

Assessment of the Level of Resistance to Bifenthrin, Cypermethrin and Methomyl Insecticides in *Bemisia tabaci* (Gennadius) and *Trialeurodes vaporariorum* (Westwood) Whitefly Populations from Selected Sites in Kenya

Thomas Njaramba Njuguna, B. Ed. (Sc) Hons., Kenyatta University.

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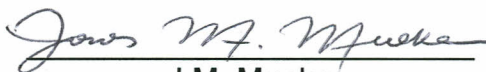


Thomas N. Njuguna

DECLARATION BY THE SUPERVISORS

This thesis has been submitted with our approval as supervisors.

Prof. Jones M. Mueke,
Zoology Department
Kenyatta University,
P.O Box 43844, Nairobi.



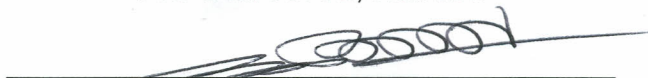
J.M. Mueke

Dr. Mohamed Ali Bob,
The International Centre of Insect Physiology and Ecology (ICIPE),
P.O Box 30772, Nairobi.



M. A. Bob

Dr. S. Sithanantham,
The International Centre of Insect Physiology and Ecology (ICIPE),
P.O Box 30772, Nairobi.



S. Sithanantham

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DEDICATION

To my Father and Mother- a wonderful couple who believe in sacrificing for a better tomorrow. They should be proud of what they have achieved.

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TABLE OF CONTENTS

Title page	i
Declarations	ii
Dedication.....	iii
Acknowledgements.....	iv
Table of contents.....	vi
List of figures.....	viii
List of tables.....	ix
Appendices.....	ix
Abstract.....	x
CHAPTER ONE	1
1.0.Introduction And Literature Review	1
1.1 Introduction.....	1
1.2. Literature review	4
1.2.1 The Biology of <i>Bemisia tabaci</i> (Gennadius) and <i>Trialeurodes</i> <i>vaporariorum</i> (Westwood).....	4
1.2.2 Whitefly taxonomy.....	6
1.2.3 Host range	7
1.2.4.Pest status	8
1.2.5 Whitefly damage	10
1.2.6 Management of whiteflies	12
1.3.0.Pesticide resistance	15
1.3.1 Development of resistance	16
1.3.2. Mechanisms of resistance to chemicals	20

1.3.3. Resistance management	21
1.4 Magnitude of whitefly management costs.....	23
1.5. Resistance monitoring.....	24
1.6. Justification	27
1.7. Hypotheses.....	28
1.8. Objectives	29
CHAPTER 2	30
2.0. Resistance levels of <i>B. tabaci</i> and <i>T. vaporariorum</i> to bifenthrin, methomyl and cypermethrin.	30
2.1.0. Materials and methods.....	30
2.1.1. Study sites and pesticides usage	30
2.1.2. Whitefly Collection.....	31
2.1.3. Species Identification	32
2.1.4. Rearing of the reference population.....	32
2.1.5. Insecticide procurement.....	33
2.1.6. Dose optimization.....	34
2.1.7. Determination of resistance levels.....	34
2.1.7.1 Glass vial bioassay	34
2.1.7.2. Leaf dip bioassay.	35
2.1.8. Data analysis.....	36
3.0. Results	40
3.1. Observed variation in pesticide usage at the study sites	40
3.2. Whitefly species.....	41
3.3.0 The glass vial bioassays.....	42

3.3.1. Responses of the whiteflies to bifenthrin.....	42
3.3.2. Responses of the whiteflies to cypermethrin.....	45
3.3.3. Responses of the whiteflies to methomyl	48
3.4.0 The leaf dip bioassays.	51
3.4.1. Responses of the whiteflies to methomyl.....	51
3.4.2 Responses of the whiteflies to cypermethrin	54
3.5. Variation in resistance factor (R.F.) values.....	57
4.0. Discussion.....	59
4.1 Conclusions.	73
5.0 Suggestions for future research work.....	74
References.....	75

List of tables

1. Percentages of whitefly species at the study sites.....	42
2. Mortality variations at different sites resulting from bifenthrin using glass vial bioassay.....	43
3. Mortality variations at different sites resulting from cypermethrin using glass vial bioassay.....	46
4. Mortality variations at different sites resulting from methomyl using glass vial bioassay.....	49
5. Mortality variations at different sites resulting from methomyl using leaf dip bioassay.....	52
6. Mortality variations at different sites resulting from cypermethrin using leaf dip bioassay.....	55

7. Resistance factors at LC50 for glass vial bioassay.....	58
8. Resistance factors at LC50 for leaf dip bioassay.....	58

List of Figures

Figure 1. Location of the study sites in Kenya	38
Figure 2. Dosage-mortality response of whitefly populations to bifenthrin using glass vial bioassay.....	44
Figure 3. Dosage-mortality response of whitefly populations to cypermethrin using glass vial bioassay.....	47
Figure 4. Dosage-mortality response of whitefly populations to methomyl using glass vial bioassay.....	50
Figure 5. Dosage-mortality response of whitefly populations to methomyl using leaf dip bioassay.....	53
Figure 6. Dosage-mortality response of whitefly populations to cypermethrin using glass vial bioassay.....	56

List of Plates

Plate 1: <i>Trialeurodes vaporariorum</i> (Westwood).....	39
Plate 2: <i>Bemisia tabaci</i> (Genn.).....	39

Appendices

Appendix I: Conversion table for percent mortality to probits.....	88
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Appendix II: A general list of commonly used pesticides against whiteflies at
the study sites 89

Appendix III. Commonly used insecticides in the study farms at Kibwezi..... 90

Appendix IV. Commonly used insecticides in the study farms at Kihara..... 90

Appendix V. Commonly used insecticides in the study farms at Mwea..... 90

Appendix VI. Commonly used insecticides in the study farms at Nguruman..... 91

Appendix VII. Commonly used insecticides in the study farms at Kitui..... 91

ABSTRACT

Despite heavy use of insecticides against whiteflies in Kenya, no information was available on their resistance to insecticides. In this study, the level of resistance of *Bemisia tabaci* (Gennadius) and *Trialeurodes vaporariorum* (Westwood) populations to methomyl (carbamate), cypermethrin and bifenthrin (pyrethroids) was assessed using leaf dip and glass vial bioassay methods. Whitefly samples were obtained from a laboratory culture and five field sites in Kenya: Kibwezi, Kitui, Mwea, Kihara and Nguruman. The samples were collected from tomatoes, eggplants (brinjals), squash (corgettes), *Dolichos*, French beans and Soya beans. The resistance levels of the field populations were determined using the probit analysis method. Resistance levels of the populations dominated by the two whitefly species as well as the expression of resistance from the two bioassay methods were compared.

Cypermethrin had the highest lethal concentration (LC) values at every site while bifenthrin had the lowest. The whiteflies from Nguruman, consisting of 94% *T. vaporariorum*, were the most resistant to cypermethrin with resistance factors (RF) of 87.971 and 10.085 for the leaf dip and glass vial methods respectively. Whiteflies from Kihara (98% *T. vaporariorum*) were the most resistant to bifenthrin (RF 6.821), and to methomyl with RF 8.087 and 7.593 for leaf dip and glass vial methods respectively. Resistance levels of the Kibwezi field population (88% *B. tabaci*) were moderately high for cypermethrin (RF 5.646) and methomyl (RF 4.168) in the glass vial bioassay. The whiteflies from Kitui (96% *B. tabaci*) were the most susceptible to the

insecticides tested. Comparison of the expression of resistance through the two bioassay methods showed the leaf dip method to be better in distinguishing the susceptible from resistant whitefly populations. On average, *T. vaporariorum* dominated populations were more resistant than those dominated by *B. tabaci*. The results indicated some positive relationship between insecticide usage at different sites and resistance to the chemicals. Nevertheless, resistance to insecticides that had never been used at some sites was also noted.

CHAPTER 1

1.0 GENERAL INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Whiteflies are small homopteran insects in the family Aleyrodidae with over 1200 species (Campbell *et al.*, 1995). These insects have a world-wide distribution, have diverse biomes and may be found in the natural fields as well as in the greenhouses (Byrne *et al.*, 1990). In spite of the wide variety of cropping systems that are affected by the whiteflies, only about 15 of the described species are actually characterised as pests. Whiteflies cause direct damage to crops by piercing and feeding on phloem sap and indirect damage of various kinds, most important of which is transmission of plant viral diseases.

Many small-scale farmers in Kenya tend to ignore the whiteflies because their initial damage does not seem to be as serious as that of other pests like borers, and therefore consider them as secondary pests. However, their role as vectors of many viral diseases, coupled with their polyphagous nature may result in devastation of a wide range of crops. Heavy whitefly infestations have been reported at least seasonally in various parts of the country (Kibata, pers. comm.). Populations of adult whiteflies in one field can exceed the economic threshold level overnight as a result of huge influx from other fields especially due to wind-assisted migration (Riis, pers. com.). *Bemisia tabaci* (Gennadius) is the leading whitefly pest species of horticultural crops in most of the hotter lowlands of Kenya, while *Trialeurodes vaporariorum* (Westwood) predominates the cooler highlands (ICIPE, 1999). Some

of the whitefly populations have been exposed to chemicals primarily targeted to other pests.

The International Centre of Insect Physiology and Ecology (ICIPE) is currently undertaking a system-wide integrated pest management (IPM) project on whiteflies in the tropics. This is being done in collaboration with other research centres including the International Centre of Tropical Agriculture (CIAT), Colombia, John Innes Centre (JIC), UK, and Asian Vegetable Research and Development Centre (AVRDC), Tanzania. One of the objectives of the system-wide project is the determination of insecticide resistance levels in target countries (CIAT, 1996).

Pesticides must remain in the arsenal of weapons to be used against pests (van Emden and Peakall, 1996). Due to overall ignorance among farmers, pesticide misuse and subsequent risks for health and environment are high in small-scale vegetable farming systems in Kenya. The major concern of the farmers is to increase/sustain the production and to maintain the damage-free quality demanded by the consumers. Forty eight percent of the farmers interviewed in a recent survey led by ICIPE in Kenya were found to use insecticides prophylactically for the whitefly control (ICIPE, 1999). Such usage encourages resistance development in the insect pests due to increased selection pressure. When insects develop resistance, the grower resorts to using higher dosage rates and more frequent applications of the pesticide to kill the pests, and eventually needs a new pesticide to replace the old one. New pesticides are extremely expensive to develop and register. Repeated applications of insecticides that do not provide adequate level of control only lead to wasteful expenditure and to

unnecessary increase in the overall pesticide load in the environment.

Resistance monitoring is necessary in situations where insect pests are managed by regular use of chemicals. The use of various types of bioassay methods is the most commonly utilized resistance monitoring strategy. Some of the most popular bioassay methods in the whitefly resistance monitoring include the leaf dip bioassay (that can be used for adults as well as nymphs), the glass vial bioassay and the insecticide coated yellow sticky cards. These methods are relatively cheap and easy to use as compared to the more recently adopted biochemical methods that require better-equipped laboratories and technical know-how. The biochemical methods are however more revealing about the level and mechanism of resistance. Two of the popular biochemical techniques in use are polyacrylamide gel electrophoresis (PAGE) of non-specific esterases and microtitre plate assay of acetylcholinesterase inhibition (Byrne and Devonshire, 1993).

The aim of this study was to detect possible whitefly resistance to some selected insecticides. Both the glass vial and leaf dip bioassays were used in the resistance assessment. The study was necessary in order to shed some light on the variations in responses of different whitefly populations in Kenya to different insecticides. This is important because despite the increased pesticide application, information on the effectiveness of the insecticides in whitefly management in Kenya was scanty and showed control failure in some areas (Kibata, pers. comm.). It is vital that the usefulness of the insecticides currently in use is not lost through resistance, hence the need for resistance management strategies.

1.2 Literature review

1.2.1 The Biology of *Bemisia tabaci* (Gennadius) and *Trialeurodes vaporariorum* (Westwood)

Certain aspects of the biology of *B. tabaci* and *T. vaporariorum* are closely related and are discussed below as those of whiteflies in general. Whitefly adults are tiny insects that feed on various plants and may occur in such large numbers as to disperse in small clouds when disturbed. Their abdomen is unique in the insect families because of the presence of the vasiform orifice, operculum and lingula (Gill, 1990). Whiteflies appear to be more active during the sunny daylight periods, and do not fly readily during early mornings, late evenings, or night hours. Adults congregate on younger leaves in most host crops and oviposition is heaviest on these leaves. They have a preference for the undersurface of leaves for feeding and oviposition. They have six life stages, the egg, the crawler, two sessile nymphal instars, the "pupa", and the adult (Gill, 1990). The fourth instar is not a true pupa because feeding occurs only in the first part of this stage and transformation into the adult takes place in the last part without molting (Nechols and Tauber, 1977a&b).

The reproduction rate in whiteflies is very high and can be bisexual as well as parthenogenetic (Byrne and Bellows, 1991). The mean egg-laying capacity of *B. tabaci* is about 160 eggs at an average daily rate of 18.8 eggs per individual (Gameel and Abdelrahman, 1978) while the average oviposition for *T. vaporariorum* at 22^o C is 5.5 eggs / individual / day (van Lenteren and Noldus, 1990). The whitefly eggs, pear-shaped and measuring about 0.2 mm, are laid on the under-surface of the leaf and are anchored by a stalk at the larger end (N.H.R.S., 1984). The

originally whitish-yellow eggs darken as they approach hatching in about five to seven days, and about two-thirds of the eggs usually hatch into female crawlers (USDA, 1995). The higher fraction of emerging females could ensure higher rate of population increase, as they are capable of parthenogenesis. On hatching, the first instar nymphs crawl for short distances before settling down to feed through their piercing-sucking mouthparts. The first stage has legs and antennae, but these are lost after the first molt and the flattened, oval-shaped larvae stay fixed at one feeding site (USDA, 1995). The second and third instars are sessile and resemble scales. The nymphs in general have colours ranging from greenish-white through yellow to brown but there are many variations with species and environmental factors (N.H.R.S., 1984; Gill, 1990). The life cycle of *B. tabaci*, from egg to adult, takes two to three weeks in warm weather, but may take as long as two months under cool weather conditions (USDA, 1995). Adult longevity of *T. vaporariorum* varies greatly with a maximum of seventy-five days at 15° C on tomatoes (van Lenteren and Noldus, 1990).

The location of the various stages of the whitefly on the plant follows the development of the plant with the older nymphs usually more numerous on older leaves. They are mainly located on the underside of leaves and can become so numerous that they almost cover the entire undersurface area (USDA, 1995). The nymphal period for *B. tabaci* lasts two to four weeks depending on the temperature. Adults emerge from the pupae through a T-shaped slit and soon mate and reproduce (USDA, 1995). More than 300 eggs per cm² and 80 million adults per hectare have been reported for *B. tabaci* (Byrne *et al.*, 1990). The adults have white waxy powder covering their bodies and their sex ratio in the field is normally 1:1

(Mound, 1983; Van Lenteren and Noldus, 1990).

