennis *et al.*, 1999).

To date, there are still several plague endemic foci in central Asia, central and southern Africa as well as northwestern India (Ramalingaswami, 1995; Bertherat *et al*., 2003; Esamaeil *et al*., 2013; Andrianaivoarimanana *et al*., 2019; Randremanana, 2019). Although relatively fewer than in the past pandemics, several cases and deaths are reported from different foci worldwide (Figure 1.1). Between 1987 and 2001, 36 876 confirmed cases of plague with 2 847 deaths were reported worldwide (Dennis *et al*., 1999; Esamaeil *et al*., 2013). In 2013, 783 cases were reported worldwide, resulting in 126 deaths (WHO, 2014). The majority (95%) of human cases, however occur in Africa; and Madagascar was one of the affected countries in African with an average 400 human cases annually between 2010 and 2015 (Shahraki, 2016; Rahelinirina, 2017). Tanzania reported a total of 8 490 plague cases with 675 (8.0%) deaths from 1980 to 2011 (Ziwa *et al.*, 2013). More recent studies indicate increasing number of plague cases in the Democratic Republic of Congo, contributing 54% cases reported from Africa over the 10 years' period between 2004 and 2014 (Abedi *et al*., 2018).

In Tanzania, the disease has been endemic in many parts of the country including Iringa, Singida, Kondoa, Rombo, Hai, Arumeru, Mbulu, Lushoto and Same districts (Ziwa *et al.*, 2013).

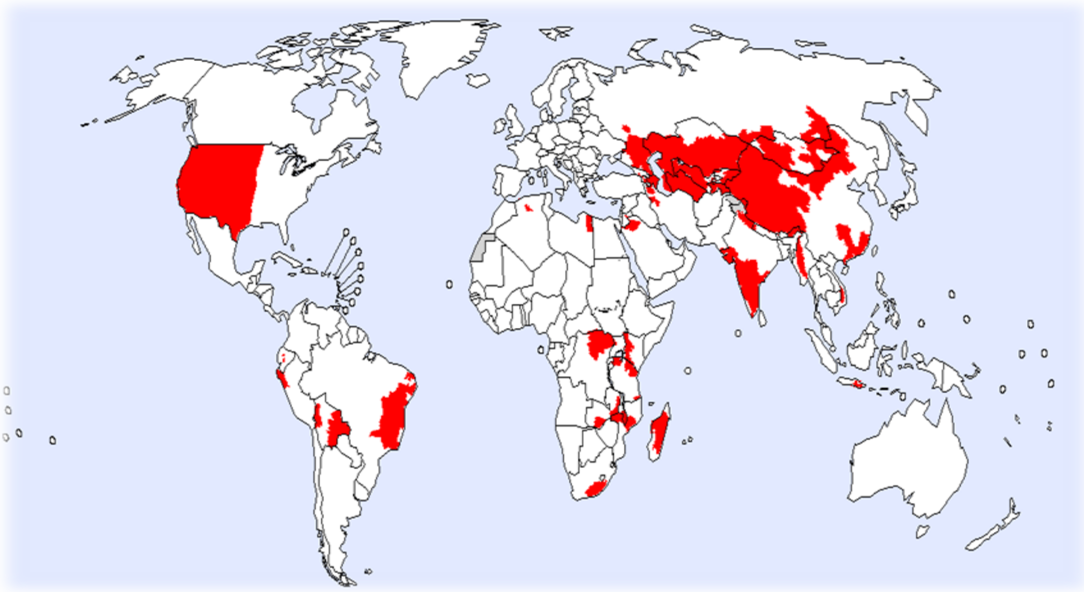

**Figure 1.1: Global distribution of natural plague foci as of March 2016**

Source: WHO/PED as of 15 March

## 1.3 Plague vector, Life cycle and Transmission

At least 80 flea species are known to carry the etiological agent of plague, although their role in disease transmission varies (Gage and Kasoy, 2005). Oriental rat flea, *Xenopsylla cheopis* (Rothschild), is considered to be the most efficient vector of plague. Other flea species have been identified as vectors in East Africa including *Ctenophtalmus bacopus, C. cabinus, Dinopsyllus, Pulex irritans* and *Xenopsyla brasiliensis* (Eisen and Gage, 2012).

Plague is primarily a disease of rodents and is transmitted between rodent hosts via flea bites. Humans and other mammals are, in fact, completely accidental hosts (Amedei *et al*., 2011; Chouikha and Hinnebusch, 2012; Gage, 2012). Life cycle of *Y. pestis* consists of a cycle between rodents and fleas.

When rodent hosts die, the fleas abandon the corpses and seek new hosts, inadvertently infecting other mammals, such as human (Anismovu and Amoako, 2006). When a flea feeds on an infected rodent, bacteria from the rodent's blood is taken into the flea's midgut. *Y. pestis* does not enter the cells of fleas or adhere to the flea's digestive tract, resulting in half of fleas, removing all of the bacteria through their feces. In order to overcome this, *Y. pestis* must build up an incredibly high density within the blood of its host rodent. When bacteria manage to persist in the flea's midgut, they rapidly reproduce and form clusters that are too large to be excreted. The bacteria proceed to form a biofilm on the proventriculus, a valve in the flea connecting the esophagus to their midgut. This causes the flea to regurgitate when it attempts to feed, spewing the bacteria into the bite and passing them onto a new host, where they once again begin to proliferate (Anismovu and Amoako, 2006; Chouikha and Hinnebusch, 2012).

Under natural environment, plague transmission is maintained primarily by the so-called sylvatic cycle, which involves wild rodents and their fleas. Many species of wild rodent and fleas have been implicated in transmission of plague in natural plague foci (WHO, 1997). Indirect transmission through the bite of flea is the most common route of transmission of plague from rodents to humans (Dennis *et al.*, 1999). The disease can also be transmitted through contaminated meat (Nyirenda, 2017). Other plague reservoirs include cats and dogs. Plague can also be transmitted through close contact with infected animals or their respiratory droplets (Gani and Leach, 2004).

## 1.4 Clinical Forms of Plague

There are three forms of plague depending on the route of infection: Bubonic, septicemic and pneumonic plague. The Bubonic form is mainly spread by infected fleas from small animals. *Y. pestis* enters at the biting site and travels through the lymphatic system to the nearest lymph node where it replicates, the lymph node becomes inflamed, tense and painful and is called a” bubo”. At advanced stages of disease, the inflamed lymph nodes turn into open sores filled with pus (WHO, 2017). Bubonic plague can advance and spread to the lungs which is the more severe type of plague called pneumonic plague.

Pneumonic plague or lung-based plague is the most life-threatening form. Its incubation can take 24hrs and transmission occurs via droplets. Symptoms of pneumonic plague include fever, headache, shortness of breath, chest pain and cough (CDC, 2004). Septicemic plague, this is the rarest form of a plague with a 100% mortality rate if untreated but ‘only’ 22% if treatment is available within the first 24 hours. When bacteria proliferate at high levels with the blood, septicemic plague arises. The blood-infection causes small blood clots to occur, cutting off circulation to parts of the body and depleting the clotting molecules in the blood. This leads to uncontrolled bleeding, nausea, fever, vomiting blood, abdominal pain and diarrhea. The blood clots result in cyanosis and necrosis due to a lack of oxygen reaching the tissues, with gangrene as a secondary effect (Amedei *et al.*, 2011; Anismov and Amoako, 2006).

## 1.5 Prevention and Control of Plague

Primary prevention of human plague mainly focuses on vector control or rodent reduction within limited areas affected by plague epizootics (Gratz, 1999). The most widely used method is to use insecticides to remove fleas from hosts. Recent studies from the West Nile region have shown that indoor residual spraying (IRS) and insecticide delivery tubes effectively reduce flea loads on rodents in the home environment where most exposures are believed to occur (Eisen *et al.,* 2014; Boegler *et al.,* 2014; Borchert *et al.,* 2014). Also, the rodent can be controlled by using lethal trapping and application of rodenticides. Secondary prevention of plague aims to reduce case fatality rates through education campaigns that emphasize recognition of signs of plague and urging persons with symptoms consistent with plague to seek care without delay (Gratz *et al.*, 1999). The control also requires investigation of animal and flea species implicated in the plague cycle in region, developing environmental management program to understand the natural zoonosis of the disease cycle and to limit spread and the surveillance of animal foci (WHO, 2017). Also, plague can be controlled by using vaccines, there are two types of vaccines currently used live vaccine which is derived from an attenuated strain related to EV76 strain and the killed vaccine uses formalin-fixed virulent strain of *Y. pestis* (Titball and Williamson, 2004).

### 1.6 Insecticide Resistance in Flea Vectors and the Mechanisms

Resistance to insecticide is defined by WHO as the ability of 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. Susceptibility is defined as the inability to withstand a pesticide at normal use rate (Biswas *et al*., 2016). The use of insecticides as the control of flea vectors has led to the great improvement of the fight against disease vectors (Hemingway, 2000; Nauen, 2007). Consequently, the intensive use of these insecticides has caused selection pressure to many populations of insects resulting to development of resistance. Currently, almost all classes of insecticides are involved in resistance (Miarinjara, 2016). There many reports of *X.cheopis* developing resistance against organochlorine, carbamates, organophosphates, pyrethroids and pyrethrins. In the beginning the rodent fleas were fully susceptible to DDT, but of recent they have developed resistance to most of the commonly used chemical insecticides (Biswas *et al.*, 2008). This is posing a threat and generates a further need to make a prior study on the insecticide susceptibility status of rodent fleas in the plague endemic areas (Gratz *et al*., 1999).

According to Miarinjara *et al*., 2017, *X.cheopis* populations from Malagasy prisons were resistant to at least seven insecticides out of twelve. Resistance to insecticides could be a serious challenge in plague foci.

The insecticide resistance can be adopted through different mechanisms such as; target size sensitivity this is based on three main insect central nervous system target sites namely; voltage-gated sodium channel, acetyl cholinesterase (AChE) in the cholinergic synapses and gamma amino butyric acid (GABA) sites in chloride channels at neuromuscular synapses.

The resistance to DDT and pyrethroids is conferred by a mutation in the voltage-gated sodium channel usually by a substitution of the amino acid leucine with phenylalanine at the same or proximate codon position in domain IIS6 of the protein. These substitutions confer knockdown resistance or, *kdr* (Davies *et al.,* 2007; Ames, 2011), also the resistance to organophosphates and carbamates is associated with insensitive AChE. This is achieved by structural modifications of the pesticide binding sites in AChE that allow at least partial binding to acetylcholine, this binding allows the AChE to break down the buildup of acetylcholine in the synapse and the synapse can function normally (Ames, 2011). Another mechanism is through metabolic resistance, this relies upon alteration of enzymes systems that arthropods use to detoxify foreign materials and/or preventing an insecticide from reaching its site of action. These occur with estarases, oxidases, oxgenases, hydrolases and glutathione-s transferases (Ferrari, 1996; WHO, 2012). Behavioral mechanism this explains the behavior of insects developing a tendency of moving away from treated surface or area, by doing so they become resistant. Resistance also can be acquired through cuticular mechanism in which the cuticle reduces the penetration and uptake of an insecticide (Ferrari, 1996).