There are between eleven and twenty one generations of *B. tabaci* per year in the field (Gameel and Abdelrahman, 1978) and up to eight for *T. vaporariorum* (Onillon, 1990). Two morphs exist within *B. tabaci*, that is, a migratory and a trivial-flying morph (Byrne and Houck, 1989). Some migrating adults can stay suspended in the air for hours and thereby carried away to great distances with a maximum of 7km recorded for *B. tabaci* (Gill, 1990).

1.2.2 Whitefly taxonomy

Whitefly taxonomy is traditionally based on morphological characters of the fourth instar 'pupa' (Martin, 1987; Gill, 1990). The pupae or pupal cases are carefully mounted as permanent slide specimens and details of their morphology examined using a key like the one given by Martin (1987). Distinguishing species by observing adult morphological features in the field is difficult. Adult *B. tabaci* are moth-like insects with white wings and a yellow body visible between the wings (USDA, 1995). Their wings are held somewhat vertically tilted like the peaked roof of a house, instead of almost flat over their bodies like the greenhouse whitefly (*T. vaporariorum*) when the insect is feeding or at rest. *T. vaporariorum* are brighter white in colour than *B. tabaci*, and neither of them have dark markings or bands on their wings (Plate 1&2).

The adult *B. tabaci* is among the smallest whiteflies with males measuring about 0.8 mm and females about 0.9mm in length (Byrne and Bellows, 1991). The *T.*

vaporariorum body length is about 0.99mm and 1.06mm for the males and females respectively. *B. tabaci* pupa has no waxy filaments around the edges as do most other species of whiteflies(Plate 2). It is thin, oval and flat with a rounded outside margin, tapering towards the leaf surface viewed from the side. In contrast, pupae of *Trialeurodes* species have distinctly ridged outside margins with flat, vertical surfaces and waxy projections (filaments) at the top of the ridges as viewed from the side (Plate 1) (USDA, 1995). *B. tabaci* pupa has very prominent red eyespots visible through pupal case and are more yellowish-green in color than those of *Trialeurodes* species. The yellowish color is due to the body pigments of the developing adult seen through the translucent pupal case.

There are A- and B-strains of *B.tabaci* which differ significantly in host range, mating behaviour, rate of sap ingestion and the number and types of viruses they transmit, though they are morphologically similar (Gerling and Kravchenko, 1995). Some scientists have classified the B-strain as a new species named *Bemisia argentifolii* Bellows and Perring (Perring, 1995), but this is still controversial.

1.2.3 Host range

Most of the whitefly host plants are in the families Cruciferae, Leguminosae, Malvaceae, and Solanaceae. Aggregation of the greenhouse whitefly (*T. vaporariorum*) is very strong, even on relatively unsuitable host plants. Crops that support large numbers of *B. argentifolii* include cotton, cucumber, squash, melons, tomatoes, eggplants, sesame, soybeans, okra, beans, peanuts, cabbage, and many ornamentals, including poinsettia (*Euphorbia pulcherrima* Willd.), *Hibiscus*

and *Lantana* species (USDA, 1995). Whiteflies switch to alternative hosts when the favored hosts are off-season and can then occur in higher numbers on a wider range of host plants especially weeds. Mound and Halsey (1978) reported that over 85% of all the whiteflies have 5 or fewer hosts. Other reports give the world record of *T. vaporariorum* host plants as 859 species (Rumei, 1995), and over 500 species in 74 families for *B. tabaci* (USDA, 1995; Dowell, 1990). Whiteflies in general have a poor sense of smell. Their host plant selection is governed mainly by visual cues and by taste (Berlinger, 1986). Adults perceive color at short distances and will preferentially select yellow/green objects (USDA, 1995).

1.2.4 Pest status

Since the 1960's, whiteflies have become increasingly important pests of cotton and vegetable crops in the whole world (Assad, 1990). In every situation where whiteflies are a serious problem, wild and cultivated host plants grow in proximity to one another, and they have little problem in finding new hosts when existing conditions become less hospitable (Byrne *et al.*, 1990). *B. tabaci* and *T. vaporariorum* have been identified as the most important whitefly pests worldwide (CIAT, 1996). Recent upsurge in pest severity of *B. tabaci* is more attributed to a new strain rather than the old one (A-strain) (Gerling and Kravchenko, 1995). The new strain is referred to as the B-strain or B-biotype and has proven competitive advantage over the A-biotype. It's extreme polyphagy and occurrence in the high value crop farming increase its world-wide exposure to insecticides, which may select for a wider range of insecticides (Cahill *et al.*, 1996a).

Whiteflies are particularly serious pests on members of the families cucurbitaceae (squash, melons, cucumbers, pumpkins), solanaceae (tomato, eggplant, potato), malvaceae (cotton, okra, *Hibiscus*), and leguminosae (beans, soybean, peanuts) (Byrne *et al.*, 1990). Cotton (*Gossypium hirsutum* L.) and tomatoes (*Lycopersicon esculentum* L.) are some of the most seriously affected crops (Perring, 1995). Other affected crops include *Brassica* spp., *Capsicum* spp., poinsettia, *Hibiscus* spp. and many other ornamental plants. *B. tabaci* and *T. vaporariorum* cause the greatest whitefly damage in Kenya which is experienced in French beans (*Phaseolus vulgaris* L.), Solanaceae (especially tomatoes), cassava (*Manihot esculenta* Crantz), brassica crops like broccoli and kales, and the commercially cultivated flowers (Kibata, pers. comm.).

In recent years, certain whitefly species, most notably *B. tabaci*, have expanded their range to new parts of the world, and have also become more important in areas where they were previously found. While the reasons remain unclear, these increases seem to be closely related to factors such as human-assisted movement, changed agronomic practices such as an extended growing season for certain crop hosts under artificial conditions, chemical destruction of the beneficial insect complex and increasing levels of pesticide resistance in the whiteflies (Byrne *et al.*, 1990). Although whiteflies are poor fliers and do not routinely engage in long distance migration, their range has expanded as man has transported them into regions to which they previously had no access. The transportation is done unintentionally as the affected plant materials bearing at least one of the life stages are taken from one locality to another. The ability to adapt to new environments and new agricultural practices, such as increased pesticide usage, identifies *B.*

tabaci as an aggressive r-strategist that rapidly fills ecological voids created through intensive agricultural practices (Riley *et al.* 1995). Simultaneity of several factors such as the following may be responsible for the rapid increase in *B. tabaci* population: (i) their rapid reproduction rate, (ii) the occurrence of most of the adults, eggs, nymphs and pupae on the undersurface of leaves where sprayed insecticides do not easily come into contact with them, (iii) reduced natural enemy populations and, (iv) resistance to insecticides, which makes chemicals intended to control them of little use (Byrne *et al.*, 1990; Horowitz and Ishaaya, 1995). The total loss resulting from the effect of whiteflies has been on a steady increase in the last two decades resulting in one of the species (*B. tabaci*) being termed as the world's most damaging insect pest (Cahill *et al.*, 1995).

1.2.5 Whitefly damage

Whiteflies cause direct damage by phloem sap ingestion, indirect damage by transmitting diseases to host plants (Perring, 1995) and decrease in photosynthesis due to sooty mould which develops on honeydew excreted by the insect (Vet *et al.*, 1980). The honeydew may also cause direct damage by making some produce such as cotton lint sticky, as well as indirect damage through the staining effect of the subsequent fungal growth. Direct crop damage occur when whiteflies feed because they injure the plant, remove sap and reduce plant vigor resulting in stunting, poor growth, defoliation, reduced yields, and sometimes death. Although damage due to sap ingestion is not readily apparent, high whitefly population may be sufficient to cause wilting of leaves. Other forms of damage include development of chlorotic spots, yellowing, blanching of vegetative structures and

irregular ripening or other abnormalities of fruiting structures. It is believed that some whiteflies inject some enzymes into the host plant while feeding, affecting the normal physiological processes (USDA, 1995). These feeding toxins cause plant disorders such as silver leaf of squash particularly by the silverleaf whitefly (B-biotype of *B. tabaci*) and irregular ripening of tomatoes (USDA, 1995).

Whitefly-borne diseases are of major importance in the tropical and sub-tropical agriculture, with more than 70 diseases being reported (Cohen, 1990). Almost all of these diseases are viral while a few are caused by bacteria and fungi. The plant viruses include the geminiviruses in tomatoes, pepper and cabbage, and certain clostroviruses like lettuce infectious yellows in lettuce and melons. Some of the whitefly-transmitted viral diseases are squash silvering, cassava mosaic, yellow mosaic in Soya beans, tomato yellow leaf curl, sweet potato sunken vein, cotton leaf curl, tobacco leaf curl, bean golden mosaic, potato yellow mosaic, lettuce infectious yellows disease and cucurbit yellow stunting disorder (Duffus, 1987; Padidam *et al.*, 1995; Fauquet and Martelii, 1995).

The whitefly-induced plant disorders and the viruses they transmit are of particular concern because they can occur even when a whitefly population is very small. Four hours of feeding by a single viruliferous whitefly are sufficient to inoculate a healthy plant with tomato yellow leaf curl virus at a probability level of 80% (Berlinger *et al.*, 1996). Five to eight viruliferous whiteflies feeding for only a few minutes can transmit the virus (Omara, 1997). It is then likely that whiteflies can spread epidemic in an area within a few days. Damage from whitefly-transmitted geminiviruses in tomatoes was estimated to be up to 50%, 75% and

100% in Kenya, Tanzania and Sudan respectively (CIAT, 1998). The disease vector potential is a notable characteristic of *Bemisia* worldwide, and it represents a serious risk in subsistence crops like cassava, tomatoes and beans particularly in the developing countries (Robinson and Taylor, 1995). The African cassava mosaic disease (ACMD) epidemic in Uganda was spreading at a rate of 10 to 20 km per year by 1996 (Otim-Nape *et al.*, 1996). In Kenya, ACMD causes a crop loss of up to 70% (Fauquet and Fargette, 1990).

1.2.6 Management of whiteflies

The need to increase food production for the ever-increasing human population has encouraged intensive agricultural practices, which require the use of more agrochemicals. The importance of whiteflies as pests of cultivated crops has led to widespread use of chemical insecticides in their control. Since plant viral diseases are incurable once established in the plant, the choice left for farmers in protecting their crop is the elimination of the vector (whitefly) through chemical use which is both expensive and increasingly difficult to achieve. Besides the cost of treatment, other factors involved in the chemical control decisions are the need for thorough coverage, the risk of secondary pest outbreaks, the risk of whiteflies developing insecticide resistance, and the regulatory restrictions on their use (USDA, 1995).

Biological control helps in the whitefly management. Parasitoids that attack *Bemisia* species occur in the three genera of Hymenoptera: *Encarsia*, *Eretmocerus* (both Chalcidoidae: Aphelinidae) and *Amitus* (Proctotrupoidae: Platygasteridae) (Hoelmer, 1995). Releases of the parasitic wasp, *Encarsia*

formosa Gahan, have been successful in controlling *T. vaporariorum* and are widely used in Europe in the greenhouse vegetable production (Onillon, 1990; USDA, 1995). However, naturally occurring parasitism in field crops is insufficient to control whiteflies on many affected crops. Cultural manipulations may help increase the parasitoids' effectiveness. Whitefly nymphs are preyed upon by *Macrolophus caliginosus* Wagner, *Crysoperla rufilabris* (Burmeister), *Delfastus pusillus* LeConte and other general predators (Heinz, 1995). *M. caliginosus* has been used in the control of *T. vaporariorum* in Europe (Van Schelt *et al.*, 1995). Entomopathogenic fungi are viewed as some of the most promising biological control agents of whiteflies (Lacey *et al.*, 1995). The most common of these are *Verticillium lecanii* (Zimm.), *Beauveria bassiana* (Balsamo) and *Aschersonia* species. The major reasons why biological control of *Bemisia* has encountered difficulties are related to poor understanding of its parasitoids as well as the complexity of the biology and host range of the genus (Hoelmer, 1995). Insecticidal soaps and oils provide varying levels of control of whiteflies and their effects on beneficial arthropods also vary (Stansly *et al.* 1995).

The main control measure for whiteflies has been by the use of different types of insecticides in different chemical classes (N.H.R.S., 1984; Prabhakar *et al.*, 1989, USDA, 1995). The method of application, frequency and choice of pesticides depend on many factors. Insecticide use in different islands of Hawaii varied from 4 to 103 insecticide sprays/site per season (Omer *et al.*, 1993a). Chemical groups used in the control of whiteflies may have related modes of action. For instance, the action of organophosphates and carbamates is to inhibit acetylcholinesterase being formed and so impulses are fired continuously. This

can lead to tremors, convulsions and death (van Emden and Peakall, 1996). However, their specific influence on different target sites in the insect body may differ.

Although natural pyrethroids have been in use since 1820s, synthetic pyrethroids have only been in wide-scale use since the early 1980s (Hirano, 1989). Pyrethroids are highly toxic to insects and dosages of the formulated material are typically 200g ha^{-1} (van Emden and Peakall, 1996). They are quickly metabolized and have low toxicity to warm-blooded animals but high toxicity to beneficial insects, fish and aquatic arthropods.

The pesticides affecting whiteflies in Kenya are usually primarily meant for the general arthropod pests. Insecticide use in most tomato production areas in Kenya has increasingly become a necessary operation despite being hazardous or ineffective and uneconomical (ICIPE, 1999). The majority of tomato farmers (86%) were found to commonly use insecticides, mostly pyrethroids and organophosphates to combat the whitefly/disease problem in their farms. Cypermethrin, karate (lambda-cyhalothrin), dimethoate, diazinon and carbosulfan were the most popular insecticides for the control of the whitefly/disease problems in the country (ICIPE, 1999). Other commonly used insecticides against whiteflies in the vegetable farms include malathion, methomyl (lannate), fenitrothion, endosulfan, omethoate, dicofol, alpha-cypermethrin and amitraz.

1.3.0 Pesticide resistance

The limitations and dangers inherent in the unrestricted use of pesticides have become apparent and are highlighted by various authors (Jepson, 1989; van Emden and Peakal, 1996). Laboratory investigation by Dittrich *et al.* (1985) showed that DDT increased oviposition of *B. tabaci* by 30% and produced a higher number of generations per period than the controls. It therefore caused pest population acceleration. This could have been because more eggs were laid when the resistant insect was under biochemical stress, or because beneficial arthropods were eliminated (USDA, 1995). There is also evidence that pyrethroids can increase populations of spider mites and aphids (hormoligosis) by causing them to reproduce faster (USDA, 1995). Resistance to insecticides, especially the pyrethroids and the reduction and/or elimination of parasitoids and predators due to overuse of non-selective insecticides has further compounded pest outbreaks. Metcalf (1980) claimed that insect resistance to insecticides is the greatest single problem facing applied entomology.