## 1.7 Contribution of Agro-Veterinary Chemicals towards the Development of Insecticide Resistance in Public Health Arthropod Vectors

Due to massive problems caused by different groups of arthropod pests, there has been excessive deployment of chemical insecticides as a strategy in control. Indeed, the control of arthropod and several other types of pests worldwide relies overwhelmingly on the use of chemical insecticides. Unfortunately, these chemicals are in many cases used injudicious, thus exerting intense selective pressure, which has in turn led to the development of resistance to many important arthropod pests (de la Fuente *et al*., 2008; Rosario-cruz *et al*., 2016).

Often the thinking has been that, many cases of development of resistance, particularly in public health disease vectors, emanates almost exclusively from their recommended control chemicals. However, there has been increasing evidence of resistance developing in public health disease vectors as a consequence of excessive use and/or misuse of agro-veterinary chemicals. The reason being that, most of the public health insecticides were reformulated from the agricultural chemicals and therefore have related chemistries. Disease vectors can be exposed to insecticide used in agriculture from contamination of nearby breeding sites and any other contaminated surfaces (Yadouleton *et al*., 2011; Nvane *et al*., 2013; Nkya *et al*., 2014). Notably, many studies have demonstrated that mosquito larvae exposed to sub-lethal doses of pollutants, herbicides or agricultural pesticides frequently appear more tolerant to insecticides (Akogbeto *et al*., 2006; David *et al*., 2010). In Tanzania, field study comparing urban and low pollution agricultural pinpointed the elevated resistance level of *An. gambiae s.s* found in proximity of intensive agricultural field (Nkya *et al*., 2014). The same study revealed candidate resistance genes associated with agricultural pesticides (Nkya *et al*., 2014). Although most of the attention is on mosquitoes, many other arthropod disease vectors, for example flea vectors of plague, increasingly threatened by the developing resistance consequent to excessive and misuse of agro-veterinary chemicals. Consequent to free market economy, there has been an increasing trend in the application of agro-chemicals, particularly in sub-Saharan Africa, Tanzania inclusive. Agro-chemicals are applied on crop fields in large amounts than required.

Concurrently, excessive use, misuse and other malpractices are increasing because most farmers lack the basic knowledge on the appropriate and safe use of these chemicals. As such, a multitude of breeding/resting habitats, substrates and other forms of surfaces, often contacted by different public health arthropod vectors, get contaminated with such chemicals. Several studies have shown the inadequate knowledge related to pesticide use, misuse of pesticides (Mixing of different pesticide to increase efficiency, over use and under use of the recommended doses) and improper storage of the pesticides as well as mishandling and disposal of pesticides. Besides, serious insufficiency of extension officers with the relevant knowledge and experience on the use of these chemicals is still a problem particularly in Tanzania and many other African countries (Mekonnen and Agonafir, 2002; Ngowi *et al*., 2007; Nonga *et al*., 2011; Okonya and Kroschel, 2015; Jallow *et al*., 2017 and Mwabulambo *et al*., 2018).

Insecticide resistance is increasingly being reported in several plague endemic countries; surprisingly even in situations where there have not been targeted flea vectors control using recommended insecticides for extremely long period of time. This strongly suggests the involvement of agro-veterinary chemicals as a driver of resistance development in plague flea vectors, thus warranting the need for research towards that direction in plague foci in Tanzania and elsewhere.

## 1.8 Methods for Resistance Detection

Accurate identification of resistance is the most important strategy in management of vector resistance (Soderlund and Knipple, 2003; Nauen, 2007). There are three categories of detection methods: bioassays, biochemical and molecular genetics.

Bioassays of agricultural pests use topical application of the toxin directly on the insect's body and mortality is assessed using a predetermined LC50 dose. Field bioassays to test for phenotypic expression of resistance also include the standard World Health Organization adult susceptibility test (WHO, 2017) originally developed in 1963 or the CDC bottle bioassay (Brogdon and McAllister, 1998) as explained in Ames (2011). Biochemical tests are rapid, multiple assays that can be performed on single insects to detect generic activity potentially correlated with metabolic resistance. They detect the generic activity of insecticide metabolic enzymes. Earlier single insects yielded one assay but during 1980s and 1990 improvement was done from using single test tube to the microplate format which allows multiple enzymes assays on single insects.

Other assays developed were microplate –spectrophotometric analysis (Brogdon, 1984; Brogdon, 1988; Brogdon and Barber, 1990), high pressure liquid chromatography (Brogdon and Dicknson, 1983) and dot blot testing on single insects (Dary *et al.*, 1991). If the insecticide resistance is due to genetic mutation, then testing for genetic mutation frequency in a flea or tick population can indirectly measure for the level of resistance in that population. Polymerase Chain Reaction (PCR) assays have been developed to test individual fleas for the presence of gene mutation associated with resistance to pyrethroids the common knockdown resistance (kdr) mutations and super-kdr mutations (Bass *et al.*, 2004).

## 1.9 Problem Statement and Justification

Although relatively fewer plague cases and deaths are experienced now than in the past, the disease remains a highly important public health concern, particularly because of its ability to reoccur after several decades of quiescence. Large and devastating plague outbreaks re-occurred after 30 years in India (Ramalingaswami, 1995), 50 years in Algeria (Bertherat *et al.,* 2007; Bitam *et al.,* 2006), 25 years in Libya (Tarantola, 2009; ProMed, 2009) and 30 years in Mbulu Tanzania (Bertharat *et al.,* 2007; Makundi *et al.,* 2008). Plague pandemics across the mediaeval Europe caused over 50 million deaths, leave alone millions of others that occurred elsewhere (Drancourt and Houhamdi, 2006; Walloe, 2008; Hufthammer *et al.,* 2013). Therefore, challenges that are potentially threatening effectiveness of flea vectors control methods, the most powerful and reliable plague control approach we have to date need to be addressed in advance.

Otherwise, plague endemic areas that have remained quiescent may face more devastating plague outbreaks than those of the past. Recently, studies in Madagascar revealed resistance in flea vectors virtually against all available classes of insecticides (Miarinjara *et al.,* 2017). Despite the fact that many endemic countries are not carrying out regular flea control using chemical insecticides as it is in Madagascar, because of the absence of plague outbreaks, they are still at risk of insecticide resistance which could be driven by agricultural and veterinary use of chemicals (Chouaïbou, 2016). Despite of, such possibility, no study has been conducted so far in the plague endemic areas in Tanzania and elsewhere to confirm such risk and/or possibilities. Lushoto and Mbulu districts are one of those plague endemic areas that are likely to face this challenge due to excessive use of chemicals against agricultural and livestock arthropod pests. Therefore, the proposed study aimed at assessing susceptibility status of plague flea vectors to recommended classes of public health insecticides and thereof the contributions of agricultural and veterinary insecticides on development of insecticide resistance in plague foci in Tanzania.

## 1.10 Objectives

### 1.10.1 Main objective

To preliminarily the potential contribution of agricultural and veterinary agro-pesticides in the development of insecticide resistance in flea vectors of plague.

### 1.10.2 Specific objectives

i. To discover misuse and other forms of malpractices which could be enhancing the exposure of flea vectors of plague to commonly used agro-veterinary chemicals in Lushoto and Mbulu districts, Tanzania;
ii. To assess the resistance status of the major plague flea vector, xenopsylla cheopis in the study districts.

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# CHAPTER TWO

## 2.0 Malpractices in the use of agro-veterinary pesticides in plague endemic foci in Tanzania: potential risk for development of insecticide resistance in flea vectors

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**Abstract**

Malpractices associated with use of agro-veterinary pesticides are increasingly associated with resistance development in arthropod vectors. We used a questionnaire and direct observations to identify malpractices in the use of pesticides in plague endemic foci, northern Tanzania, which could predispose plague-flea vectors to resistance development. Malpractices in the use of pesticides were common in both districts. More than 80% of the respondents were applying agro-pesticides over 3-times per cropping season, did not adhere to manufacturers’ recommendations and had limited/no knowledge on safety procedures and adverse effects of the pesticides. Up to 49% of the respondents were applying pesticides over twice the recommended doses. The 3 out of 14 most commonly used agro-pesticides in Lushoto were master kinga72WP (mancozeb+cymoxanil), suracron720EC (profenos) and Sumo 5EC (lambda-cyhalothrin). The 3 out of 17 most commonly used agro-pesticides in Mbulu were Dursban50W (Chlorpyrifos), Duduban 450EC (Cypermethrin+chloropyrifos) and Dursban+farmerzeb. Cybadip (Cypermethrin) and paranex (alphacypermethrin) were the most commonly used veterinary pesticides. In conclusion, these results suggest high risk of contamination of environments or surfaces and exposure of flea vectors with agro-pesticides. Thus, warrant studies to show a causal link between misuse of agro-veterinary pesticides and development of resistance in fleas in endemic areas across Tanzania.

**Keywords:** fleas, malpractices, pesticides, resistance

## 2.1 Introduction

Plague is a life-threatening disease caused by a highly infectious bacterium, *Yersinia pestis*. The disease is transmitted to humans from infected rodents primarily by fleas [1]. More than eight flea species can transmit the bacteria [2]. The most common and widely distributed species include *Xenopsylla cheopis*, *Xenopsylla brasiliensis* and *Dinopsyllus lypusus* [3]. The first two species have played a significant role in most plague outbreaks due to their high transmission efficiency and broad host preference [4].

Measures targeted on flea vectors, mostly chemical insecticides, remain the most effective and widely used approach for controlling plague. This approach has significantly reduced plague in most if not all endemic countries; however, its long-term effectiveness is challenged by the development of resistance in flea vectors. Some countries have reported resistance in plague flea vectors virtually against all recommended classes of insecticide [5, 6].

Based on classical thinking, all cases of insecticide resistance in arthropod vectors including fleas are exclusively attributed to selection pressure from the public health pesticides. Implying that, all quiescent plague endemic areas without long-standing application of public health pesticides are not at risk of insecticide resistance. This assertion might put such areas in great danger during plague outbreaks, which are often unpredictable because there are increasing reports associating agrochemicals to development of resistance in disease vectors [7-10]. To the best of our knowledge, no study has been conducted to assess malpractices of agro-veterinary pesticides in plague endemic foci in Tanzania which potentially predispose plague flea vectors to resistance development. Small-scale farmers in different parts of Tanzania are increasingly reported to misuse agro-veterinary pesticides, thus predisposing non-targeted organisms and ecosystems to pesticides-associated negative impacts [9, 10, 11-12]. Therefore, this study identified malpractices in the use of agro-veterinary pesticides which potentially enhance contamination of environments/surfaces and exposure of flea vectors to such pesticides. The long-term exposure of plague flea vectors to agro-veterinary pesticides could purportedly select for insecticide resistance. The results would warrant for subsequent studies to confirm a causal link between identified malpractices and development of resistance in such vectors under realistic settings.

## 2.2 Materials and Methods

### 2.2.1 Study area

This study was done in the selected villages of Lushoto and Mbulu district, Southern Tanzania (Figure 2.1). Lushoto district in Tanga region is situated at the West Usambara Mountains, which forms part of the Eastern Arc Mountains (04$^{o}$22'-05$^{o}$08'S, 038$^{o}$05'-038$^{o}$38'E). The district lies at the altitude of 900 to 2,250 m above the sea level. The long rain season runs from March to May; and short rain season runs from November to December, with a mean annual rainfall of 1070mm and temperature of 17$^{o}$C. The dry season runs from July to October. The district has a population of 492,441 people. Mbulu District in Manyara region is bordered to the north by Arusha region and lake Eyasi; and to the west by Singida region (3$^{o}$57'1"S, 35$^{o}$18' 40"E). It covers a total surface area of approximately 3,800km$^2$; and lies between 1000-2400 m above the sea level. The long rain season runs from January to May; and short rain season runs from November to December, with a mean annual rainfall of 994mm and temperature of 17.5$^{o}$C. The dry season runs from June to October. The district has a population of 320 279 people.

### 2.2.2 Study population

The study was done in four (Viti, Lukozi, Ndabwa and Mavumo) and two (Arri and Mongahay) villages in Lushoto and Mbulu district respectively. All the six villages had a history of a plague outbreak. Crop cultivation is the main economic activity both in Lushoto and Mbulu district. The main crops in Lushoto district include maize, beans, potatoes, vegetables and fruits. The main crops include maize, beans, potatoes, wheat and vegetables. Besides, the communities in Mbulu district are actively involved in livestock keeping.

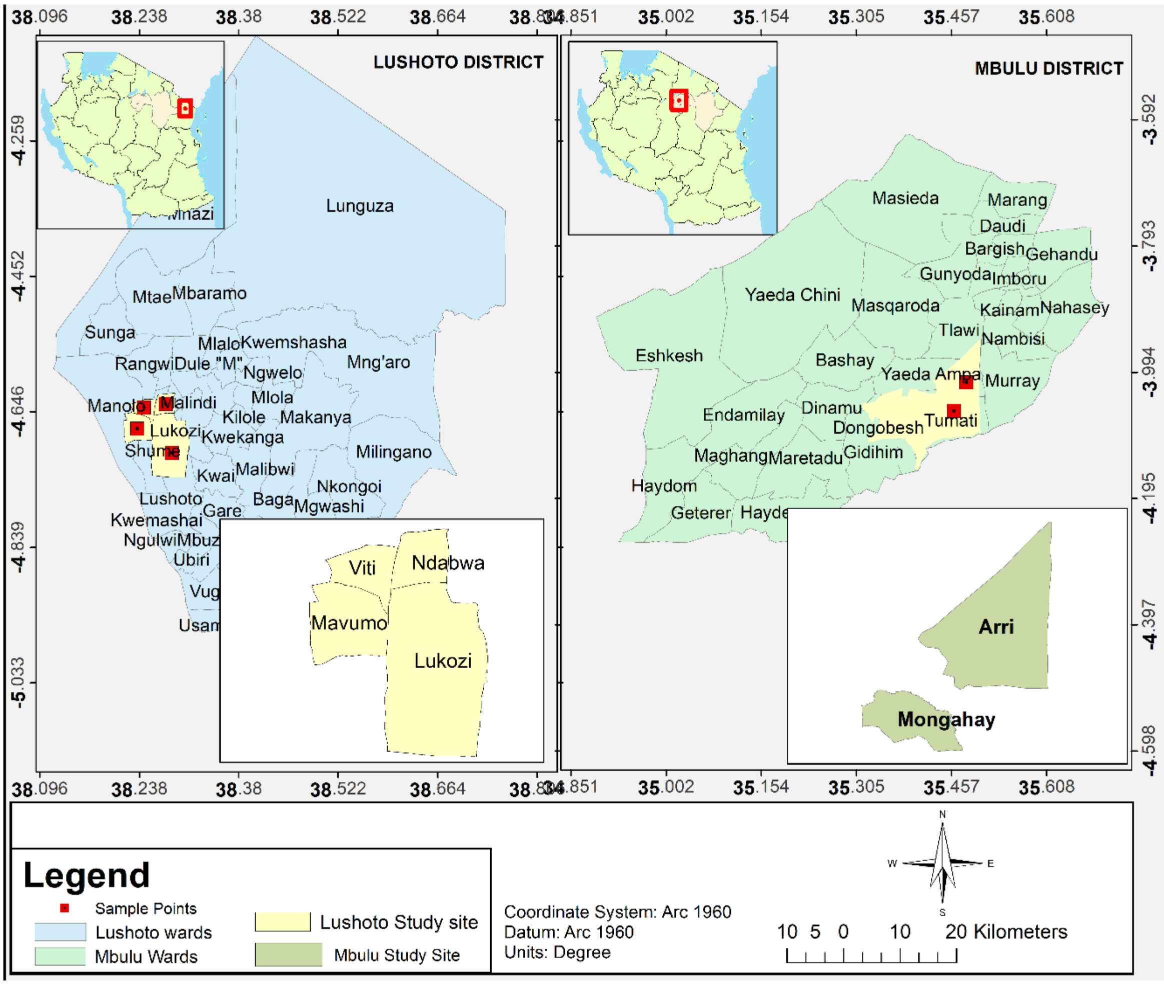


**Figure 2.1:** A map of Lushoto (left) and Mbulu district (right) showing villages in which the study was conducted

### 2.2.3 Data collection

The study villages were selected using a purposeful sampling technique based on two main criteria: having experienced plague outbreaks at least twice and proximity to active and/or fallow agricultural fields. Two hundred (200) randomly selected households, 100 per district, were used for this study. Salespersons from four agro-vet shops within each district were selected based on their involvement in such business within the last two years and above. Data collection was done using a standard questionnaire with structured and semi-structured questions.

The questionnaire was written in English and translated into Kiswahili, a national language which was understood by all of the respondents in the study areas. Only one person per household/agro-vet shop was interviewed after a verbal consent. The questionnaire was supplemented with direct observations to confirm certain responses where participants could provide evidence, for example, presenting containers of the pesticides they were using and demonstrating the preparation of spray solutions. The survey aimed at gathering information related to the use of agro-veterinary pesticides: following manufacturers' application instructions and safety procedures, and routine practices in terms of commonly used pesticides, frequency of applying pesticides, use of agricultural pesticides on livestock and vice versa, compliance with applicable safety procedures, adherence to manufacturers recommended doses and keeping animals inside living houses.

#### 2.2.4 Data analysis

The data were summarized and analyzed by using Epidemiological Information Epi Info™ version 7.2.3.1(CDC) and Excel to obtain appropriate percentages and frequencies. These descriptive results are presented in figures and tables.

### 2.3 Results and Discussion

#### 2.3.1 Socio-demographic characteristics

Majority of the respondents were males, 55(55%) in Lushoto and 58(58%) in Mbulu. Majority of the respondents were below 35 years of age followed by those of 36 and 45 years of age in Lushoto and Mbulu respectively. Most respondents in Lushoto (n = 71, 71%) and Mbulu (n = 83, 83%) had primary education. The proportion of respondents with secondary education was higher in Lushoto (24%) than Mbulu (14%). Only three (n=3) respondents across the study districts had a college education. The remaining respondents, 3(3%) in Lushoto and 2(2%) in Mbulu had formal education (Table 2.1). Farmers with primary education only are mostly unable to understand the manufacturer's labeling and instructions, particularly because they are written in English. Possibly due to illiteracy and cost, farmers in certain parts of Tanzania agree to buy and use pesticides without seeing the original packages, as pesticides were usually sold by weight or already diluted without labeling [13]. These increase the likelihood of pesticides misuse and risk of undesirable effects to the environment and non-targeted organisms. Arguably, the assurance that farmers and other end-users acquire the desirable knowledge and instructions from salesmen and extension workers would minimize the chances of malpractices and anticipated risks. Unfortunately, such extension services are either lacking or untimely across many rural communities including the current study area due to shortage of extension officers [14, 15]. Also, studies have reported poor quality services in most salesmen and extension offices across the country because they are either incompetent or did not undergo relevant training [15].

| **Category** | **Percentage of respondents in Lushoto district (%)** | **Percentage (%) of respondents in Mbulu district (%)** |
|---|---|---|
| Male | 55 | 58 |
| Female | 45 | 42 |
| Age categories | | |
| Less than 35 | 41 | 20 |
| 36 to 45 | 25 | 52 |
| 46 to 55 | 21 | 18 |
| More than 55 | 13 | 10 |
| Education level | | |
| Formal | 3 | 2 |
| Primary | 71 | 83 |
| Secondary | 24 | 14 |
| College/University | 2 | 1 |

**Table 2.1:** Socio-demographic characteristics of respondents from Lushoto and Mbulu district (each n=100)

### 2.3.2 Common agricultural pesticides and malpractices

Most respondents, 91% in Lushoto and 93% in Mbulu reported using pesticides against crop pests during the 3-months cropping season. The rest, 9% in Lushoto and 7% in Mbulu reported that they were not using pesticides at all. The overreliance in chemical pesticides against insect pests of crops, livestock and humans is common in Tanzania and the rest of the world [16-18]. However, this is not surprising because pesticides remain the most efficient, cheap and widely accessible measure for control of insect pests [18, 19-20]. About one-third of agricultural, forestry and livestock production losses worldwide annually are associated with insect pests [19]. Because of that concern, crop production in Tanzania has correspondingly increased in tandem with the use of large quantities of pesticides.