Pesticide resistance is the ability of an insect or mite to survive the rate of pesticide that other individuals in the population cannot survive (USDA, 1995). The resistant pest is not usually immune, for it can still be killed, if enough of the chemical is applied. It can also be said to occur when a pest can no longer be controlled by the treatment that was previously effective (Johnes and Johnes, 1984). Resistance is a dynamic phenomenon in which levels of tolerance, cross-resistance patterns, gene frequencies and the overall response of field populations

alter ceaselessly as pests come into contact with pesticides (Sawicki, 1985). This characteristic of resistance is inherited and the survivors pass the gene(s) for resistance to the next generation. The first case of resistance to pesticides was detected in 1914 (Melander, 1914) and by 1991, 506 species of resistant insects and mites could be listed, most being of agricultural importance (Georghiou and Lagunes, 1991).

1.3.1 Development of resistance

Resistance to pesticides is an almost inevitable evolutionary consequence of man's activities. Insecticide applications often fail to provide adequate control because of resistance development and whiteflies are able to rapidly increase in numbers when conditions are ideal. Overuse and misuse of insecticides is the most important single factor contributing to the emergence of resistant insect strains (Georghiou, 1983). Resistance is not acquired by an organism during its lifetime. Denholm *et al.* (1995) reports that genes capable of conferring resistance to novel insecticides are present in the natural field populations, and in some cases there are already reports of such resistance compromising control efficacy. The genes normally arise due to mutation, which need not be triggered by pesticides. The inherited change favours the mutants in the face of increasing exposure of the population to insecticidal chemicals (USDA, 1995). Spraying selects for insects with genes for resistance and these can provide the insect protection in many ways. The most common way is increasing enzymes in their body to break down the pesticides into chemicals that do not kill them.

When insecticides are sprayed, the resistant arthropods will survive better. However, often there is a cost to the insect to have extra enzymes ready to resist the pesticide. Consequently, in the absence of the pesticide spray, susceptible individuals may reproduce faster or survive better (USDA, 1995). Thus, there is a biological trade-off for an arthropod to be resistant so that it is not stronger in all situations. The more often a population is sprayed, the faster it is expected that the susceptible individuals will be removed and selection for a population that has mostly resistant individuals will take place (USDA, 1995). It is no wonder, therefore, that the earliest cases of resistance reported in the literature usually come from cropping systems of high intensity. Very often glasshouse crops, which are grown under conditions of the highest intensity, are amongst them (Dittrich *et al.*, 1990). The artificial conditions in the greenhouses have been found to favour resistance development in whiteflies (Rumei, 1995). Comparison of resistance to deltamethrin, a synthetic pyrethroid, showed a 6290-fold increase between 1983 and 1988 in Sijiqing greenhouse area of China, yet only a 4-fold increase was detected at Tangshan where horticulture is not practised (Rumei, 1995).

The development of resistance due to the extensive pesticide use has been investigated in different parts of the world and the findings documented (Cahill *et al.*, 1996a). For example, *B. tabaci* had been a minor pest of cotton in the Gezira (Sudan) before 1970. By late 1970s, it had become the number one pest in the Gezira cotton zone, replacing *Helicoverpa armigera*, Hubn. Repeated sprays, eight times or more per season, did not prevent the populations from increasing steadily to as much as ten times the economic threshold. Factors responsible might have been expansion and diversification of cropping systems, intensified agronomic

practices and wide-scale use of conventional insecticides (Eveleens, 1983). A 240-fold resistance to dimethoate was detected in the Sudan Gezira where dimethoate had been used continuously for over 20 years against the whitefly (Dittrich and Ernest, 1983). Dittrich *et al.* (1990) reported resistance in *B. tabaci* in Sudan to be high against organophosphates and synthetic pyrethroids, and moderate against carbamates and endosulfan.

Studies from different parts of the world have shown that under increasing selection pressure from insecticides, whiteflies have developed resistance to all major chemical groups of insecticides including carbamates, pyrethroids, organophosphates and cyclodienes (Denholm *et al.*, 1995). Whiteflies have become resistant to insecticides throughout the U.S., threatening the success of traditional chemical control techniques in some other areas (USDA, 1995). It is easy to envision whitefly crisis situations developing wherever irrigated crops are grown in arid conditions using organophosphate and pyrethroid insecticides (Robinson and Taylor, 1995). Varying resistance mechanisms, levels and frequencies have been documented in various parts of the world (Dittrich and Ernest, 1983; Dittrich *et al.*, 1990b).

Trialeurodes vaporariorum became an economically important insect pest of greenhouse vegetable and ornamental crops in the middle 1970's in Beijing, China. The synthetic pyrethroids were the most effective insecticides for greenhouse whitefly control, when they were first introduced at the end of the 1970's (Zou and Zheng, 1988). After several years of application, whitefly control with both fenvalerate and deltamethrin became very difficult in the greenhouse

and in the field. Resistance levels for fenvalerate and deltamethrin reached 405.6 and 1,941.7-fold respectively, based on the dipping bioassay recommended by FAO in 1988 (Zheng and Rui, 1992). *T. vaporariorum* does not only show serious resistance to insecticides but also a very high rate of colonization. For instance, it rose from a newly recorded insect to a serious pest in the greenhouses throughout Japan in a period of four years (Nakazawa, 1981). It is also among the earliest whiteflies to be reported as showing resistance in the greenhouse (Wardlow *et al.*, 1972).

Resistance to dimethoate, endosulfan, methomyl and amitraz, which are commonly used insecticides against the aleyrodids on cotton in Sudan, was investigated in the adults and nymphs of *B. tabaci* (Ahmed *et al.*, 1987). Compared with a susceptible strain, resistance levels differed greatly with the chemical and the highest resistance was recorded against dimethoate at 454-fold in adults and 257-fold for nymphs.

Studies of the development of resistance to insecticides by *T. vaporariorum* in Bulgaria revealed that with the exception of deltamethrin (Decis) and permethrin (Ambush) which are still in common usage, a general tendency was noted from 1983 towards greater susceptibility to the majority of preparations used for controlling the pest, such as endosulfan (Thiodan), propoxur (Unden), pirimiphos-methyl (Actellic), bioresmethrin (Izatrín) and methomyl (Lannate). This could be explained by the discontinuation of their use against the pest and also by the interruption of its life cycle by the change to winter planting of the greenhouse crops, which has reduced the number of chemical treatments (Natskova, 1987).

Determination of the susceptibility of *T. vaporariorum* and *B. tabaci* from Hawaii showed varying levels of resistance to acephate, methomyl and permethrin (Omer *et al.*, 1993a). Significant and positive associations between the LC50 for each insecticide and the frequency of application of the same insecticide across the sites, suggested that local variation in insecticide use was an important cause of the observed differences in susceptibility (Omer *et al.*, 1993a and b).

Sivasupramaniam *et al.*, (1997) determined that resistance of the B-biotype of *B. tabaci* (*B. argentifolii*) to the widely-used mixture of Danitol (fenpropathrin) and Orthene (acephate) confers cross-resistance to Asana (esfenvalerate), Capture (bifenthrin), Danitol, Decis (deltamethrin), Decis+Orthene and Karate (lambda-cyhalothrin). Additionally, selection with the mixture resulted in statistically significant reductions in susceptibility to Curacron (profenofos), Lannate (methomyl), Monitor (methamidophos) and Ovasyn (amitraz).

1.3.2 Mechanisms of resistance to chemicals

The mechanisms of resistance can be morphological, behavioural or biochemical (Green *et al.*, 1977; Hassal, 1982). The morphological mechanism involves the development of some structures in the insect which reduce the dose transferred to the vital site of action. For instance, increased thickness of the epicuticle or the wax layer can result in reduced permeability to the chemicals. The behavioural resistance is due to a change in the behaviour of the insect; for example, increased ease of detecting and avoiding the chemical. Biochemical resistance include

means by which the insect reduces the toxic effect of the chemical due to:

- (1) Increased metabolic breakdown of the pesticide. This metabolic resistance is the most common mechanism of resistance. The insect uses various enzymes to detoxify the pesticide before poisoning could become effective. Many insects use esterase or mixed function oxidase enzymes to break down pesticides.
- (2) Increased elimination of the chemical from the body.
- (3) Decreased or complete loss of susceptibility at the target sites, as can happen due to enzymatic or functional change of part of the nervous system.
- (4) A combination of two or more of the above (Hassal, 1982; Prabhaker *et al.*, 1988).

Some insect pests use the same enzymatic pathway for the detoxification of host-plant allelochemicals and insecticides (Sivasupramaniam *et al.*, 1997). Because *Bemisia* and *T. vaporariorum* are highly polyphagous, they could possess an extensive array of detoxifying enzymes developed to survive on a wide diversity of host plant species. These same enzyme complements may provide a greater tolerance to many of the pesticides in use today.

1.3.3 Resistance management

While insecticides will be required in most whitefly control programs, they should be selected carefully and used only when shown to be needed by a regular monitoring program. Problems of resistance, which appear to be critical, should be tackled through an integrated resistance management program (IRM) which is a part of the integrated pest management (IPM). IPM is the careful integration of a

number of available pest control techniques that discourage the growth of pest populations and keep pesticides and other interventions to levels that are economically justified and safe for human health and the environment (van der Graaff, 1997). Resistance to pesticides has been the driving force behind the introduction of IPM methods in the developed countries (van Emden and Peakall, 1996).

The IRM program includes:

- (a) minimal use of pesticides restricted to highest infestation periods or when other control measures fail
- (b) use of non-chemical insect pest control measures, for instance, by utilizing resistant cultivars, biological and cultural control
- (c) alternation of classes of the control chemicals used, for example., organophosphates may alternate with pyrethroids in different application periods within a growing season
- (d) use of synergists or cotoxicants to enhance the pesticides activities
- (e) use of non-persistent pesticides which do not continue exerting selection pressure on the insects for long periods (Sawicki, 1985).
- (f) Stopping insecticide spraying at 40% flowering is ideal to get the maximum control of the whitefly and have a crop free of insecticidal residues which may contribute to resistance development (Ahmed and Wani, 1997).

These IRM measures aim at preventing and/or delaying resistance development which could otherwise lead to reduced pesticide effectiveness and an increase in the number of sprays needed. This directly increases the cost/benefit ratio of the pest control, hence the need for resistance monitoring. Monitoring of insecticide

resistance of major pests is critical for any integrated resistance management strategy (Horowitz and Ishaaya, 1995). Accurate and regular monitoring for changes in susceptibility is essential for anticipating and contending with the insecticide resistance problems (Denholm, 1990). To be fully effective, monitoring tests should ideally conform to several criteria relating to their precision and/or practical utility. Above all they should be as rapid and simple as possible, yield repeatable results with an unambiguous endpoint, and be sufficiently sensitive to detect any differences in tolerance likely to be of significance under field conditions (Denholm *et al.*, 1995).

1.4 Magnitude of whitefly management costs

In spite of about US\$25 billion worth of pesticides being used in the world annually, pests remain a drawback to agriculture (van der Graaff, 1997). Losses related to the whitefly menace and management costs around the world appear to have been on the increase. The primary loss incurred by melon producers from foregone sales and wasted production expenditures in the Imperial Valley of California amounted to \$27.7 million in 1991 and \$22.1 million in 1992 (Birdsall, 1992, 1993). It was estimated that each \$1 million of primary loss created secondary ripple effects amounting to an additional \$1.2 million in lost personal income and 42 lost jobs (Gonzales *et al.*, 1992). These short-run estimates of economic impacts illustrate the dramatic impact that whiteflies have had in agricultural systems. Yield losses due to B-biotype of *B. tabaci* in the United States alone was estimated at \$500 million in 1991 (Perring *et al.*, 1993) and this can rise up to \$1 billion annually (USDA, 1995).

1.5 Resistance monitoring

The resistance status of pest populations can be determined through bioassays. These can give a direct measure of an insect's response to a toxicant, although these responses can vary depending on the test used (Dennehey *et al.*, 1983). A bioassay is a test of the ability of a living insect or mite to survive a pesticide. If more than a few individuals survive a given dosage, then appropriate analysis may show that population has some resistance to the pesticide (USDA, 1995). They can therefore help determine the level of failure of a chemical in pest control. However, for detecting low resistance frequencies, bioassays are limited by the large sample sizes required and usually provide little information on the genotypic composition of the test insects. Biochemical methods that accurately identify resistant genotypes are better than the bioassay in this respect (Byrne *et al.*, 1994). Two of these techniques, polyacrylamide gel electrophoresis (PAGE) of non-specific esterases and microtitre plate assay of acetylcholinesterase inhibition by organophosphates and carbamates, have yielded biochemical markers associated with resistance in *B. tabaci* (Byrne *et al.*, 1992; Byrne and Devonshire, 1993). Polymerase chain reaction (PCR)-based molecular diagnostics are also being used to detect insecticide resistance. They include the PCR amplification of specific mutations (PASA), PCR product digestion with appropriate restriction endonucleases (PCR/REN) and single-stranded conformational polymorphism analysis (SSCP) (French-Constant *et al.*, 1995). SSCP can detect novel mutant alleles overlooked by either PASA or PCR/REN. These techniques can more readily address several fundamental issues relating to the evolution and inheritance of specific resistance alleles in insect populations. The techniques are applicable to any life stages from which a sufficient quantity of DNA can be

extracted (French-Constant *et al.*, 1995). A microtitre plate assay for characterizing insensitive acetylcholinesterase genotypes of insecticide-resistant insects can also be used in monitoring resistance (Moore *et al.*, 1988).

Leaf-dip and glass-vial techniques are two of the most commonly used bioassay methods. Resistance assessment through bioassay methods requires one to have baseline data or a reference/control population to be compared with the field samples. The reference population is normally a strain that is known to be susceptible but whiteflies from an area where there has been limited or no pesticide usage can also be used (Busvine, 1971). In using a certain strain as reference in resistance testing, care should be taken not to cross species boundaries, because different whitefly species respond very differently to various insecticides (Castle, pers. comm.).

The most widely used type of bioassay for *B. tabaci* is based on the adult leaf-dip test where adults are exposed to leaf material dipped in formulated insecticides (Denholm *et al.*, 1995). Where systemic insecticides are tested, only the petiole of a freshly detached leaf need to be in the chemical. The method is applicable even in the use of slow-acting pesticides as the insects continue feeding. The glass-vial bioassay is a simple, inexpensive, and effective technique for monitoring and determining resistance in field populations of adult *B. tabaci* (Sivasupramaniam *et al.*, 1997). In addition, this method provides the user with results quickly and does not require the studied insects to be reared. However, this method is applicable only to fast-acting contact insecticides as there is no food for the insects. The use of adults is preferred because the effect of the chemicals on their mobility is easily

observed unlike that of the sessile immatures. The adults are also easier to collect and transfer from the field without any damage than the nymphs that stick on to leaf surfaces due to the wax they produce (Gill, 1990). However, the nymphs can also be treated with chemicals while still attached to the growing leaves and the effect of the chemical monitored till hatching.