Since 1992, the use of pesticide has rapidly increased following agrochemical trade liberalization in the country [11]. From then on, the availability and access to pesticides including unregistered products have erratically increased throughout the country [11]. This study documented over 14 brands of agro-pesticides. These agro-pesticides belonged to five main classes including carbamates, pyrethroids, organophosphates, organochlorines and avermectins. However, pyrethroids were the most predominant pesticides. In Lushoto, the most commonly used pesticides were master kinga 72WP (mancozeb 640g/kg+cymoxanil 80g/kg) (44%), suracron 720EC (profenos 500g/l EC) (25.3%) and Sumo 5EC (lambda-cyhalothrin) (18.7%) (Table 2.3). In Mbulu, the most commonly pesticides were Dursban50W (chlorpyrifos) (29%), Duduban 450EC (Cypermethrin 10g/lt+chlorpyrifos 35g/lt) (18%) and Dursban+farmerzeb (Chlorpyrifos 48%, Mancozeb 80%WP) (Table 2.4). These and other pesticides documented in the present study are also common elsewhere in the country [15, 17, 21].

Most chemical products used against fleas and other arthropod vectors belong to the first four classes, but pyrethroids are the most widely spread and used. This suggests a high likelihood of cross-resistance in flea vectors emanating from agro-pesticides commonly used in the study area, particularly when such chemicals are applied in ways that enhance exposure. Most public health pesticides are reformulations of pesticides that were once used in agriculture [22]. This emphasizes the assertion that on-going use of agricultural and veterinary chemistries similar to those used against fleas poses a threat to resistance development.

This study documented several malpractices related to the use of agro-veterinary pesticides, all of which enhance the possibility of exposing fleas to those chemicals. Excessive use of pesticides within a 3-month cropping season was common across the study districts. In Lushoto, the majority (n=73; 80%) applied pesticides more than three times per cropping season. In Mbulu likewise, the majority applied pesticides two (n=39; 42%) or more than three times (n=35; 38%) per cropping season (Fig. 2.2). Although all respondents reported to had acquired the necessary knowledge on the type, handling and applications of pesticides from the salesmen and extension officers, the majority did not adhere to the application and safety instructions indicated by the manufacturers. 49 (53.4%) respondents in Lushoto and 39 (42%) respondents in Mbulu applied more than twice the recommended doses. The rest, 18 (19.8%) in Lushoto and 25 (27%) in Mbulu (Fig. 2.2), claimed to apply recommended doses; however, they were unable to demonstrate that. As such, agricultural fields are excessively contaminated with such pesticides thus potentially increasing the likelihood of exposing flea vectors while on contaminated rodents and/or burrows. During rains, soils in rodent burrows and other habitats become contaminated via run-off of pesticides. Studies have reported alarming contamination of soils, water, air and other types of environments with agricultural chemicals [23-25]. Larval and adult fleas are predominantly associated with rodents and therefore they are mainly found on loose soils of rodent burrows in agricultural fields and other environments. Consequently, the fleas coincidentally contact untargeted pesticides and other control agents. Resistance to various chemicals in rodent fleas may be selected either at the larval or adult stage. Gratz [26] reported that indoor residual spraying (IRS) with DDT against mosquitoes affected the susceptibility of plague flea vector *Xenopsylla cheopis*. Oftentimes farmers get tempted to use agricultural pesticides as alternatives against fleas and other public health pests because they are comparatively cheaper. Our co-workers witnessed such practices during their previous visits to the present study areas. Switching of pesticides to unintended uses is also common elsewhere [27], and thus increases the risk of exposure and selection for resistance in fleas.

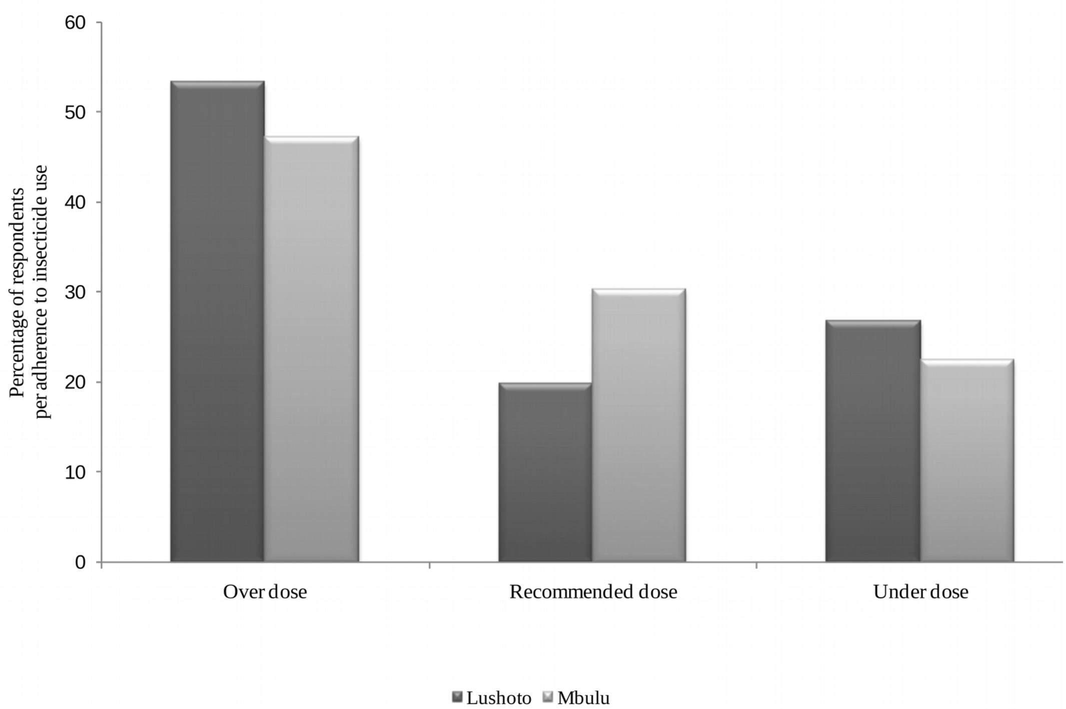


**Figure 2.2:** The respondents' adherence to manufacturers recommended doses of agricultural pesticides in Lushoto and Mbulu district

**Table 2.2:** Commonly used agricultural pesticides in Lushoto district (n = 91)

| Class of pesticide | Trade name | Active ingredient | Percentage of respondents (%) |
|---|---|---|---|
| Carbamates | Master kinga72WP | Mancozeb 640g/kg+Cymoxanil 80g/kg | 44 |
| Organophosphates | Suracron 720EC | Profenos 500g/l EC | 25.3 |
| Pyrethroids | Sumo 5EC | Lambda-cyhalothrin | 18.7 |
| Organophosphates | Profecron 720 EC | Profenos 500g/l EC | 15.4 |
| Pyrethroids | Karate 5EC | Lambda-cyhalothrin | 15.4 |
| Pyrethroids | Ninja plus 5%EC | Lambda-cyhalothrin | 14.3 |
| Avermectins/neonicotinoids | Dudumectin | Emamectin 4.8% + acetameprid 6.4% | 14.3 |
| Carbamates | Farmerzeb 80WP | Mancozeb 80%WP | 11 |
| Carbamates | AMSAC | Indoxacarb 14.5%SC | 7.6 |
| Carbamates | Indofil M45 | Mancozeb 80% | 4.4 |
| Pyrethroids | Kungfu | Lambda-cyhalothrin | 4.4 |
| Organochlorines | DDT | | 3.3 |
| Organophosphates | Wilcron | Profenos 720EC | 3.3 |
| Carbamates | Dithane M55 | Mancozeb 80% | 2.1 |

| Class of pesticide | Trade name | Active ingredient | Percentage of respondents (%) |
|---|---|---|---|
| Organophosphates | Dursban | Chlorpyrifos48% | 29 |
| Pyrethroids/ organophosphates | Duduban | Cypermethrin 10g/lt+chloropyrifos 35g/lt | 18 |
| Organophosphates/Carbamates | Dursban, farmerzeb | Chlorpyrifos48%, Mancozeb 80%WP | 9 |
| | No pesticide use | | 9 |
| Pyrethroids/Organophosphates | Duduban, ninja | Cypermethrin 10g/lt+chloropyrifos 35g/lt | 8 |
| Carbamates | Farmerzeb | Mancozeb 80%WP | 6 |
| Pyrethroids | Ninja plus | Lambda-cyhalothrin 50g/lt | 5 |
| Pyrethroids/Organophosphates | Duduban, dursban | Cypermethrin 10g/lt+chloropyrifos 35g/lt, Chlorpyrifos 48% | 2 |
| Pyrethroids/Organophosphates /Carbamates | Duduban, farmerzeb | Cypermethrin 10g/lt+chloropyrifos 35g/lt, Mancozeb 80%WP | 2 |
| Pyrethroids/organophosphates | Karate, Dursban | Lambda-cyhalothrin, Chlorpyrifos 48% | 2 |
| Avermectins/neonicotinoids | Dudumectin | Emamectin 4.8%+ acetamiprid 6.4% | 1 |
| Organophosphates/Pyrethroids | Dursban, karate | Chlorpyrifos48%, Lambda-cyhalothrin | 1 |
| Carbamates/ Organophosphates | Farmerzeb, duduban Dursban | Mancozeb80%WP,cypermethrin 10g/lt+chloropyrifos 35g/lt, Chlorpyrifos48% | 1 |
| Carbamates/Pyrethroids | Farmerzeb, ninja | Mancozeb 80%WP, Lambda-cyhalothrin | 1 |
| Pyrethroids | Karate | Lambda-cyhalothrin | 1 |
| Pyrethroids/Organophosphates | Karate, duduban | Lambda-cyhalothrin, cypermethrin 10g/lt+chloropyrifos 35g/lt | 1 |
| Pyrethroids | Karate, ninja | Lambda-cyhalothrin | 1 |
| Carbamates | Thionix | Zinc dimethyl dithiocarbamate 98.2% | 1 |

**Table 2.3:** Commonly used agricultural pesticides against crop pests in Mbulu district (n = 93)

### 2.3.3 Common veterinary pesticides and risk of flea exposure

Three veterinary pesticides in Lushoto and five in Mbulu district were used for control of livestock pests and all of them were pyrethroids. In Lushoto, the most commonly used pesticides were cybadip (cypermethrin) followed by paranex (alphacypermethrin) and tick-tick (permethrin) (Fig. 2.3). The first two were by far the most commonly used pesticides in Mbulu (Fig. 2.4). These pesticides were mainly used on goats and cattle against ticks. Most respondents (95%) did not adhere to the manufacturer's recommended doses, safety procedures and frequency of application. These chemicals were only applied during excessive infestation and in doses far less than recommended, possibly due to their high cost. Like elsewhere in Tanzania, cans and other leftovers of these pesticides were disposed of haphazardly [15] thus enhancing contamination of the environment and other surfaces. As such, fleas can be exposed to those chemicals, most likely on lower doses than required, either on treated animals and/or contaminated soils/surfaces. Livestock serve as alternative blood-meal sources for fleas [28]. Exposure through animals is potentially enhanced by frequent interactions between commensal rodents and domestic animals. These interactions are frequent in areas where animals are kept inside living houses during the night. The tendency of keeping livestock inside living houses during the night was observed in Mbulu during the current study.

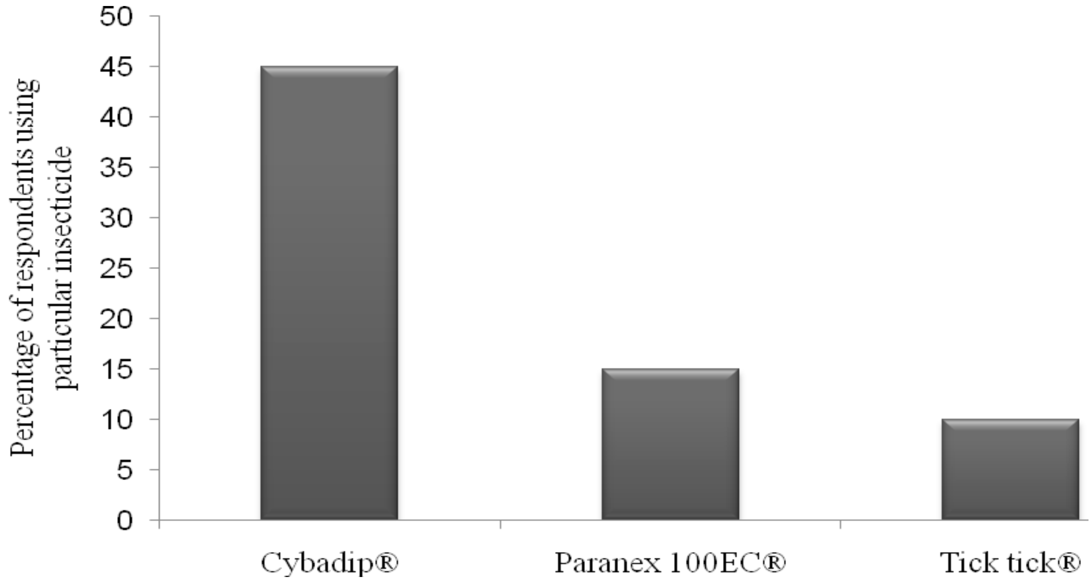


**Figure 2.3:** Veterinary pesticides commonly used against livestock pests in Lushoto district: Cybadip® (cypermethrin 15%m/v), Paranex100EC® (alphacypermethrin) and Tick tick® (permethrin). All of them are pyrethroids.

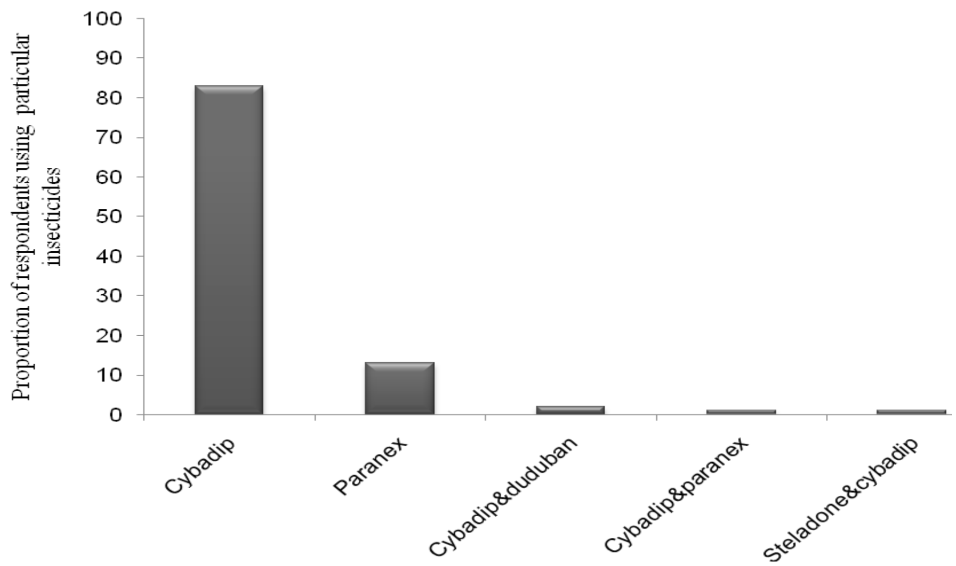

**Figure 2.4:** Veterinary pesticides commonly used against livestock pests in Mbulu district: Cybadip® (cypermethrin 15%m/v), Paranex® (alphacypermethrin), Cybadip® & Paranex® and Steladone® & cybadip®. The last two sets of insecticides represent cases where respondents reported to be using the two insecticides interchangeably.

### 2.3.4 Common pesticides against fleas

The majority of pesticides that respondents reported to use in Lushoto and Mbulu belonged to two main classes: pyrethroids and carbamates (Table 2.4). However, like in agro- and veterinary-pesticides, pyrethroids were the most predominant class. Synthetic pyrethroids have become the most popular and prevalent active ingredients for public health use due to their relatively low mammalian toxicity but high invertebrate potency at low levels, resulting in rapid immobilization ('knockdown’) and killing [29].

The animals, mainly calves and goats, were only sprayed on body parts preferred by the fleas using small portable hand sprayers. The powder formulation of carbamates was sprinkled inside living houses with hands. The application of flea control pesticides in study districts was restricted indoors on a few parts of the house and was done exclusively during the dry season when flea abundance was intolerably high. Even during that period of high infestation, only a few respondents reported affording chemical pesticides. Alternatively, they poured hot water on the floors of their living houses. Therefore, the use of deployment of specific flea control pesticides was probably inadequate to select for resistance.

**Table 2.4:** Commonly used pesticides against fleas in Mbulu and Lushoto district

| District | Class of pesticide | Trade name | Active substance | Percentages of respondents (%) |
|---|---|---|---|---|
| | Pyrethroids | Cybadip | Cypermethrin | 42 |
| | | No Pesticide use | | 16 |
| | Carbamates | Dudu dust | Carbaryl 75g/kg | 14 |
| | Organophosphates | Dursban | Chlorpyrifos 48% | 12 |
| Mbulu | Pyrethroids | Paranex | Alphacypermethrin | 8 |
| | Carbamates | Akheri powder | Carbaryl5%w/w+Lambdacyhalothrin 0.1%w/w | 5 |
| | Pyrethroids | Cybadip, dudu dust | Cypermethrin, Carbaryl 75g/kg | 1 |
| | Pyrethroids/Organophosphates | Cybadip Dursban | Cypermethrin, Chlorpyrifos 48% | 1 |
| | Carbamates/Pyrethroids | Akheri powder | Carbaryl 15%w/w + Lambda cyhalothrin 0.1w/w | 4 |
| Lushoto | Pyrethroids | Paranex100EC | Alphacypermethrin | 2 |
| | Pyrethroids/Organophosphates | Duduban | Cypermethrin 10g/lt+chloropyrifos35g/lt | 1 |

## Conclusions

Any forms of malpractices which enhance the exposure of plague vectors to agro-veterinary pesticides constitute risk factors for resistance development. The present study documented a suite of malpractices including abnormally high frequency of agro-pesticide application, use of abnormally high doses of agro-pesticides and poor adherence to the application, disposal and safety procedures for agro-veterinary pesticides. These malpractices/injudicious uses are potentially enhancing contamination of environments/surfaces and exposure to flea vectors (thereof). As such, flea vector populations across the two plague endemic districts are purportedly in a high risk of developing insecticide resistance from agro-veterinary chemicals.

The results warrant studies to confirm a causal link between malpractices on agro-veterinary pesticides and the development of resistance in fleas across plague endemic foci across Tanzania.

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# CHAPTER THREE

## 3.0 Susceptibility of *Xenopsylla cheopis* (Family: Pulicidae) to agro-veterinary and public health insecticides commonly used in plague endemic foci in Tanzania

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**Abstract**

The control of rodent-borne fleas, which play major role in plague transmission, is the mainstay of plague control. The application of chemical insecticides forms the most powerful and widely used control approach. The reliability and continued use of this approach however is dictated by the insecticide susceptibility status of the targeted populations of main flea vectors. Unfortunately, no survey has been conducted across plague endemic foci in Tanzania, despite the increasing risk of insecticide resistance emanating from the injudicious deployment of use of agro-veterinary and public health insecticides. We assessed the susceptibility of *Xenopsylla cheopis*, originating from the wild population in Mbulu district, to commonly used agro-veterinary and public health insecticides by using bioassay test. The percentage mortality after exposure to recommended doses of nine insecticides tested was strongly suggestive of resistance

(100% 24 h mortality, 93–96%). The fleas confirmed resistance to lambdacyhalothrin &carbaryl, with 90% mortality.

The reference ‘susceptible’ colony *Xenopsylla Cheopis* was fully susceptible (100% 24 h mortality) to all tested insecticides. Similarly, the field *Xenopsylla Cheopis* was fully susceptible (100% 24 h mortality) to 5× and 10× the recommended doses of all insecticides indicating low resistance intensity. In conclusion, results of the present study indicate resistance in wild population of *Xenopsylla cheopis* from Mbulu district. Although these fleas were fully susceptible at higher doses of the insecticides, the observed resistance status warrants further extensive research in Mbulu and other plague endemic areas in the country to understand the distribution, involved resistance mechanisms and confirm the contribution of agro-veterinary insecticides.

**Keyword:** Agro-veterinary insecticides, resistance, *Xenopsylla cheopis,* plague, Mbulu district.

### 3.1 Introduction

The interactions that rodents and other wildlife carnivores have with livestock and humans enhance transmission of many pathogens including bacteria, viruses, helminthes and protozoa (Cleaveland et al. 2001; Sathaporn et al. 2008; Mikhali et al. 2016). Several of these pathogens are potentially transmissible to humans via rodent-borne fleas and other ectoparasites. Plague, caused by a gram negative bacterium *Yersinia pestis,* is one of the most severe diseases spread by fleas (Neerinck et al. 2010; Abedi et al. 2018). Plague is one of the few diseases that are still subject to international health regulations and notifiable to the WHO (Lotfy, 2015). The disease is normally associated with devastating health and socio-economic consequences; more so when it is not intervened timely. In the past, plague caused millions of deaths. Plague caused 50 million deaths across medieval

Europe. Although fewer cases are currently reported, plague could cause outbreaks that are as devastating as those of the past if control challenges are not addressed appropriately. With the growing trend of climate change, land-use change and other anthropogenic changes, the role of fleas in transmitting zoonotic diseases other than plague could be increasing, particularly in Africa. On-going studies, more so in wildlife-human interfaces, are unveiling novel rodent-borne viruses, which could be passed to susceptible hosts by fleas and/or other rodent-borne ectoparasites.Although human plague could also be transmitted by other means, majority of human infections occurs through flea bites (Tikhomirov, 1999; WHO, 2008; Ziwa et al. 2013); and rodents form major source of the plague bacteria. Over 80 flea species are capable of harboring plague bacteria; however, their inherent efficiency in transmitting the pathogen varies considerably (Gage et al. 2005). The oriental rat flea, *Xenopsylla cheopis,* is considered the most efficient vector throughout plague endemic regions. This species also serves as the main plague vector across plague endemic foci in Tanzania (Ziwa et al. 2013). Other important flea vectors in Tanzania and elsewhere are *Xenopsylla brasiliensis, Dinosyllus lypusus and Pulex irritans* (Eisen et al. 2012; Ziwa et al. 2013). Due to the overwhelming role of fleas in transmitting plague, control of the disease relays on interventions which target such vectors (Borchet et al. 2010; Halos et al. 2014; Rust 2014; Durden and Hinkle, 2019). Despite the commendable efforts, there is no vaccine available yet that is effective against plague. Similar to other important arthropod disease vectors, the control of fleas is almost exclusively reliant on the use of chemical insecticides (Dryden et al. 2013; Coles and Dryden, 2014; Halos et al. 2014; Naqqash et al. 2016).