Toxicological work is complemented by biochemical analyses of resistance mechanisms (Devonshire and Denholm, 1998). Whilst bioassays must remain at the center of all resistance studies, they are now complemented by other methods that can throw light on the underlying biochemical and genetic changes responsible, and on how these changes develop within the insect populations. For instance;

- (a) The use of synergists in bioassays can give indications of the biochemical mechanisms involved.
- (b) Formal genetic studies establish inheritance patterns and can isolate resistance genes for detailed toxicological analysis.
- (c) Population genetics and modeling describe and predict the buildup of resistance.
- (d) Studies of insecticide metabolism can identify the type of enzyme(s) involved in resistance.
- (e) Protein purification and characterization can establish whether insecticide-degrading enzymes change qualitatively or quantitatively in the resistant insects.
- (f) Electrophysiological and enzyme kinetic studies describe changes in the interaction between insecticides and their targets.

- (g) Molecular biological techniques elucidate the changes in DNA underlying all the above observations
- (h) Biochemical, immunological and molecular biological studies can provide accurate and sensitive methods for monitoring resistance genes or their protein products in insect populations (Brown and Brogdon, 1987; Devonshire, 1987).

1.6 Justification

Whiteflies are widespread pests in cropping systems throughout Kenya. Although they are still considered as secondary pests in almost all areas where they occur in the country, they can easily and rapidly change from secondary to primary pests and may cause considerable loss in the horticulture industry especially due to their role as vectors of many plant diseases. Pesticide usage remains highest in the developed countries, but these markets are stagnating or contracting while those in developing countries like Kenya are expanding. Many insecticides are used for the control of whiteflies, but their effectiveness has so far not been assessed in Kenya. Fifty two percent of the farmers in a recent survey estimated incurring costs ranging from \$100-400 per hectare of tomatoes for the management of whitefly/disease problem (ICIPE, 1999). Farmers' reports of chemical control failure of varying degrees in various parts of the country suggested that there was a possibility of presence of some resistant whitefly strains. Despite the suspected resistance and the seriousness of whitefly-transmitted diseases in some parts of the country, no documented records are available on the whitefly pest status and its resistance to pesticides. In view of the

considerable importance of whiteflies as pests in vegetable cropping systems and their high potential to develop resistance to insecticides, there was need to carry out dose-mortality tests so as to understand the resistance scenario for the whiteflies in Kenya. This would help to identify insecticides on which resistance may have developed and also to compare the resistance status of the country's topmost aleyrodid pests such as *B. tabaci* and *T. vaporariorum*. The former species is a vector of a high number of viruses while the latter is a cosmopolitan plant pest that is considered one of the most important greenhouse pests. It therefore warrants close monitoring in the field to forestall possible catastrophe in the Kenyan vegetable farming with particularly serious threat to the budding greenhouse farming for the export market.

1.7 Hypotheses

- (i) The level of whitefly resistance to insecticides does not vary with localities in Kenya.
- (ii) The level of resistance of *B. tabaci* to insecticides is the same as that of *T. vaporariorum*.
- (iii) The level of whitefly resistance to cypermethrin, methomyl and bifenthrin is the same.
- (iv) The results from glass vial bioassay and those from leaf dip bioassay do not differ in indicating the levels of resistance to insecticides in *B. tabaci* and *T. vaporariorum*.

1.8 Objectives

1. To assess the resistance level of *B. tabaci* and *T. vaporariorum* to cypermethrin, bifenthrin and methomyl in selected parts of Kenya.
2. To determine the resistance factors (R.F.) of *B. tabaci* and *T. vaporariorum* populations in selected areas in Kenya.
3. To compare results of leaf dip bioassay to those of glass vial bioassay in the assessment of the level of whitefly resistance to selected insecticides.

CHAPTER 2

2.0 RESISTANCE LEVELS OF *B. TABACI* AND *T. VAPORARIORUM* TO BIFENTHRIN, METHOMYL AND CYPERMETHRIN.

2.1.0 Materials and methods

2.1.1 Study sites and insecticide usage

Adult whiteflies were collected from the following agricultural areas of Kenya where a variety of vegetable crops are grown: Kihara (1800m a.s.l.) in Nairobi province, Kibwezi (700m a.s.l.) and Kitui (1000m a.s.l.) in Eastern province, Nguruman (800m a.s.l.) in Rift Valley province and Mwea (1260m a.s.l.) in Central province (Fig. 1). Kihara and Mwea are situated in the central highlands of Kenya while Kitui, Kibwezi and Nguruman are in the hotter southern lowlands where long dry periods are common. Cultivation of various types of vegetable crops takes place in each of the target sites. In Mwea, Nguruman, Kibwezi and Kihara, some farmers carry out irrigation of horticultural crops for all year round production. Only a few farmers in Kitui practise irrigation and this result in the area having limited cultivated host plants particularly during the dry seasons.

Farmers at the study sites were requested to supply information on the commonly used insecticides and the general frequency of applications. The chemical names and classes of the trade names of insecticides used by the farmers were later identified based on information from Royal Society of Chemistry (1991) and

Research Information (1999).

2.1.2 Whitefly Collection

Adult whiteflies were collected from the field study sites on different days between October 1998 and May 1999. Most of the adults were collected from tomato, brinjal and French bean crops and a few were collected from *Dolichos* and Soya beans. Choice of the host source was dependent on the availability of the adult whiteflies on the crop. High whitefly populations were found on *Dolichos*, tomatoes and cotton in a few scattered farms in Kitui especially where bucket irrigation was practised along the Kalundu stream and near wells. Collection of samples from Kihara was restricted to two farms that had the highest whitefly populations because nearby farms had very low numbers. Common vegetable crops from the target farms were tomatoes, eggplants (brinjals), squash (corgettes), kale and French beans. The host plants from which whiteflies were collected at Mwea were tomatoes and French beans. At Nguruman whiteflies mainly infested tomatoes and eggplants although few adults and nymphs were also found on okra. Whitefly samples from Kibwezi were collected from two large farms: one owned by the University of Nairobi and the other by the Tana and Athi River Development Authority (TARDA). Tomatoes, eggplants and Soya beans in the two farms were seriously attacked by whiteflies particularly during the months of December to March. Other vegetable crops that were infested with whiteflies at Kibwezi were melons, pumpkins and okra.

Collection of the whitefly adults from host plants was by mouth aspirators. It was carried out in the mornings before adults become more active. The reservoir

glass vial was replaced with a fresh one when necessary. The vials were covered with plastic caps that had many small holes for ventilation during transportation. They were then placed in a cool box containing ice to make the insects inactive thereby saving energy. Cotton wool that was used as a filling material was placed on top of a polythene paper covering the ice before putting the insects in so as to avoid the increase of mortality through freezing. The whiteflies were later removed from the cool box and released into small perspex glass cages measuring 25 by 20 by 20cm. Dead and very weak adults remained on the cage floor while the active ones flew to the top due to positive phototaxis and were used in the bioassays. Leaves bearing the whitefly pupae were picked from the crops at the study sites and preserved in 70% alcohol for identification.

2.1.3 Species Identification

External morphological features of adults and nymphs were used for the initial identification in the field as described by USDA (1998). Pupae collected from the study sites were later processed and examined at the ICIPE laboratories for confirmation of identification as described by Martin (1987). The permanent slides of the specimens were stored at ICIPE. Percentages of species present were calculated from the identification of 50 pupae from each study site.

2.1.4 Rearing of the reference population

There was no history of insecticide usage against whiteflies or other pests within ICIPE's Duduville campus (Nairobi) and its precincts. A laboratory culture that

originated from tomatoes in the field at Duduville provided the baseline as the presumed susceptible *T. vaporariorum* population. Few adult whiteflies were collected from the field and reared to produce five generations in the laboratory before comparison with the field samples. The whiteflies were reared in cages measuring 40cm by 40cm by 60cm at the ICIPE laboratories for about six months (early Sept. 1998 to end Feb. 1999) before being used in the bioassays.

Glass panes were used to cover the front, back and top sides of the cages while the sides were covered with fine meshed net. The front side was fitted with a black cotton sleeve to allow for passage into the cage and the floor of the cage was made up of aluminium. The room temperature conditions were maintained at $25\pm 2^{\circ}\text{C}$ and humidity at 50%. The whiteflies were reared on brinjals (eggplant), French-beans, tomato and tobacco plants grown in plastic pots with a diameter of 15 cm and a depth of 14cm and no insecticide was used on the plants. The reference population for *B. tabaci* was from the Kitui fields. A general survey on the insecticide usage at the study site revealed that there was relatively low application of chemical pesticides for the control of whiteflies and other agricultural pests.

2.1.5 Insecticide procurement

Two pyrethroids (cypermethrin and bifenthrin) and a carbamate (methomyl) were used to assess levels of resistance of whitefly populations to pesticides. Technical grade and 5% emulsifiable concentrate of cypermenthrin were procured from Rhoune poulenc (Kenya). The bifenthrin technical grade was donated together with vials already coated with ten different dosages by Steve Castle of USDA. The

methomyl technical grade and 20L emulsifiable concentrate were obtained from Du Pont (SA), France through Thomas Frey.

2.1.6 Dose optimization

In the absence of a baseline population or data at the start of this work, whiteflies from the field sites were tested to determine optimal dose levels that gave percent mortality ranging from 5-95% for both the glass vial and leaf dip bioassays. Glass vial bioassay dosages were in micrograms of active ingredients per ml of acetone, used to coat one vial. The range used for bifenthrin was 0.01 to 3, methomyl 0.125 to 4 and cypermethrin 3 to 200 micrograms of active ingredient per vial. Dosages for the leaf dip bioassays were prepared in parts per million (ppm) of active ingredient in distilled water. The range used for methomyl was 15.63 to 500 and cypermethrin 15.63 to 1000 ppm active ingredient.

2.1.7 Determination of resistance levels

2.1.7.1 Glass vial bioassay

20-ml vials coated with six different concentrations of technical grade samples of cypermethrin, methomyl and bifenthrin, which are commonly used insecticides against whiteflies were used. Each of the concentrations was replicated 3 times for each test. To coat the vials, the insecticides were dissolved in acetone at the insecticide concentration levels that would provide mortality range of 5-95%. 1 ml of the dissolved insecticide was added into each vial. The vials were rotated slowly until the inner surface was uniformly coated with the insecticide. The control vials

were coated with acetone only. The vials were left open to air dry for 20-30 minutes for all the acetone to evaporate. Once confirmed dry, the vials were closed with plastic caps and kept in a cold room till whiteflies were available for use. Approximately 25 active whiteflies from the small perspex cages were introduced into each of the coated vials that were then closed and left for 3 hours. The total number of adult whiteflies per vial was counted and recorded. The vials were then opened to let out the live adult whiteflies and those that remained in the vials were removed and left on a black cloth for about 30 minutes to allow those which were not dead to recover. A soft camel brush was used to remove those that stuck on the inner surfaces of the vials. Adults that had not flown away and could still not walk in a well co-ordinated manner were then counted and recorded as dead.

2.1.7.2 Leaf dip bioassay.

The leaf dip bioassay was carried out on the whitefly populations from Kihara, Nguruman and Kitui as well as on a laboratory culture. French beans (*Phaseolus vulgaris* L.) that provided leaves for the bioassay were grown in the greenhouse without use of any insecticides. Fresh leaves were detached, washed with distilled water, air-dried and a cork-borer used to get discs of about 3.5cm diameter. Emulsifiable concentrates of cypermethrin (Sherpa/Polytrin) and methomyl (Lannate/Methomex) were each made into six concentrations giving 5-95% mortalities.

Three replications of each dosage were used for every complete test and this was repeated on different days. Leaf-discs were dipped in an aqueous solution of

the insecticide for 20 seconds and allowed to air dry on filter paper for 15-30 minutes. The control had leaf-discs dipped in distilled water. The treated leaf-discs were placed on inverted plastic petri dish covers containing a thin layer of 2% agar solution with the upper leaf surface on the agar. Approximately 25 adult whiteflies were placed in petri dishes, which were immediately covered with petri dish lids having the leaf discs. The dishes were then placed in the upright position. Adults moved upwards and fed on the provided leaf materials. Ventilation was provided by small holes drilled on the sides of each dish. The petri dishes were opened after 24 hours and the insects placed on black pieces of cloth for about 30 minutes to allow for possible recovery. Those whiteflies that remained on the cloth showing no well-coordinated movement after the 30 minutes were recorded as dead.

2.1.8 Data analysis

Percent mortality was calculated for every test. The collected data was then adjusted using the Abbott formula (Abbott, 1925); that is,

$$PT = \frac{Po - Pc}{100 - Pc} \times 100$$

In the equation, PT is the corrected mortality, Po is observed mortality, and Pc is control mortality. (All the mortalities are in percentage). The conversion to PT aims at adjusting mortalities to the levels they could have reached if all factors other than the toxicants were uniform (Busvine, 1971). Sums of the totals for the number and adjusted percent mortality were used in the probit analysis in which SAS software 6.12 was employed to assess variations between the study sites.

The lethal concentration values of 50 (LC50) and 90 (LC90) for each insecticide were determined. The LC50 is the median dosage level that gives a response of 50 percent mortality in the insects tested, while LC90 gives a response of 90 percent. The LC values were converted to probits and the log dosage figures plotted against these. Resistance factors were calculated by dividing LC50s of each sample from the field by the LC50 of the reference population (that is, Kitui for Kibwezi and Laboratory culture for Nguruman and Kihara). LC50 or LC90 values were considered significantly different if their 95% fiducial limits did not overlap.

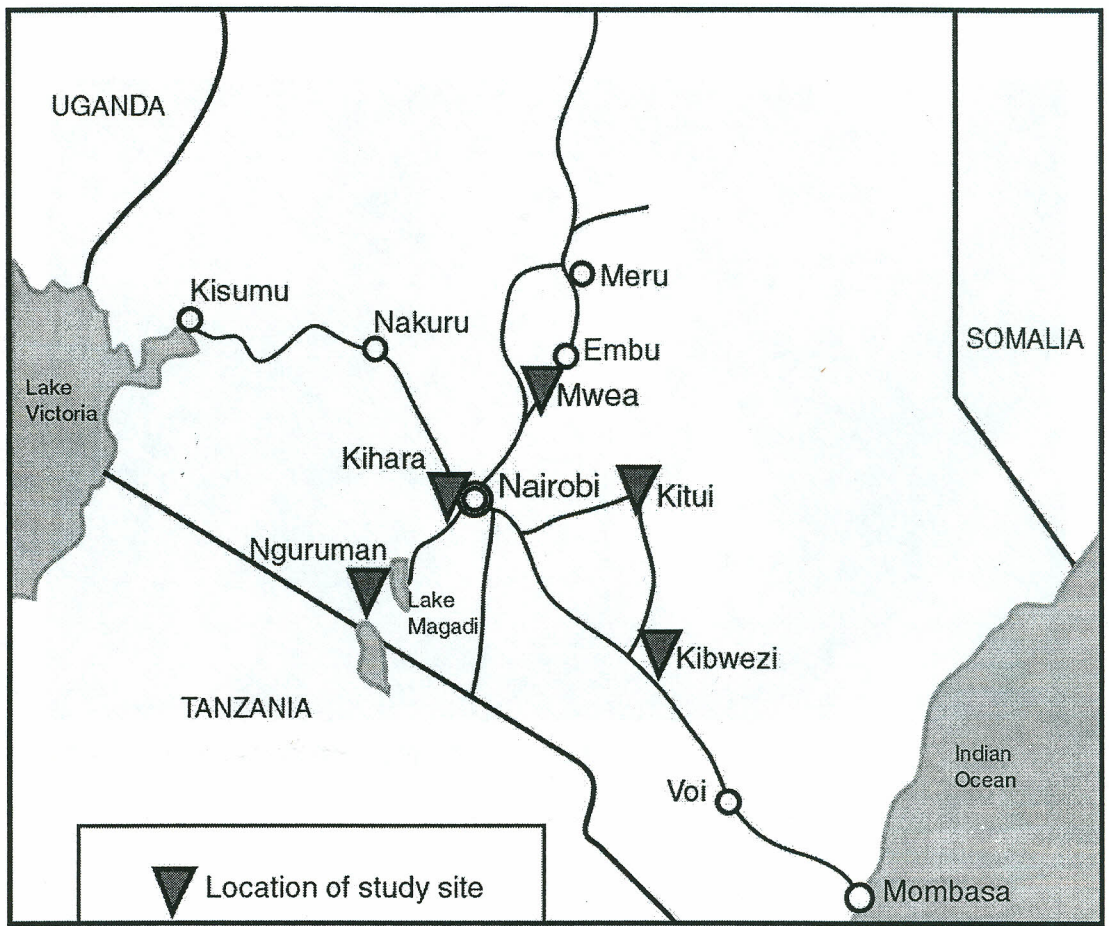


Fig. 1. Location of the study sites in Kenya

3.6 Results

3.7 Observed variation in pesticide usage at the study sites



Plate 1. *Trialeurodes vaporariorum* (Westwood)



Plate 2. *Bemisia tabaci* (Genn.)