Although the deployment of chemical insecticides has remarkably contributed to the reduction of vector-borne disease burden including plague, the effectiveness of this approach is constrained by several challenges, the most important of which is the development of resistance (Heimbach and Muller, 2013; Miarianjara and Boyer, 2016) The intensive use of insecticides cause selection pressure on insect populations, which correspondingly develop mechanisms to survive insecticide treatments.

To date, all classes of insecticides are threatened by development of resistance (Biswas et al. 2008; Wojciechowska et al. 2016; Reid and Mckenzie, 2016; Rosario-Cruz and Dominguez, 2016).

The lack of new insecticidal compounds and the misuse or overuse of insecticides is among the reasons for development of insecticide resistance in pests (Chareonviriyaphap et al. 2013; Sparks, 2014). The first cases of insecticide resistance in *X. cheopis* to DDT were described in Madagascar in 1965 (Boyer et al. 2014). Another case of *X. cheopis* resistance to Malathion, fenitrothion and propoxur was reported in 1984 (Boyer et al. 2014). Following the long-term use of pyrethroids and other classes of insecticides recent reports have shown resistance in *X. cheopis* to 12 insecticides belonging to organophosphates, organochlorine; carbamates and pyrethroid families (Boyer et al. 2014; Miarinjara and Boyer, 2016). The insecticide resistance reported so far is entirely associated with indiscriminate use of insecticides against flea vectors. Studies in other arthropods, however, are increasingly associating evolution of resistance with agricultural and veterinary use of chemicals (Reid and McKenzie, 2016). Despite the possibility of similar scenario in flea vectors of plague, no study has been conducted so far in the plague endemic areas, to confirm such risk. Besides, no study has been conducted in the country after many years to determine resistance in field populations of flea vectors in plague endemic foci in Tanzania. The proposed study aims at assessing susceptibility status of plague flea vectors to commonly used agro-veterinary and public health insecticides in view of establishing at least presumptively whether or not agro-veterinary pesticides could be playing a role in the development of resistance.

## 3.2 Materials and Methods

### 3.2.1 Establishment of flea colony for susceptibility testing

Fresh colony of fleas used in this study was established from eggs of wild fleas collected from Mongahay and Arri village within Mbulu district. The two villages were selected based on previous history of plague outbreak and active involvement in agriculture and/or livestock keeping. The wild fleas were obtained from rodents collected from the agricultural fields and inside houses. Rodents from the agricultural fields were collected by means of baited Sherman traps (H.B. Sherman traps, Inc., USA). Rodent trapping was done in 8 agricultural fields, 4 per village, for three consecutive nights (Table 3.1). Domestic rodents were collected from 10 purposefully selected households in each village using box traps, one per house. Fleas were combed off rodents using fine hand brushes. The collected fleas were then placed transparent plastic containers (3000mls) containing sterile sand, dry cattle blood and live laboratory white mice; and maintained under room conditions for a maximum of three days to allow them to lay eggs. Afterwards, the containers with flea eggs were transported and reared at the Sokoine University of Agriculture, Pest Management Centre (SPMC) insectary. The field collected adults were stored in 70% alcohol and identified to species level by means of stereo-microscope with the Duchemin systematic (Duchemin, 2003). In the insectary, the fleas were maintained at 70%-85% relative humidity and 25°C to 31°C temperature until we obtained the required number of experimental adult fleas. Adult fleas were fed on laboratory reared white mice after every 3-4 days.

**Table 3.1**: Location, approximate sizes of field and number of traps distributed per field

| **Agricultural field** | **Size l/w(feet)** | **No. of traps distributed (5ft between traps)** |
|---|---|---|
| Fields in Mongahay | | |
| Field 1 | 50/20 | 25 |
| Field 2 | 40/18 | 25 |
| Field 3 | 65/20 | 30 |
| Field 4 | 35/18 | 20 |
| Fields in Arri | | |
| Field 1 | 50/25 | 25 |
| Field 2 | 40/20 | 20 |
| Field 3 | 40/50 | 25 |
| Field 4 | 60/30 | 30 |

### 3.2.2 Agro-veterinary and public health insecticides used for susceptibility testing

Fleas were tested against different agro-veterinary and public health insecticides which are commonly used in the study districts (Table 3.2). We selected insecticides that are most commonly used across the villages where the fleas were collected from (Rugalema and Mnyone, unpublished data). The selected insecticides belong to three main classes: pyrethroids, organophosphates and carbamates. These pesticides were tested at three different doses: recommended dose, 5× and 10× the recommended dose (Table 3.2).

**Table 3.2:** List of agro-veterinary and public health insecticides tested against adult fleas

| Chemical classes | Chemical name(s) | Active substance(s) | Rec. dose | 5 times rec. dose | 10 times rec. dose |
|---|---|---|---|---|---|
| Pyrethroids | Diknet® | Lambdacyhalothrin | 17.5µl/5ml | 87.5µl/5ml | 175µl/5ml |
| Pyrethroids & Organophosphates | Duduban® | Cypermethrin and Chlorpyrifos | 7.5µl/5ml | 37.5µl/5ml | 75µl/5ml |
| Organophosphates | Dursban® | Chlorpyrifos | 25µl/5ml | 125µl/5ml | 250µl/ml |
| Avermectins & neonicotinoids | Dudumectin® | Abamectin and Acetamiprid | 0.75µl/5ml | 3.75µl/5ml | 7.5µl/5ml |
| Phenylpyrazole | Fiprofarm® | Fipronil | 10µl/5ml | 50µl/5ml | 100µl/5m |
| Pyrethroids | Akheripowder® | Lambdacyhalothrin and Carbaryl | 2.5g/5ml | 12.5g/5ml | 25g/5ml |
| Carbamates | LAVA® | Dichlorvos | 25µl/5ml | 125µl/5ml | 250µl/5ml |
| Pyrethroids | Paranex® | Alphacypermethrin | 2.5µl/5ml | 12.5µl/5ml | 25µl/5ml |
| Pyrethroids | Cybadip® | Cypermethrin | 5µl/5ml | 25µl/5ml | 50µl/5ml |

### 3.2.3 Treatment of glass tubes with test insecticides

The test insecticides were dissolved in 5 ml acetone inside the sterilized glass tubes (50.3 mm long by 30 mm wide). The different doses of each insecticide (recommended dose, 5× and 10× the recommended dose) were prepared in three replicates. After obtaining homogeneous solutions, the tubes were hand rotated gently until their entire inner surfaces became coated with the respective insecticide. Afterwards, the glass tubes were maintained in upright position for about 15 min until acetone evaporated and left uniformly distributed residues of the pesticides. The control vials were treated with acetone, rotated and left to dry the same way as above.

### 3.2.4 Exposure of fleas to insecticide-treated glass tubes

Adult *Xenospylla cheopis* were introduced into insecticide-treated and control glass tubes, 10 fleas per tube, left for 1 hour and thereafter transferred to clean glass tubes (50.3 mm long by 30 mm wide) where they were monitored for 24-hour mortality. During the 1-hour exposure, fleas were monitored for knockdown. During and after exposure, the tubes containing fleas were maintained in upright position under ambient temperature and humidity. All pesticides were tested against the reference ‘susceptible’ colony and fresh field colony of *Xenopsylla cheopis*. The reference colony originated from Lushoto district, Tanzania and was established about 12 years ago. Mortality was assessed after 24 hours. A flea was considered dead if it was not moving or could not right itself when probed.

### 3.2.5 Interpretation of results of insecticide susceptibility tests

All data were entered into Microsoft excel and percentage mortalities of fleas exposed to individual insecticides and controls calculated. All fleas that died within 24 hours after exposure to insecticide-coated glass tubes were considered susceptible to the test insecticide. The fleas that still survived after 24 hours were assumed to have resistant traits. Specifically, the data were interpreted as follows: deaths ≥ 98% indicates susceptibility; deaths between 90% and 97% suggests presence of resistance; and deaths below 90% confirmed resistance (Brogdon and Chan, 1998; WHO, 2016). The Chi square to compare the percentage mortalities across the flea species (laboratory and field colony) and different insecticide was run.