3.0 Results

3.1 Observed variation in pesticide usage at the study sites

Cypermethrin and lambda-cyhalothrin were commonly used insecticides against whiteflies and other pests at each of the study sites (Appendix III-VII). Some farmers were found to apply insecticides in alternation without realizing that they were re-using the same chemical sold under different trade names (Appendix II). Such application was most common at Nguruman and Mwea for cypermethrin. The only farmer who used cypermethrin at the Kihara study farms reported that he applied it very rarely. The following were other frequently applied insecticides that were used at all but the study farms at the sites placed in brackets: alpha-cypermethrin (Kitui), dimethoate (Kihara), carbosulfan (Kihara and Mwea), and endosulfan (Kihara and Kibwezi). The use of bifenthrin was reported only in some of the study farms in Kibwezi and Mwea while methomyl was used in Kibwezi, Nguruman and Mwea (Appendices III-VI). Mixtures of detergents with materials like tobacco, ash, crushed onions and pepper were reported to be used by a few farmers in Kitui, Kihara and Mwea. Farmers in Kitui reported the least usage of chemical pesticides against whiteflies with the frequency of application being very low and erratic. The types of pesticides used by various farmers in Kitui were more than in Kihara, but the frequency was higher at the latter study site where the pesticides recorded were only used by two farmers (Appendix IV and VII).

Various insecticides were applied regularly on the vegetables for commercial purposes in Kihara (Appendix IV). However, in one of the study farms, homemade botanicals were used to complement occasional chemical pesticides for the

management of whiteflies on vegetables grown for home consumption. Some farmers in Nguruman reported that polytrin (cypermethrin) had declined in its effectiveness as a control agent for whiteflies while lannate (methomyl) gave better results. Farmers in Mwea frequently applied the insecticides shown in Appendix V. Very frequent spraying using different insecticides was reported by farmers in Nguruman, Kibwezi and Mwea where the surveyed crops are primarily grown for the export market that requires unblemished produce. Many of the pesticides listed in Appendix III were used at least once every season in Kibwezi. There were times when collective spraying of all the crops at the University farm (Kibwezi) was carried out to kill as many of the insect pests as possible.

3.2 Whitefly species

The percentages of the whitefly species from the study sites were calculated and are presented in Table 1. *T. vaporariorum* was the predominant whitefly species in Nguruman (94%), Kihara (98%) and in the Laboratory culture (87%) while *B. tabaci* predominated in Kitui (96%) and Kibwezi (88%) areas. Whitefly samples identified from the Mwea farms consisted of 49% *T. vaporariorum*, 44% *B. tabaci*, 6% *Bemisia afer* (Priesner & Hosney) and 1% *Trialeurodes ricini* (Misra). During the identification of the samples it also became evident that there were sharp variations in the ratio of the species in the farms at different localities in Mwea.

Table: 1 Percentages of whitefly species at the study sites

Site	<i>T. vaporariorum</i>	<i>B. tabaci</i>	<i>Bemisia afer</i>	<i>Trialeurodes ricini</i>
Nguruman	94%	6%	0	0
Kihara	98%	2%	0	0
Kibwezi	12%	88%	0	0
Kitui	0	96%	4%	0
Mwea	49%	44%	6%	1%
Laboratory	87%	13%	0	0

3.3.0 The glass vial bioassays

3.3.1 Responses of the whiteflies to bifenthrin

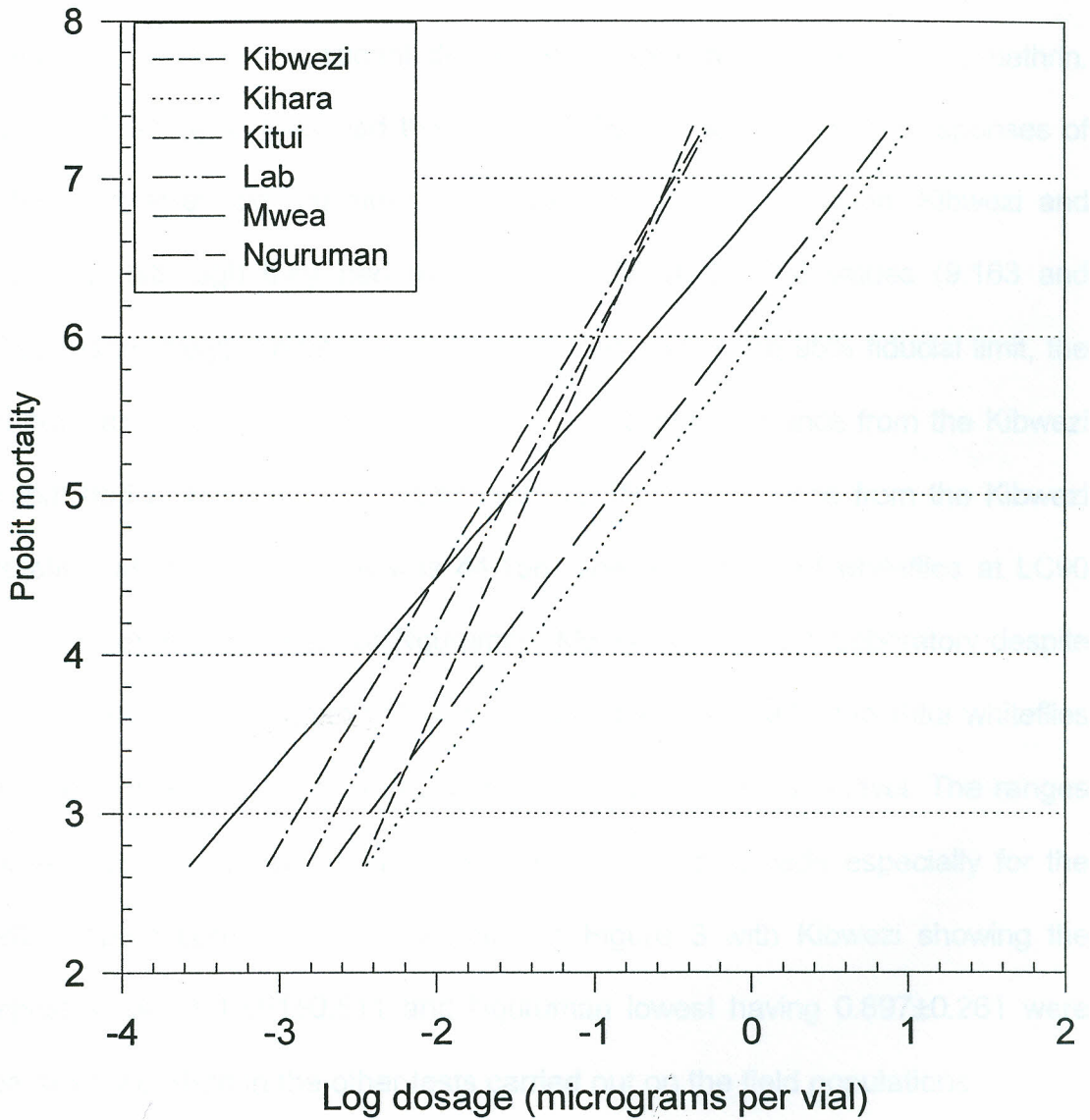
Toxicological responses of the whiteflies to bifenthrin at LC50 varied from 0.021 to 0.191 micrograms of active ingredients while the LC90 values range was 0.120 to 1.700 (Table 2). The LC50 values for Nguruman and Kihara did not differ significantly at the 95% fiducial limit (F.L.) but they were significantly higher than those of Mwea, Kitui, Kibwezi and the Laboratory ones, which showed no significant differences. There was no significant difference between the Nguruman and Kihara whitefly responses at LC90 and also between the Kitui, Laboratory and Kibwezi whitefly responses. The observation that the LC90 for Mwea (0.375) did not differ significantly from the Kitui (0.120) and Kibwezi (0.144) but differed from the laboratory whitefly response (0.143) was unexpected. The slope values for the dosage-response curve were lowest in the Mwea population (1.149 ± 0.069) and highest in the Kibwezi population response (2.222 ± 0.356) (Table 2; Fig. 2).

Table 2: Mortality variations at different sites resulting from bifenthrin using glass vial bioassay

Site	LC50	95%F.L.	LC90	95%F.L.	Slope	N
Nguruman	0.128 b	0.078-0.206	1.227 c	0.654-3.310	1.306± 0.142	3502
Mwea	0.029 a	0.023-0.035	0.375 b	0.292-0.506	1.149± 0.069	1329
Kitui	0.021 a	0.011-0.032	0.120 ab	0.072-0.300	1.674± 0.226	1146
Kihara	0.191 b	0.100-0.373	1.700 c	0.759-7.528	1.350± 0.190	4308
Kibwezi	0.038 a	0.022-0.064	0.144 ab	0.082-0.465	2.222± 0.356	596
Laboratory	0.028 a	0.023-0.035	0.143 a	0.108-0.208	1.824± 0.172	556

Values followed by the same letters at either the LC50 or LC90 level are not significantly different at 95%FL. The values are in micrograms of active ingredient per vial.

Fig.2. Dosage-mortality response of whitefly populations to bifenthrin using glass vial bioassay



3.3.2. Responses of the whiteflies to cypermethrin

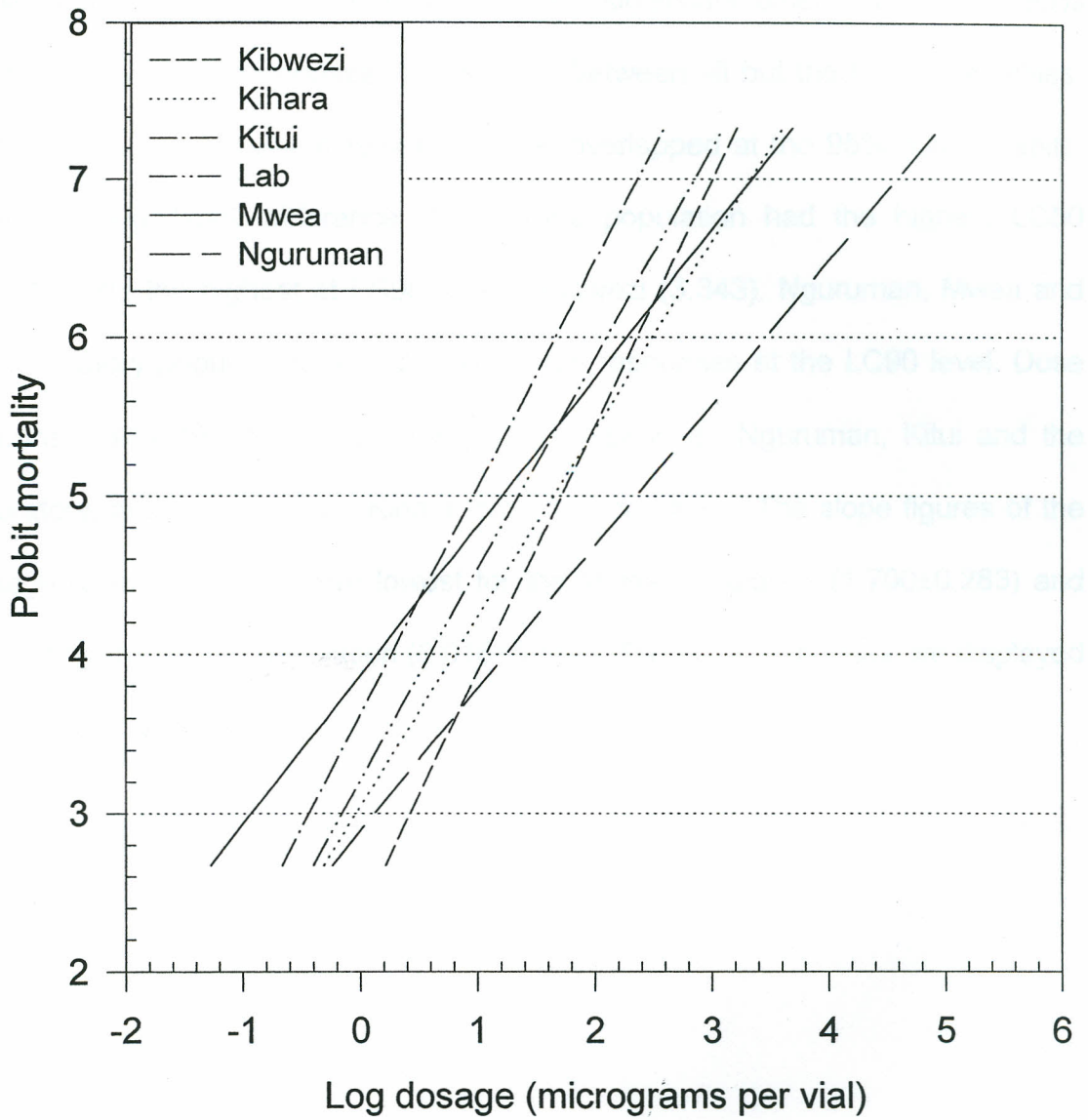
Results summarised in Table 3 show the LC50 and LC90 variations at 95% fiducial limits for the study sites. At the LC50, whiteflies from Nguruman, Kihara and Kibwezi did not show significant differences in their responses to cypermethrin. Whiteflies from Nguruman had the highest LC50 value (223.895). Responses of the Kitui whiteflies did not differ significantly from those in Mwea, Kibwezi and Laboratory, although they had the lowest LC50 and LC90 values (9.163 and 72.228 respectively). An unexpected finding was that at the 95% fiducial limit, the Kitui population (LC50 9.163) did not show significant difference from the Kibwezi one that had LC50 of 51.730 but showed significant difference from the Kibwezi population whose LC50 value was 44.158. The responses of whiteflies at LC90 overlapped at the 95% F.L. for Nguruman, Mwea, Kihara, and Laboratory despite the wide range in the mean LC90 values obtained. Similarly, the Kitui whiteflies only differed significantly from the Nguruman ones at the LC90 level. The ranges between upper and lower fiducial limits were extremely wide especially for the LC90. Slope values that are illustrated in Figure 3 with Kibwezi showing the steepest slope of 1.551 ± 0.511 and Nguruman lowest having 0.897 ± 0.261 were generally lower than in the other tests carried out on the field populations.

Table 3: Mortality variations at different sites resulting from cypermethrin using glass vial bioassay

Site	LC50	95%F.L.	LC90	95%F.L.	Slope	N
Nguruman	223.895 c	90.658-21349	5998 b	724.320- 8401340447	0.897± 0.261	1504
Mwea	16.024 ab	3.711-33.475	373.195 ab	123.367- 17884	0.937± 0.218	1172
Kitui	9.163 a	1.548-19.328	72.228 a	32.795- 704.248	1.429± 0.317	1081
Kihara	44.158 bc	24.451-97.022	529.503 ab	185.380- 11621	1.188± 0.240	2859
Kibwezi	51.730 abc	11.902-306.090	346.800 ab	113.492- 23541798	1.551± 0.511	643
Laboratory	22.200 ab	8.215-40.830	204.189 ab	94.548-1492	1.330± 0.267	617

Values followed by the same letters at either the LC50 or LC90 level are not significantly different at 95%FL. The values are in micrograms of active ingredient per vial.

Fig.3. Dosage-mortality response of whitefly populations to cypermethrin using glass vial bioassay



3.3.3. Responses of the whiteflies to methomyl

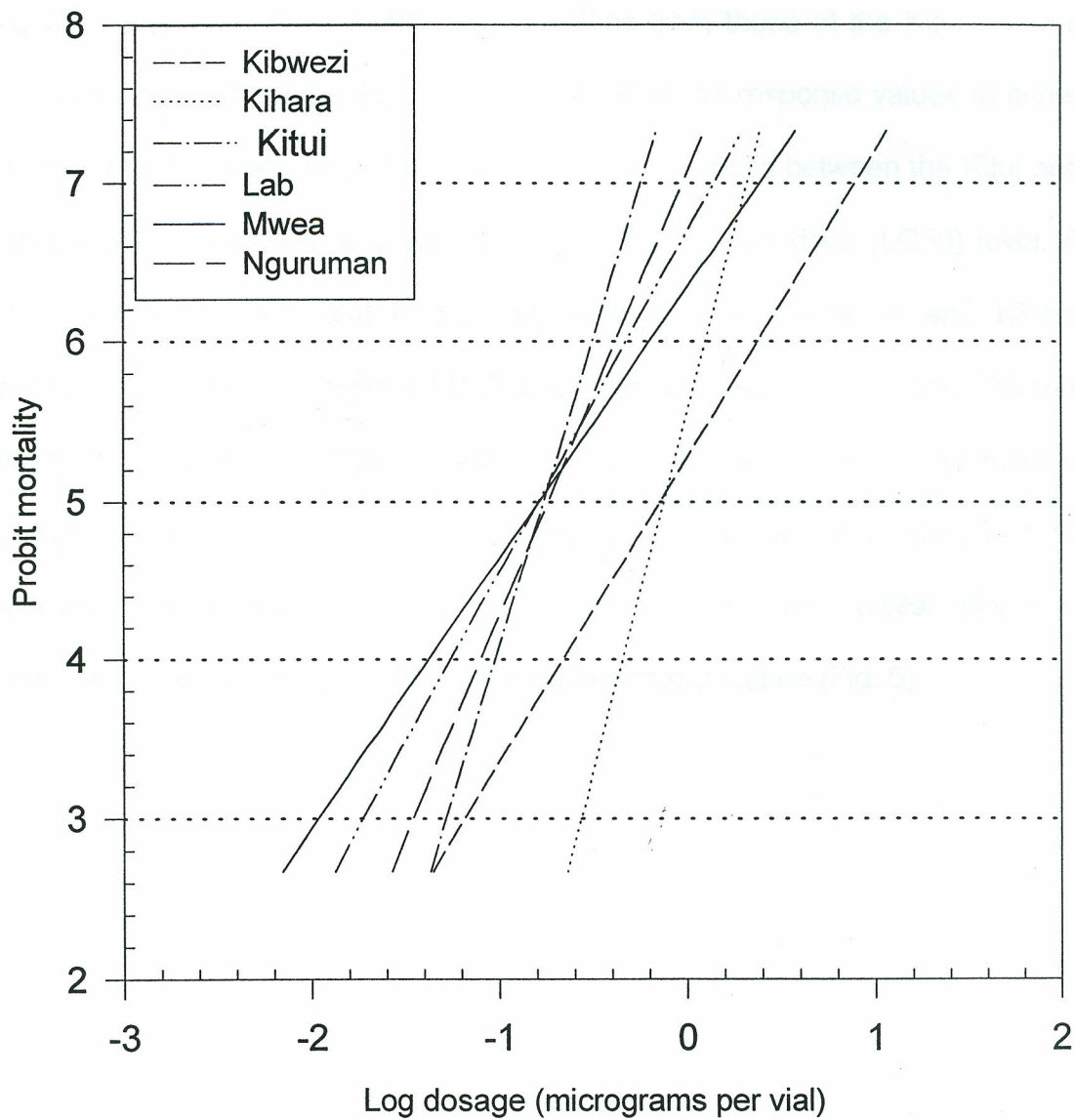
Table 4 shows that at LC50 there were no significant differences at the 95% fiducial limits in the responses to methomyl between all but the Kihara whiteflies. The Kibwezi and Kihara whitefly responses overlapped at the 95% fiducial limits, hence no significant difference. The Kihara population had the highest LC50 (0.754) while the highest at LC90 was at Kibwezi (3.343). Nguruman, Mwea and the laboratory populations had closely related responses at the LC90 level. Dose responses at LC90 did not differ significantly between Nguruman, Kitui and the laboratory, and also between Kihara, Kibwezi and Mwea. The slope figures of the dosage response curve were lowest for the Mwea population (1.700 ± 0.283) and highest for the Kihara population (6.016 ± 3.557). The dose responses are displayed graphically in Figure 4.

Table 4: Mortality variations at different sites resulting from methomyl using glass vial bioassay

Site	LC50	95%F.L.	LC90	95%F.L.	Slope	N
Nguruman	0.183 a	0.160-0.205	0.525 ab	0.452-0.638	2.790± 0.236	851
Mwea	0.163 a	0.067-0.257	0.925 bc	0.573-2.495	1.700± 0.283	1041
Kitui	0.173 a	0.150-0.195	0.371 a	0.319-0.458	3.863± 0.450	536
Kihara	0.754 b	0.548-0.977	1.449 c	1.099-2.493	4.517± 0.727	492
Kibwezi	0.721 ab	0.195-1.906	3.343 c	1.433- 320.522	1.924± 0.523	782
Laboratory	0.162 a	0.082-0.235	0.643 ab	0.433-1.417	2.140± 0.348	563

Values followed by the same letters at either the LC50 or LC90 level are not significantly different at 95%FL. The values are in micrograms of active ingredient per vial.

Fig.4. Dosage mortality response of whitefly populations to methomyl using glass vial bioassay



3.4.0 The leaf dip bioassays

3.4.1 Responses of the whiteflies to methomyl

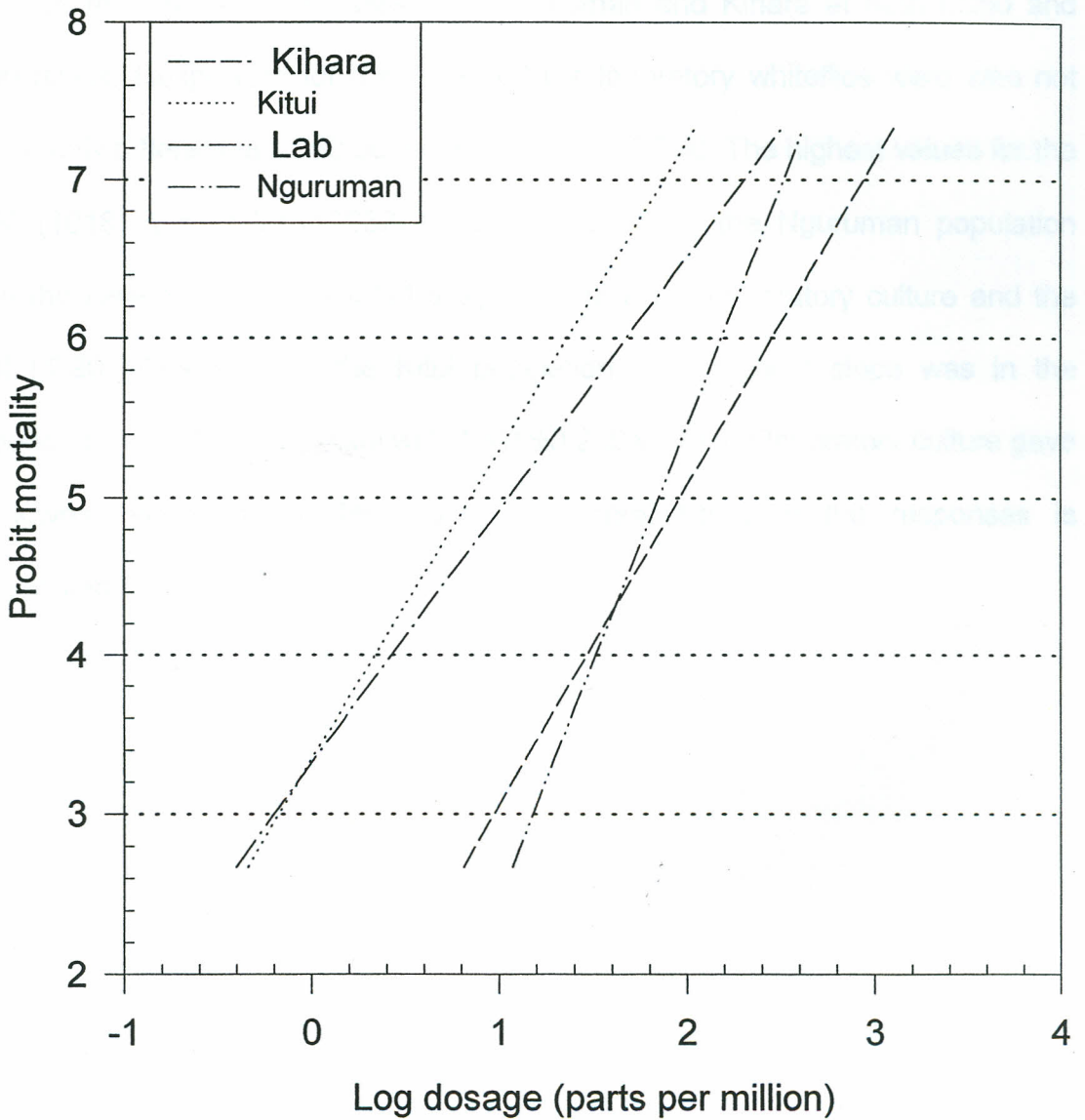
Methomyl leaf dip bioassay produced results that clearly discriminated responses of the Nguruman and Kihara whitefly populations from those of the Kitui and the laboratory populations at the 95% fiducial limits. Dosage-response values at either the LC50 or LC90 levels did not show significant differences between the Kitui and the laboratory populations, and were closer at the median dose (LC50) level. A similar trend was also found in the responses of the Nguruman and Kihara whiteflies. Kihara had the highest LC50 and LC90 values of 90.530 and 385.594 respectively, while Kitui had the lowest values of 7.047 and 31.834, respectively. The highest slope of 3.014 ± 0.299 was produced by the Nguruman whitefly population followed by Kihara with 2.036 ± 0.158 , and the lowest slope of 1.603 ± 0.501 was given by whiteflies from the laboratory culture (Fig. 5).

Table 5: Mortality variations at different sites resulting from methomyl using leaf-dip bioassay

Site	LC50	95%F.L.	LC90	95%F.L.	Slope	N
Nguruman	70.094 b	56.562-86.447	186.591 b	142.733-278.237	3.014± 0.299	1087
Kitui	7.047 a	4.207-9.712	31.834 a	26.795-38.562	1.957± 0.257	1115
Kihara	90.530 b	72.489-113.258	385.594 b	277.688-617.842	2.036± 0.158	1257
Laboratory	11.195 a	0.002-26.050	70.535 a	32.365-4639	1.603± 0.501	533

Values followed by the same letters at either the LC50 or LC90 level are not significantly different at 95%FL. The values are in parts of active ingredient per million.