### 3.3 Results

Susceptibility status of reference 'susceptible' and field colony of *Xenopsylla cheopis* was assessed against 9 commonly used insecticides. The percentage mortality 24 hours after exposure ranged from 90% to 100% (Table 3.3). The reference 'susceptible' colony *Xenopsylla Cheopis* was fully susceptible (100% 24 hours mortality) to all tested insecticides. Similarly, the freshly established 'field' colony of *Xenopsylla Cheopis* was fully susceptible (100% 24 hours mortality) to 5× and 10× the recommended doses of all insecticides. However, the percentage mortality of this field colony of *Xenopsylla Cheopis* after exposure to recommended doses of nine insecticides were suggestive of resistance (100% 24 hours mortality, 93 - 96%). Out of these eight insecticides, the percentage mortality was lower (93% mortality) in fleas exposed to three insecticides (chlorpyrofos, fipronil, and cypermethrin) relative to those exposed to the remaining five insecticides (96% mortality, lambdacyhalothrin, cypermethrin&chlorpyrifos, abamectin and acetameprid, Dichlorovos - LARVA and Dichlorovos-Paranex). The percentage mortality of 90% in fleas exposed to lambdacyhalothrin&carbaryl confirmed resistance. The results are summarized in Table 3.3. There was significant difference between percentage mortality of the reference colony and that of the field colony ($X^2$=10.8, d.f.=1, p=0.0128). There was no significant difference across the insecticides, ($X^2$=18, d.f.=8, p= 0.803).

| Insecticide | Test dose | Percentage Mortality (%) | | Resistance status of field colony |
|---|---|---|---|---|
| | | Reference colony | Field colony | |
| Lambdacyhalothrin | Recommended dose | 100 | 96 | R |
| | 5× | 100 | 100 | S |
| | 10× | 100 | 100 | S |
| Cypermethrin and Chlorpyrifos (Duduba) | Recommended dose | 100 | 96 | R |
| | 5× | 100 | 100 | S |
| | 10× | 100 | 100 | S |
| Chlorpyrifos (Dursban) | Recommended dose | 100 | 93 | R |
| | 5× | 100 | 100 | S |
| | 10× | 100 | 100 | S |
| Abamectin and Acetameprid (Dudumectin) | Recommended dose | 100 | 96 | R |
| | 5× | 100 | 100 | S |
| | 10× | 100 | 100 | S |
| Fipronil (Fiprofam) | Recommended dose | 100 | 93 | R |
| | 5× | 100 | 100 | S |
| | 10× | 100 | 100 | S |
| Lambdacyhalothrin and Carbaryl | Recommended dose | 100 | 90 | R |
| | 5× | 100 | 100 | S |
| | 10× | 100 | 100 | S |
| Dichlorvos (LAVA) | Recommended dose | 100 | 96 | R |
| | 5× | 100 | 100 | S |
| | 10× | 100 | 100 | S |
| Dichlorvos (Paranex) | Recommended dose | 100 | 96 | R |
| | 5× | 100 | 100 | S |
| | 10× | 100 | 100 | S |
| Cypermethrin (Cybadip) | Recommended dose | 100 | 93 | R |
| | 5× | 100 | 100 | S |
| | 10× | 100 | 100 | S |

**Table 3.3:** Percentage mortality and resistance status of *Xenopsylla cheopis* to different agro-veterinary and public health insecticides

### 3.4 Discussion

This is the first study in Tanzania assessing the susceptibility of plague flea vectors to agro-veterinary and public health insecticides commonly used in plague endemic areas. The involvement of agro-veterinary chemicals in the emergence of resistance in human disease vectors is apparently increasing. Therefore, targeted and regular resistance monitoring in such vectors including fleas is extremely necessary. Like many other parts of the country, plague endemic communities rely almost exclusively on agriculture, which is also accompanied with indiscriminate use of chemicals (Nonga et al., 2011; Rajabu et al., 2017).

The susceptibility tests conducted in the present study are suggestive of resistance in *X. cheopis* from Mbulu district. Whereas the reference colony of *X. cheopis* was 100% susceptible to all tested insecticides and doses, mortality rates in field fleas at recommended doses ranged from 90%–96%. The first three are agricultural insecticides, the second two are veterinary insecticides and last three are public health insecticides (Table 3.3). All the tested insecticides are commonly used in the study district from which the wild fleas originated (Rugalema and Mnyone, Unpublished data). Low mortalities, indicative of resistance, against agro-veterinary-insecticides that led to 100% mortality in reference colony, suggest the involvement of such chemicals in the observed resistance. As revealed from our recent study in the districts, most of the commonly used agro-veterinary and public health insecticides fall within similar classes, indicating that they are biochemically related and thus likely to suffer from cross resistance. Insecticide selection pressure from agriculture is generally regarded as an important early driver of insecticide resistance in disease vectors (Diabate et al., 2002; Hien et al., 2017). Nevertheless, follow up studies to confirm observations of the present study are inevitable before a sensible and reliable conclusion is made.

Lambdacyhalothrinandcarbaryl, commonly used for flea control, recorded the lowest mortality rate (90%), confirming resistance to the respective mixture of insecticides. This mixture of a pyrethroid (lambdacyhalothrin) and carbamate (carbaryl) is branded as ‘Akheri powder’ and is manufactured in the country. This product was exclusively used in flea control campaigns during the recent (2008) plague outbreaks in Mbulu district. Subsequently, ‘Akheri powder’ became the most favorite and used flea control insecticide in Mbulu and Lushoto district. Carbaryl 5% has also been widely used in these districts long before. The prolonged use of these pesticides alongside their injudicious uses, like in many other chemicals, could have selected for resistance. It is admitted that frequent and prolonged use of same insecticides lead to establishment of resistant population (Corbel et al., 2003; Corbel et al., 2007). Several studies in many parts of Tanzania are increasingly reporting injudicious uses like overuse of insecticides, non-adherence, poor storage and disposal of pesticide containers, use of banned and counterfeit pesticides and the use of a mix of pesticides in a single spray (Spiewak, 2001; Ngowi et al., 2007; Nonga et al., 2011; Lema et al., 2014). Most of these and other forms of misuses were revealed during our recent study in Lushoto and Mbulu district (Rugalema and Mnyone, Unpublished data). These injudicious uses of agro-veterinary chemicals within similar classes as lambdacyhalothrin and carbaryl could have also played complementary role in the observed resistance (indicative and confirmed).

Fortunately, the test insecticides with indicative and confirmed resistance killed 100% of the fleas at 5× and 10× the recommended dosages. This indicates that the current level of resistance is still insufficient to compromise the operational effectiveness of flea control interventions. Normally, the findings on resistance intensity may provide information on whether or not the recorded level of resistance has an operational impact (WHO, 2016). Such studies should be particularly keen at unraveling the levels and mechanisms of resistance as well as their distribution; and confirm beyond doubt the contribution of agro-veterinary insecticides. These kinds of findings will help inform immediate development of insecticide resistance management strategies before the situation worsens. Efficient resistance management needs to depend not only on the information about the insect population, but also consideration of the main source of selection pressure (Helps et al. 2017). Without these actions, local communities would very likely switch to increasing the dosages in an attempt to maintain efficacy. This option is strongly discouraged because of environmental and safety concerns, increased cost of insecticide and the resistance genes can be driven to even higher frequencies (Aïzoun et al., 2013).

### 3.5 Conclusion

This study indicates resistance in flea species, *Xenopsylla cheopis*, from Mbulu district, to recommended doses of commonly used agro-veterinary and public health insecticides. However, these fleas were susceptible to the tested insecticides at 5× and 10× the recommended dose, indicating low, but yet important, resistance intensity. Nevertheless, the observed level of resistance calls for more extensive research in Mbulu and other plague endemic in the country to understand the distribution, involved resistance mechanisms; and confirm the contribution of agro-veterinary insecticides. This will eventually inform the development of early detection and insecticide resistance management strategies.

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**Author's Contributions**

LLM and GPR conceptualized and designed the study. GPR conducted field work. LLM and GPR conducted data analysis and interpreted the results. GPR wrote first draft of the manuscript. LLM conducted a series of revisions on the manuscript and produced the version submission. All authors read and approved final version of the manuscript.

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# CHAPTER FOUR

## 4.0 Summarizing Discussion, Conclusion and Recommendations

### 4.1 Summarizing Discussion

Generally, the study aimed at assessing the risk associated with the ongoing use of agro-veterinary and public health insecticide commonly used in plague endemic areas in Tanzania, in selecting for insecticide resistance in main plague vector, together with assessing the susceptibility status of plague flea vector collected from one of the endemic areas.

Lushoto and Mbulu districts are dominated by small-scale farmers and livestock keepers; in both districts people control pests almost exclusively using pesticides. Over 91% of the respondents across both districts reported using pesticides to control agricultural pests. Using pesticides as control agents in agriculture and in livestock is a common practice throughout Tanzania (Nonga *et al.,* 2011; Rajabu *et al*., 2017). Similarly, the use of pesticides is one among the most widely used and powerful means of controlling fleas and other public health pests (Boyer *et al.,* 2014; Biswas *et al*., 2015; Katakweba *et al*., 2015).

Excessive and injudicious use of agricultural and veterinary pesticides was common across the study districts. Most of the farmers reported applying agriculture pesticides up to 4 times per cropping season, particularly on horticultural crops. These farmers also did not adhere to manufacturers recommended doses and had limited or no knowledge on safety procedures and adverse effects associated with such pesticides. The same malpractices have been reported from Tanzania and elsewhere in Africa as it is shown in other studies (Spiewak, 2001; Ngowi *et al.,* 2007; Nonga *et al.,* 2011; Lema *et al.,* 2014). Farmers reported over fourteen agricultural and three veterinary pesticides, most of which were pyrethroids, carbamates, organophosphates, organochlorines and avermectins. Because of the aforesaid injudicious uses and other malpractices indoor and outdoor environments/surfaces get contaminated with chemicals, thus posing high risk of insecticide resistance to plague flea vector. Many studies have reported alarming contamination of soils, water, air and other type of environments with agriculture chemicals (Yadav *et al.,* 2015; Mahamood *et al.,* 2016; Carvalho, 2017; Kibblewhite *et al.,* 2018). Furthermore, fleas are exposed to chemicals via insecticide-treated livestock because these animals also serve as alternative blood meal sources. The study also revealed the tendency of some communities still sharing the same house with domestic animals within Lushoto and Mbulu district. With this, fleas are constantly exposed to veterinary chemicals on top of exposure to agricultural chemicals.

Insecticide selection pressure from agriculture is generally regarded as an important early driver of insecticide resistance in disease vectors (Diabate *et al*., 2002; Hien *et al*., 2017. Lambdacyhalothrin and carbaryl, commonly used for flea control, recorded the lowest mortality rate (90%), confirming resistance to the respective mixture of insecticides. This mixture of a pyrethroid (lambdacyhalothrin) and carbamate (carbaryl) is branded as ‘Akheri powder’ and is manufactured in the country. This product was exclusively used in flea control campaigns during the recent (2008) plague outbreaks in Mbulu district. Subsequently, ‘Akheri powder’ became the most favorite and used flea control insecticide in Mbulu and Lushoto district. Carbaryl 5% has also been widely used in these districts long before. The prolonged use of these agro-veterinary pesticides alongside their injudicious uses, like in many other chemicals, could have led to the observed resistance. It is admitted that frequent and prolonged use of same insecticides lead to establishment of resistant population (Corbel *et al*., 2003; Corbel *et al*., 2007). Fortunately, the test insecticides with indicative and confirmed resistance killed 100% of the fleas at 5× and

10× the recommended dosages. This indicates that the current level of resistance is still insufficient to compromise the operational effectiveness of flea control interventions. Nevertheless, the observed state of resistance in main plague flea vector calls for more extensive research in Mbulu and possibly other plague endemic areas in the country.