Fig.5. Dosage-mortality response of whitefly populations to methomyl using leaf dip bioassay



3.4.2 Responses of the whiteflies to cypermethrin

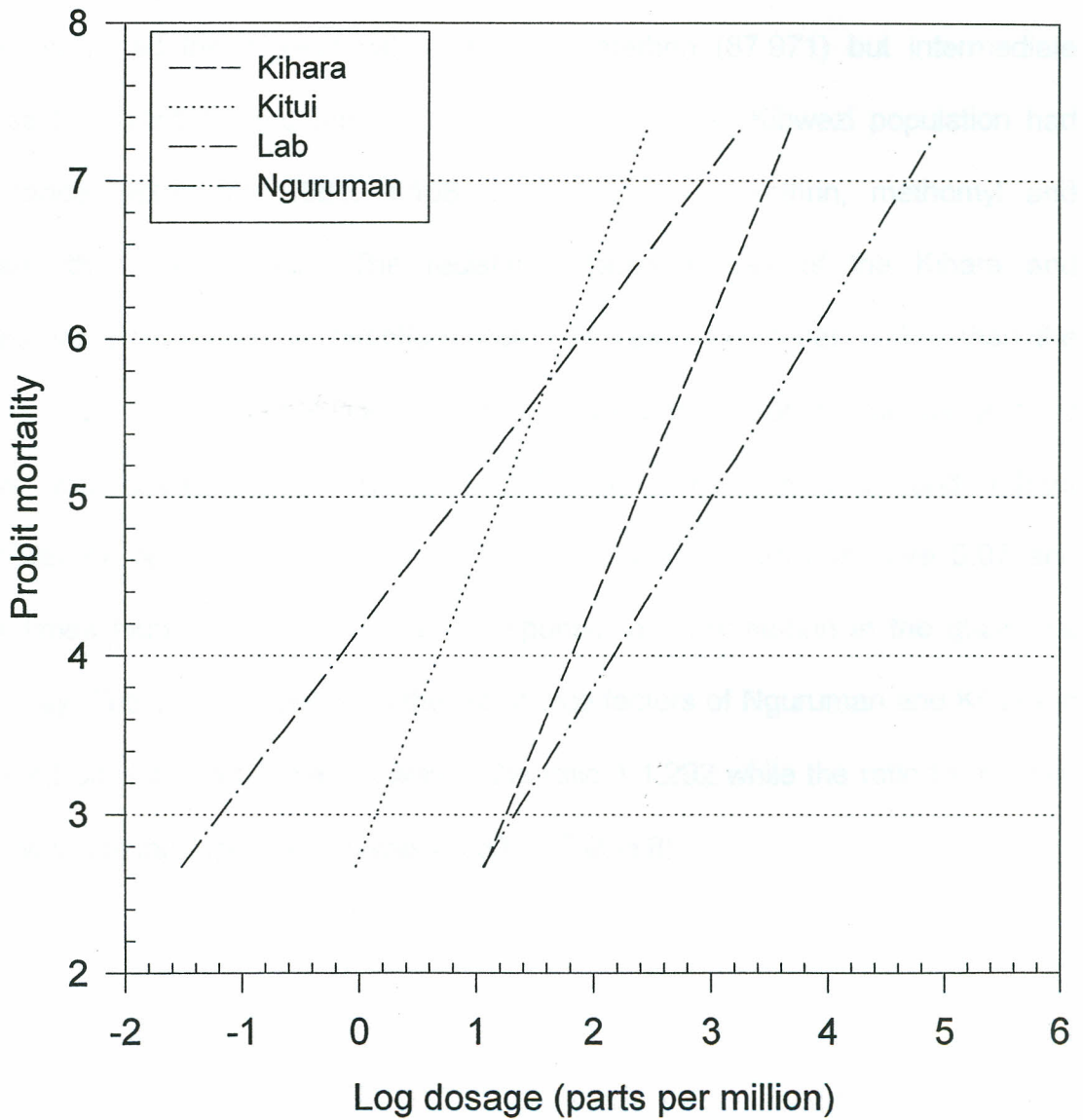
The results in Table 6 show that no significant differences were detected between the dose responses of whiteflies from Nguruman and Kihara at both LC50 and LC90 levels. Responses for the Kitui and the laboratory whiteflies were also not significantly different at LC50 but were different at LC90. The highest values for the LC50 (1018) and LC90 (12032) were obtained from the Nguruman population while the lowest LC50 values (11.572) were from the Laboratory culture and the least LC90 (79.915) from the Kitui population. The highest slope was in the response of whiteflies from Kitui with 1.870 ± 0.209 while the laboratory culture gave the lowest slope of 1.094 ± 0.133 . The general trend of the responses is represented by Figure 6.

Table 6: Mortality variations at different sites resulting from cypermethrin using leaf-dip bioassay

Site	LC50	95%F.L.	LC90	95%F.L.	Slope	N
Nguruman	1018 b	592.805-2763	12032 c	3929-130267	1.195± 0.174	1326
Kitui	16.495 a	11.886-20.965	79.915 a	63.938-107.305	1.870± 0.209	623
Kihara	238.798 b	125.706-646.823	1249 c	511.450-23834	1.783± 0.404	993
Laboratory	11.572 a	6.245-17.533	171.590 b	123.498-269.059	1.094± 0.133	611

Values followed by the same letters at either the LC50 or LC90 level are not significantly different at 95%FL. The values are in parts of active ingredient per million.

Fig.6. Dosage-mortality response of whitefly populations to cypermethrin using leaf dip bioassay



3.5 Variation in resistance factor (R.F.) values

There were distinct variations in the resistance factor (RF) values at LC50 for the field populations in the glass vial bioassay for the pesticides used (Table 7). Resistance factors from the glass vial bioassay for bifenthrin and methomyl were the highest in the Kihara population at 6.821 and 8.087 respectively, while Nguruman had the highest values for cypermethrin (87.971) but intermediate values for bifenthrin and the lowest for methomyl. The Kibwezi population had resistance factors of 1.081, 4.168 and 5.646 for bifenthrin, methomyl and cypermethrin, respectively. The resistance factor values of the Kihara and Nguruman populations for bifenthrin were 6.31 and 4.23 times higher than the values of the Kibwezi population. The ratio of resistance factors for methomyl in the glass vial bioassay was 1:3.69:6.72 for Nguruman, Kibwezi and Kihara respectively. Nguruman and Kibwezi had resistance factors that were 5.07 and 2.84 times higher than for Kihara in response to cypermethrin in the glass vial bioassay. The variation between the resistance factors of Nguruman and Kihara in the methomyl leaf dip bioassay was in the ratio 1:1.292 while the ratio for the two sites when using cypermethrin was 4.263: 1 (Table 8).

Table 7. Resistance factors at L50 for the glass vial bioassay

Site	Bifenthrin	Methomyl	Cypermethrin
Nguruman	4.571	1.130	10.085
Kihara	6.821	7.593	1.989
Kibwezi	1.081	4.168	5.646

Table 8. Resistance factors at L50 for the leaf dip bioassay

Site	Methomyl	Cypermethrin
Nguruman	6.261	87.971
Kihara	8.087	20.636

4.0 Discussion

Although the word population refers to members of one species living together, it is repeatedly used in this discussion to refer to whiteflies from the same site, that is, "field population" that may consist of more than one species. Every study site had more than one species and the percentage constitution of the species was found to differ. Altitude and environmental temperatures are likely to have been partly responsible for the observed species diversity. *B. tabaci* was found to be the dominant whitefly species in the hotter lower altitude areas of Kibwezi (700m a.s.l.) and Kitui (1000m a.s.l.) while *T. vaporariorum* was predominant at the cooler higher altitude (1800m a.s.l.) area of Kihara. However, *T. vaporariorum* was also predominant in Nguruman, which is hotter and low-lying (800m a.s.l.). Mwea (1260m a.s.l.) was the only site with four whitefly species identified from the vegetable crops surveyed. Although the host preference and range have been found to differ with whitefly species (Greathead, 1986; Mound and Halsey, 1978), variation in types of vegetable crops might not account for the observed levels of species diversity at the sites because similar crops at some of the sites were found to harbor different species. Non-cultivated alternative host plants might have been more influential in determining the species of adult whiteflies collected from the crops. This is likely because weeds and wild plants near the study farms were observed to differ with sites and could have been the source of re-infestation after every cycle of insecticide application or at the start of a new season. The crops at different sites could, however, be similar due to the preference by farmers, and the use of irrigation to cultivate crops that could otherwise not have grown at

certain sites in particular times of the year.

The number of adult whiteflies tested (N) for each chemical varied due to factors highlighted later in this discussion. The numbers used in the bioassays were however satisfactory as Robertson *et al.* (1984) found that 120 was the minimum number necessary for reliable estimation, but that precision was better with the number (N) being 240 and above. The results at the extremes of the LC range are less reliable than at the LC50 (Busvine, 1971), hence the LC90 values were not used in calculating resistance factors. The Kibwezi whitefly population gave moderately high resistance factors to cypermethrin (5.646) and methomyl (4.168) but showed no resistance to bifenthrin with a resistance factor of only 1.081. Resistance to bifenthrin was also lower than to cypermethrin in Nguruman, and the LC50 values for bifenthrin were lower than for the other insecticides at every study site. These results compare well with those by Dittrich *et al.* (1990 a & b) on the Sudanese *B. tabaci* populations which also showed high resistance to cypermethrin but not to bifenthrin. Cypermethrin was more frequently applied under various trade names at the study sites than bifenthrin and this could have contributed to the development of resistance against the insecticide. Use of bifenthrin was not reported in the Nguruman, Kitui and Kihara study farms. Notwithstanding, it was noted that the resistance factor for bifenthrin in Kihara was high (6.821) while that for cypermethrin at the same site was 1.989 in the glass vial results. The pyrethroid (cypermethrin) was reportedly rarely used in the target farms, consequently, cross-resistance is unlikely to account for resistance to bifenthrin. The whiteflies at every study site responded very differently to cypermethrin and bifenthrin as indicated by the great differences between their LC

and RF values, despite the two insecticides belonging to the same chemical group. Resistance can develop to insecticides that have never been applied to a population (Abdeldaffie *et al.*, 1987) and this appears to be true for bifenthrin in Kihara. The high level of resistance could have been due to factors other than selection by exposure to pesticides.

It was noted that resistance to methomyl in Kihara appeared to be moderately high and consistent from both the glass vial and leaf dip methods (RF 7.593 and 8.087 respectively) yet the chemical was not reported to have been in use at the target farms. It is likely that methomyl could have been in use in the nearby farms and whiteflies from there could have occasionally migrated to the target farms. However, such influence could have been trivial, as the immediate neighbors were not found to carry out pest management using insecticides. Resistance to methomyl (a carbamate) in the *T. vaporariorum* predominated field population in Kihara could therefore have been due to a mechanism that is not triggered by exposure to the insecticide or other carbamates as there was none in use at the farms.

The overall response of each category of the field populations revealed that the *T. vaporariorum* dominated populations showed greater tolerance to the three pesticides than the *B. tabaci* ones as indicated by higher LC values. This collective comparison was taken to explore possible influence of body size on resistance expression as had been highlighted by Busvine (1971) regarding insects in general. The larger body size of *T. vaporariorum* compared to that of *B. tabaci* provide the latter species with a larger surface area to volume ratio. This

implies that *B. tabaci* could have had greater exposure to the chemicals per unit body size in the glass vial tests and the subsequently higher concentration of absorbed insecticides could have increased their mortality. Once a given amount of toxins was taken in, it was more likely to spread to a higher percentage of the body organs in the smaller insect within a specific period of time. Such effect could have been more pronounced in the leaf dip tests where the insects not only came into contact with the toxins but also fed on leaf materials that were previously soaked in solutions of the insecticides. Differences in the genetic make-up of the two species could also have contributed to the observed variations. Genetic make-up, ease of penetration/absorption and body size are some of the causes of species specificity in responses to pesticides discussed by Busvine (1971).

Resistance levels of the populations predominated by either of the two species varied between the study sites. In spite of the possible influence of size discussed above for the two categories of sites, the *B. tabaci* dominated population of Kibwezi was more resistant to cypermethrin and methomyl than the predominantly *T. vaporariorum* whiteflies from Kihara and Nguruman respectively. The heavy and very frequent spraying of cypermethrin and methomyl that was reported at the Kibwezi farms could have exerted selection pressure on the whiteflies resulting in the observed levels of resistance to the insecticides. None of the sites dominated by *T. vaporariorum* (Kihara and Nguruman) gave consistently higher LC50 values than the other for all the insecticides, but the predominant *B. tabaci* whiteflies from Kibwezi had consistently higher LC50 values unlike the ones from Kitui. The latter observation could have been due to the heavier insecticides usage reported at the Kibwezi farms selecting for resistance as had been observed elsewhere (Cahill *et*

al., 1995). The Kitui whiteflies were generally more susceptible to the three pesticides than any from the other field sites. This was most likely due to low selection pressure from the low insecticide applications reported in the area compared with the other areas that had much higher pesticide usage. The low tolerance of the Kitui whiteflies could in part have been due to ecological stress as the tests were carried out during the dry season when the temperatures in the semiarid area were constantly high and the host crops were also ecologically stressed. Castle *et al.* (1996) reported higher susceptibility of whiteflies to insecticides at the end of each season suggesting ecological stress factors leading to physiologically weakened whiteflies.

Low slope values in the dose-mortality results may indicate heterogeneity in the population (Cahill *et al.*, 1995). The highest slope value in this study was for the Kihara whiteflies (4.517 ± 0.727) in response to methomyl and the lowest was for the Nguruman whiteflies (0.897 ± 0.261) in response to cypermethrin, both of which were in the glass vial bioassay. Steeper slopes in response to a pesticide indicate the chemical to be more toxic to the pests and there is a bigger change in kill over small changes in dosage (Yassin *et al.*, 1989; Busvine, 1971). Overall, cypermethrin had the lowest slopes for both bioassay methods while methomyl had the highest. A comparison of the dosage-mortality curves for all of the field sites in the glass vial results showed that the populations with relatively low slopes were Kibwezi for methomyl, Nguruman for cypermethrin and Mwea for the three insecticides. Such slopes show that the insects tested had wide variations in their susceptibility to the insecticides. The low slopes could indicate genetic heterogeneity that may allow for development of resistance (Prabhaker *et al.*,

1985; Abdeldaffie *et al.*, 1987). The three populations therefore have greater potential for developing higher levels of resistance to the corresponding insecticides. This could be due to the selection pressure exerted by the chemicals that have been in use at the study sites.

Comparing results of different types of bioassays is a tricky task but it is acceptable to examine resistance levels that were revealed by one bioassay and compare them to resistance levels determined by the other bioassay (Castle, pers. comm.). The bioassay method used has some impact on the insecticide resistance expression (Dennehey *et al.*, 1983). The leaf dip and glass vial methods have been widely used to discriminate between resistant and susceptible whitefly strains elsewhere (Cahill and Hackett, 1992; Prabhaker *et al.*, 1996). The results on resistance factor (Tables 7 and 8) and the subsequently calculated ratios comparing the two methods indicate that the leaf dip bioassay provided greater discrimination between the reference and field populations. This type of difference between bioassays for whiteflies was reported by Dr. Dennehey from the University of Arizona (Castle, pers. comm.). The RF values from the leaf dip bioassay were much higher than the ones from the glass vial bioassay and these results agree well with those of Cahill and Hackett (1992) on bifenthrin and cypermethrin. Response to bifenthrin in the leaf dip bioassay was not carried out in this study because the emulsifiable concentrates required for the tests could not be procured. Cahill and Hackett (1992) also reported the leaf dip bioassay as giving the greatest discrimination between pyrethroid susceptible and resistant strains, and stated that the best method is that which gives the best discrimination.

When the whiteflies were removed from the test vials/petri dishes after exposure to the insecticides, more were observed to recover in the glass vial bioassay than in the leaf dip bioassay. It seems that the leaf dip tests exposed the whiteflies more to the insecticides than the glass vial tests, possibly due to the longer duration the insects stayed in the experimental petri dishes. Part of the insecticides could also have been consumed by the whiteflies as they fed on the treated leaf materials and this could have added to the negative effect of the toxins penetrating through the cuticle. The glass vial method also had one shortcoming in that the whiteflies were sticking on the inner vial surfaces especially at higher concentrations. Other researchers have reported similar observation and attributed it to the pesticide residues on the glass surface (Cahill and Hackett, 1992; Sanderson and Roush, 1992). Cypermethrin was the chemical with the highest number of whiteflies sticking, and this could have been due to the gelatinous nature of its technical grade that was used to coat the vials. Variation in the number of the insects that died as a result of this entrapment could have contributed to the very wide fiducial ranges produced for cypermethrin. More whiteflies from the *B. tabaci* predominated populations were observed to stick to the vials than those from the *T. vaporariorum* populations, possibly because the smaller body size of *B. tabaci* brought it into closer contact with the coated glass surface. Their slanting tent-like wings were also more likely to come into contact with the sticky surface than the horizontally spread wings of *T. vaporariorum* (Plate 1&2).