## 4.2 Conclusion

The present study pinpointed a suite of either injudicious uses and/or malpractices; excessive use of agricultural chemicals, maluse of agro-veterinary chemicals as well as poor adherence to the application and safety procedures, all of which potentiate contamination of environments/surfaces and exposure of the chemicals to fleas thereof. Flea vector population across Lushoto and Mbulu districts are putatively under intense risk of resistance development emanating from the indiscriminate use of agro-veterinary chemicals. Lambdacyhalothrin&carbaryl, commonly used for flea control, recorded the lowest mortality rate (90%), confirming resistance to the respective mixture of insecticides. However, the test insecticides with indicative and confirmed resistance killed 100% of the fleas at 5× and 10× the recommended dosages indicating that the current level of resistance is still insufficient to compromise the operational effectiveness of flea control interventions.

## 4.3 Recommendations

The observed level of resistance calls for more extensive research in Mbulu and other plague endemic in country to understand the distribution, involved resistance mechanisms and confirm the contribution of agro-veterinary insecticides. This will eventually inform the development of early detection and insecticide resistance management strategies.

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# APPENDICES

## Appendix 1: DODOSO KUHUSU MATUMIZI YA DAWA ZA KILIMO NA MIFUGO

### SEHEMU A. MKULIMA

**a. Taarifa binafsi**

Jinsia ……………………..
Umri ……………………
Kiwango cha elimu ………………
Mahali unapoishi (kijiji)…………………

**b. Taarifa za matumizi ya dawa za kuulia wadudu**

1. Ni aina gani ya kilimo unayojishughulisha nayo? Chagua jibu sahihi

   a) Kilimo cha bustani
   b) Kilimo cha mazao

2. Ni aina gani ya mazao/ mboga unazolima?
   …………………………
   …………………………
   ………………………...

3. Ni aina gani ya mazao/mboga kati ya zilizotajwa hapo juu zinashambuliwa sana na wadudu Tafadhali taja aina mazao/mboga hizo
   ………………………….
   …………………………..

4. Je kuna juhudi zozote unazo fanya kudhibti wadudu hao? Chagua jibu sahihi.
   a) Ndio b) Hapana

5. Kama jibu ni ndio, taja njia unazotumia kudhibiti wadudu hao waharibifu wa mazao/mboga
   …………………………………………………….
   ……………………………………………………
6. Je huwa unatumia dawa za kilimo kudhibiti wadudu wanaoharibu mazao? Chagua jibu sahihi
   a) Ndio b) Hapana

7. Kama jibu ni ndio tafadhali taja dawa unazotumia kudhibiti wadudu hao,

   ……………………………….

………………………………
………………………………

8. Kati ya dawa ulizozitaja ni ipi unaipendelea zaidi kutumia?

   ………………………….
   …………………………..

9. Kwanini unapendelea kutumia dawa hiyo? Chagua jibu sahihi

   a) Bei yake ni nafuu
   b) Inafanya kazi kwa ufanisi
   c) Upatikanaji wake ni rahisi

10. Tafadhali unaweza kunionyesha kifungashio au kopo la dawa unayotumia?
11. Je unatumia kemikali zozote kudhibiti mazao dhidi ya wadudu na panya wakati yamehifadhiwa?
12. Kwa mazao ya muda mrefu kama mahindi, kwa wastani unanyunyizia kemikali mara ngapi kabla ya kuvunwa?
13. Kwa mbogamboga unanyunyizia kemikali mara ngapi kabla ya kuvuna?
14. Kwa kutolea mfano wa kemikali mojawapo, unachanganya kwa viwango gani kati ya maji na kemikali kabla ya kutumia?

**Jina la kemikali kiasi cha dawakiasi cha maji**

15. Je unafahamu athari zozote zinazosababishwa na matumizi ya kemikali kupita kiasi kwa afya ya binadamu?

    Kama jibu ni ndio, tunaomba utaje walau tatu

    i)………………………….

    ii)…………………………

    iii)…………………………

16. Je huwa unatumia vifaa vya kukukinga na madhara ya kemikali wakati wa kupulizia dawa shambani?

    a) Ndio b) Hapana

**SEHEMU B. MFUGAJI**

**Taarifa binafsi**

Jinsia ………………….

Umri ………………….

Kiwango cha elimu ……………

Mahali unapoishi (Kijiji)………….

**a. Taarifa kuhusu matumizi ya dawa za kudhibiti wadudu wa mifugo**

1. Ni aina gani ya wanyama unaowafuga? Chagua jibu sahihi
   a) Ng'ombe
   b) b) mbuzi
   c) c) mbwa
   d) d) paka

2. Je kuna baadhi ya mifugo yako inalala ndani ya nyumba yako unamolala? itaje

3. Ni aina gani ya wadudu wanaoshambulia mifugo yako? Zungushia duara kila kundi la wadudu wanaoshambulia mifugo,

a) Kupe

b) Viroboto

c) Utitiri

d) Chawa

4. Je kuna juhudi zozote unazofanya kudhibiti wadudu wanaoshambulia mifugo? Chagua Jibu sahihi.

a) Ndio

b) Hapana

5. Kama jibu ni ndio tafadhali taja njia unazotumia kudhibiti wadudu hao?
……………………………….
……………………………….
6. Taja dawa unazotumia mara kwa mara kwa ajili ya kuogeshea mifugo yako.
………………………...
…………………………
…………………………
7. Je viroboto pia ni kati ya wadudu wanaosumbua mifugo yako?
8. Kama jibu ni ndio, ni dawa gani unazitumia mahususi kwa ajili ya kudhibiti viroboto wa mifugo yako? zitaje tafadhali
9. Unaweza kunipatia kifungashio/ kopo la dawa unayotumia kuogeshea mifugo yako tafadhali.

**SEHEMU C. WENYE MADUKA YA DAWA ZA KILIMO/MIFUGO**

**a. Taarifa binafsi**

Jinsia …………………
Umri ………………….
Kiwango cha elimu………….
Mahali duka lilipo (kijiji)……………………………

**b. Taarifa juu ya uuzaji wa dawa za kilimo**

1. Ni dawa gani ambazo unauza hapa dukani kwako zinazodhibiti wadudu wanaoshambulia mazao/mboga, tafadhali taja dawa hizo,
……………………………….
………………………………

2. Kati ya dawa ulizotaja hapo juu ni zipi zunanunuliwa sana? Taja dawa hizo
……………………………
……………………………
3. Unafikiri ni kwanini dawa hizo zinanunuliwa sana? Chagua jibu sahihi

a) Bei yake in nafuu
b) Inafanya kazi kwa ufanisi
c) Inapatikana kwa urahisi

4. Tafadhali unaweza kutuonyesha baadhi ya dawa za kudhibiti wadudu wanaoharibu mazao/mboga?

**b. Taarifa juu ya uuzaji wa madawa ya kudhibiti wadudu wa mifugo**

5. Ni dawa gani ambazo unauza dukani kwako zinazodhibiti wadudu wanaoshambulia mifugo kama ng’ombe, mbuzi, mbwa na paka, tafadhali taja dawa hizo
…………………………
…………………………
6. Kati ya dawa ulizozitaja hapo juu ni zipi zinazonunuliwa sana?
…………………………..
…………………………
7. Ni dawa zipi ambazo unaziuza mahususi kwa ajili ya kudhibiti viroboto? Tafadhali taja dawa hizo.
8. Huwa mna kawaida ya kupeleka dawa zenu minadani kutafuta wateja siku za minada?

**ASANTE KWA USHIRIKIANO WAKO**

**THE USE OF AGRICULTURAL AND VETERINARY CHEMICALS**

**SECTION A. FARMER**

Sex………….

Age…………….

Village………….

Education level………………

1. Which type of farming are you involved with? Choose the correct answer below
   a) Vegetables farming
   b) Crop cultivation

2. Mention the types of Vegetables/crops that you cultivate

……………………….
……………………….
……………………….

3. Among the mentioned crops/vegetables above which one is much destroyed by pests? List them,
   ………………………
   ………………………

4. Are there any efforts made to control pests in your farm?
   a) Yes
   b) No

5. If the answer is yes mention the ways used to control pests in your farms.
   ………………………………..
   ………………………………..

6. Do you use pesticides to control pests in your farm?
   a) Yes
   b) No

7. Mention the pesticides that you use in controlling pesticides in your farm,
   …………………………….
   …………………………….

8. Which pesticide do you prefer to use among of the mentioned above?
   ………………………………
   ………………………………

9. Why do you prefer that type of pesticide? Choose the correct answer
   a) Cheap price
   b) Most efficient
   c) Easy availability
10. Can you show the pesticides containers, if you have any?
11. Do you use any pesticides to control pests during crops storage?
    ………………………………………………………………….

12. For the long-term crops such as maize, how many times do you spray before harvesting?
13. For the vegetables how many times do you spray before harvesting?
14. Giving example of one pesticide, how do you mix with water to attain the appropriate mixture?

15. Do you know the effects of excess use of pesticides to human being? Mention 3 of

    them.
    ………………………………..
    ………………………………..

16. Do you use the personal protective equipments during spray of pesticides;
    a) Yes b) No

**SECTION B. LIVESTOCK KEEPER**

Sex……………

Age…………….

Village………….

Education level………………

1. Which type of domestic animals do you keep? Choose the correct answer
   a) Cow
   b) Goat
   c) Dog
   d) Cat

2. Do you share the same house with some of your domestic animals?
3. What type of animal pests are found on your domestic animals? Choose the correct

   answer
   a) Ticks
   b) Fleas
   c) Mites
   d) Lice

4. Are there any efforts which are made to control animal pests? Choose the correct

   answer
   a) Yes
   b) No
5. If the answer is yes please mention the ways used to control animal pests
   ………………………………………………..
   ………………………………………………..

6. List the pesticides you use in during livestock dipping.
   …………………………..
   ………………………….
   ………………………….
7. Are fleas one of animal pests that are found on your domestic animals?

a) Yes
b) No

8. If the answer is yes what is the name of the pesticides specifically used to control fleas, mention them.
9. Can you show the bottle/container of any animal pesticides?

**SECTION C. AGROVET SHOPS**

Sex……………

Age…………….

Village………….

Education level………………

1. What type of pesticides which are used to control crop and vegetables pests that are sold in your shop? List down
……………………………
……………………………
2. Among the pesticides mentioned above which of them is mostly liked by customers?
…………………………
…………………………
3. Why do you think they are most preferred? Choose the correct answer below
a) The price is cheap
b) They are more efficient
c) They are most available
4. Can you show us the common pesticides used in crop/vegetable farming?
5. Can you mention the pesticides which are used to control animal pest that are available in your shop?
………………………
………………………
6. Among the mention above which of them are preferred most?
……………………….
………………………..

7. What are the pesticides commonly used to control fleas?
………………………….
………………………….
8. Do you normally sell the pesticides in the market?
………………………….
………………………….