An overview of the averages of the resistance factors obtained for different insecticides in this study indicated that the whiteflies tested had close

susceptibility levels to methomyl and bifenthrin in the glass vial bioassay (Average R.F.s of 4.297 and 4.158 respectively), but not to cypermethrin that gave a higher average R.F of 17.72. Cypermethrin also produced the highest of all resistance factor values in the leaf dip bioassay. The higher the resistance factor, the higher the level of resistance of the field samples to the tested insecticide (Prabhaker *et al.*, 1985). This implies that resistance to cypermethrin was generally higher than to either bifenthrin or methomyl, both of which had higher toxicity as indicated by the lower LC values and higher slope figures. Low tolerance to bifenthrin and methomyl have been reported in earlier work (Prabhaker *et al.*, 1996; Cahill *et al.*, 1995). The highest LC values for the two bioassay methods were for cypermethrin at Nguruman. This is the site where some farmers had reported that polytrin (cypermethrin) was giving poor results in whitefly management and the observed results could therefore have been due to prolonged selection by the insecticides.

A fair comparison of response of the predominant *B. tabaci* and *T. vaporariorum* populations to the insecticides in the leaf dip bioassay could not be carried out. This is because only the reference *B. tabaci* population in Kitui was tested using leaf dip method and no resistance factors could therefore be calculated. Kibwezi was the only other studied site with a clear majority of the whiteflies identified as *B. tabaci*. The long distance from Kibwezi to Nairobi and logistic problems made it impossible to carry out all the expected tests at the site. The timing of the Kibwezi whitefly collection trips was critical not only because of the seasonality of the whiteflies (see section 2.2) but also because of the crop protection practices carried out there (see section 3.1). The LC50s of the Kitui population that was constituted of 96% *B. tabaci* were far below those of the field populations with high

percentages of *T. vaporariorum*. The LC50s were close to those of the laboratory culture but the LC90s were less than half those of the *T. vaporariorum* dominated culture.

The fiducial limits in most of the data sets analyzed were very wide especially for the LC90 because some of the sets did not fit well in the probit model. The probit analysis program automatically includes a heterogeneity factor in the analysis of data sets in which heterogeneity is detected and this also contributes to wide FL ranges (Finney, 1964; SAS, 1994). The fiducial limits about a log dose-probit line are curvilinear, being narrowest at the midpoint (LC50) and widest near both ends (LC10 and LC90). A response at a single dose in which the mortality is a little below or above the expected value can have a strong effect on the fiducial limits at the LC90 (Castle, pers. comm.). This might be the reason for the extremely wide FL ranges observed in the LC90 limits. Assessment of mortality after 24 hours in the leaf dip tests could have contributed to wide variations in the response, resulting to the poor fit in the probit model. Some of the whiteflies recorded as dead were still responsive to stimulation and there is a possibility that a number of them could finally have recovered enough to fly away. It is also likely that some whiteflies recorded as being alive were only displaying some final spasms of hyperactivity and they could have died within a very short time. The possible influence of the experimental duration was reported by Cahill *et al.* (1996b) who got erratic and unrepeatable results with poor dose-response relationship when assessment was done after 24 hours, but more consistent results after 48 hours. However, there are researchers who assessed the mortality after 24 hours and got consistent results with good dose-response relationship

(Omer *et al.*, 1992). The probit statistical model is constrained for use on field populations because of their natural heterogeneity that can produce wild fiducial limits (Castle, pers. comm.). The lack of fit in the probit model could have been due to great differences in diet, sex and age of the insects tested, environmental temperatures and humidity as well as some possible variations in the preparation of individual test units. Busvine (1971) noted interaction between environmental conditions and the physiological state of the insect in influencing tolerance to insecticides. He also reported that males of most species of insects were more susceptible to contact insecticides than the females, and this implies that sex ratio variation in the replicates could have had significant influence on the fiducial limits. MacCuaig (1968) reported that locusts fed before treatment with diazinon tolerated nearly double the dose of those unfed. Although the whiteflies tested in this study were free to feed in the fields, differences in the feeding prior to the tests could have resulted in differences in susceptibility. The poor fit in the model could also have been due to heterogeneity in the field population due to the presence of other species or biotypes in some replicates and not in the others as tolerance varies with species. The recorded relative proportions of species tested were based on the pupae collected from crops and this does not necessarily mean that the adults collected from the crops were of that/those same species, as an insect may prefer a host plant for feeding but not for breeding. Variation in mortality for different replicates as a result of whiteflies sticking onto the vial surface or the treated leaf discs was also a likely contributory factor. The differences as a result of diet were likely because data from different host crops were pooled. Another possible cause could have been the pooling of replicates for data collected on different days, as there could have been very great variations

between the days. Seasonal changes have been found to affect whitefly susceptibility to insecticides (Castle *et al.*, 1996). The pooling of data reduced replication, yet, greater replication would have acted to dampen variation, thus producing a data set that conforms better to the probit statistical model (Castle, pers. comm.). The great difference in the fiducial ranges accounts for the unexpected results like the one for cypermethrin (glass vial bioassay) in which Kitui and Kibwezi did not show significant difference but the Kihara site, that had an LC50 value higher than for Kitui but lower than that for Kibwezi, differed significantly from the Kitui site. The Kibwezi fiducial range was very wide and so overlapped with the lower figures for Kitui unlike the narrow range for Kihara. A similar explanation suffices for the unusual observation on the levels of *significance between LC50 for Mwea, Kitui and Kibwezi in the bifenthrin glass vial results*. Other researchers have used the overlap of fiducial/confidence limits to show possible significant differences in insecticide resistance testing (Sivasupramaniam *et al.*, 1997).

The probit analysis used sums of the data for every dose level of each insecticide for every site. Other researchers have previously used percentage sums of the dead insects to compare resistance of different whitefly strains (Cock *et al.*, 1995). *Pooling of the data helped to get a general idea on resistance level variations between the sites*. It however failed to show possible influence of the host crops in the five sites, yet diet may have a strong influence on the susceptibility of insects to pesticides (Busvine, 1971, Sivasupramaniam *et al.*, 1997). The influence of host plants was overlooked because:

1. The number of whiteflies collected from different crops per site varied greatly,

and the number from some of the crops was hardly enough for a few replicates. This was partly because of the preference of farmers to grow certain crops at particular times of the year, and the whiteflies' host preference and availability. Logistics limitations could not allow for bioassays of individual sites at the most opportune time.

2. This study targeted small holder vegetable farmers and most have small pieces of land in which inter-cropping is done, or different crops are grown in small strips /plots adjacent to one another in the open fields. The whiteflies moved freely from one host plant to another and the influence of each host could therefore not be easily assessed.
3. The logistics problems and seasonality of whiteflies in some sites also hampered the possibility of assessing the influence of environmental factors, particularly those related to the distinct dry and wet seasons in Kenya. The results therefore could not show possible variation in resistance levels due to the environmental temperature and humidity, both of which contribute to variation in tolerance to toxins and recovery after poisoning (Busvine, 1971).

Whitefly adults from Mwea had to be transported back to Nairobi but those from the other sites were tested close to the collection site. The cool box used for carrying whiteflies from the field to the laboratory did not have temperature regulatory system. The conditions in the cool box were therefore not standardized yet there is need to standardize experimental conditions so as to get comparable results (Busvine, 1971). It is likely that these conditions contributed to whiteflies from the Mwea fields having the highest number of replicates that were discarded as a result of having mortality values higher than 20% in the controls. Such tests

should be repeated but this was not always possible with the Mwea whiteflies because by the time the first test was completed, most of the remaining adults were even weaker than the first lot. The prolonged stay in the cool box while in the field and on transit could therefore have affected dose-mortality responses in the tests. The insects could have been very weak by the time of testing and therefore succumbed more readily to the insecticides, hence the relatively low LC50 values for all the insecticides tested. The Mwea whiteflies had LC50 values that were only slightly higher than those of Kitui for bifenthrin and cypermethrin in the glass vial method despite there being heavier pesticide usage at the former site. The LC90 values were however higher for the three insecticides, possibly due to the presence of some few resistant whiteflies that were less adversely affected by extraneous factors. The Mwea whiteflies gave relatively low slope values for all the chemicals and this indicated heterogeneity in the population. Heterogeneity in the Mwea whiteflies could have been due to the presence of a higher number of whitefly species as shown in the species identification results, and extraneous mortality factors such as the temperature variation could also have acted to dampen homogeneity.

The two reference whitefly populations in this study were basically field populations as specified in section 2.1.4. This is because clearly susceptible laboratory cultures were not available for use as the controls. Using a field population as a reference strain is justifiable, and in many respects is superior to using a laboratory colony that has been maintained for an indefinite number of years (Castle, pers. comm.). Often, such laboratory-maintained colonies give results that overestimate the resistance factor because they are genetically unfit

and show no tolerance to insecticides in a bioassay (Castle, pers. comm., Sawicki, 1987). On the other hand, the resistance factors obtained in this study could have been underestimated by the use of field populations as the control. The Mwea population did not have a reference population and so no resistance factors were calculated for the site. It was realized after identification of the pupae samples that this field population was constituted of four whitefly species with *T. vaporariorum* and *B. tabaci* being the majority, and as such neither the laboratory culture nor the Kitui field population could fit to be used as its control.

4.1 Conclusions

- (i) The level of whitefly resistance to insecticides varied with localities in the selected vegetable growing areas of Kenya and was not consistent for all the insecticides tested.
- (ii) Bifenthrin was the most effective insecticide against the whiteflies in the glass vial bioassay followed by methomyl while cypermethrin was the least effective. Cypermethrin may therefore be less effective in the management of whiteflies in Kenya.
- (iii) Overall comparison showed that the *T. vaporariorum* dominated populations were generally more resistant to methomyl, bifenthrin and cypermethrin than those dominated by *B. tabaci*. However, the *B. tabaci* dominated sites with heavy pesticide usage recorded higher resistance than some *T. vaporariorum* dominated sites.
- (iv) There was an overall indication of some positive relationship between pesticide usage and resistance to the chemicals but there were some exceptions, as a few cases of resistance to some of the insecticides were found at sites where they were reportedly never used.
- (v) The glass vial bioassay was found to be less effective than the leaf dip bioassay in discriminating between the susceptible and resistant whiteflies.

This study has provided, for the first time, information on the resistance status of some field populations of whiteflies in Kenya. The findings can be used as a basis

for further work on resistance and in selecting suitable insecticides for integrated pest management of whiteflies in vegetable cropping systems in Kenya.

5.0 Suggestions for future research work

1. There is need to carry out further research to compare toxicological responses of whiteflies to all major classes of insecticides commonly being used. The future resistance assessment in Kenya should rely not only on bioassays but also on the modern molecular and genetic techniques that can disclose the underlying resistance mechanisms in the whiteflies.
2. Variations in susceptibility of the whiteflies to insecticides due to the influence of host plants need to be investigated.
3. Investigation on resistance of the different life cycle stages of the whiteflies should be carried out as their change in anatomy, physiology and size could influence susceptibility to insecticides.
4. Investigation on whitefly resistance to insecticides in cotton and floriculture farms (where pesticide application is high) should also be carried out.

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Appendix I: Conversion table for percent mortality to probits

Percent	Probit	Percent	Probit	Percent	Probit	Percent	Probit
1	2.67	26	4.36	51	5.03	76	5.71
2	2.95	27	4.39	52	5.05	77	5.74
3	3.12	28	4.42	53	5.08	78	5.77
4	3.25	29	4.45	54	5.1	79	5.81
5	3.66	30	4.48	55	5.13	80	5.84
6	3.45	31	4.5	56	5.15	81	5.88
7	3.52	32	4.53	57	5.18	82	5.92
8	3.59	33	4.56	58	5.2	83	5.95
9	3.66	34	4.59	59	5.23	84	5.99
10	3.72	35	4.61	60	5.25	85	6.04
11	3.77	36	4.64	61	5.28	86	6.08
12	3.82	37	4.67	62	5.31	87	6.13
13	3.87	38	4.69	63	5.33	88	6.18
14	3.92	39	4.72	64	5.36	89	6.23
15	3.96	40	4.75	65	5.39	90	6.28
16	4.01	41	4.77	66	5.41	91	6.34
17	4.05	42	4.8	67	5.44	92	6.41
18	4.08	43	4.82	68	5.47	93	6.48
19	4.12	44	4.85	69	5.5	94	6.55
20	4.16	45	4.87	70	5.52	95	6.64
21	4.19	46	4.9	71	5.55	96	6.75
22	4.23	47	4.92	72	5.58	97	6.88
23	4.26	48	4.95	73	5.61	98	7.05
24	4.29	49	4.97	74	5.64	99	7.33
25	4.33	50	5	75	5.67		

This conversion table was adapted from Finney, 1964.

Appendix II: A general list of commonly used pesticides against whiteflies at the study sites

Common/trade names	Chemical name	Chemical group
Rogor	Dimethoate	Organophosphate
Polytrin	Cypermethrin	Pyrethroid
Ripcord	Cypermethrin	"
Ambush	Cypermethrin	"
Cymbush	Cypermethrin	"
Barricade	cypermethrin	"
Sherpa	Cypermethrin	"
Marshal	Carbosulfan	Carbamate
Fastac	Alpha-cypermethrin	Pyrethroid
Celecron (<i>Curacron??</i>)	(<i>Profenofos??</i>)	(Organophosphate)
Lybacid/ Lebaycid	Fenthion	Organophosphate
Azocord	?	(<i>Petroleum spray oil?</i>)
Thiodan	Endosulfan	Organochlorine
Brigade	Bifenthrin	Pyrethroid
Diazinon	Diazinon	Organophosphate
Niocidol	Diazinon	organophosphate
Kayazinon	Diazinon	organophosphate
Diazol	Diazinon	Organophosphate
Danadim	Dimethoate	Organophosphate
Malathion	Malathion	Organophosphate
Karate	Lambda-cyhalothrin	Pyrethroid
Sumithion	Fenitrothion	Organophosphate
Thionex	Endosulfan	Organochlorine
Dimethoate	Dimethoate	Organophosphate
Lannate	Methomyl	Carbamate
Methomex	Methomyl	Carbamate
Evisect	Thiocyclam-hydrogen oxalate	Trithiane
Trigard	Cyromazine	Triazine
Ekalux	Quinalphos	Organophosphate
Ekatin	Thiometon	Organophosphate
Dipel	<i>B. thuringiensis</i>	Biopesticide
Amitraz	Amitraz	Amidine
Folimat	Omethoate	Organophosphate
Others: 1. <i>Dithane, Antracol, thiovit and ridomil</i>		1. Fungicides
2. <i>Mixtures of detergent and tobacco/onions/pepper/ash</i>		2. <i>Homemade botanicals?</i>

References for the identification of chemical names and classes in the above table were Royal society of chemistry 1991. *The agrochemical handbook*. 3rd edition, and Research information, 1999. *International pesticide directory*, 17th edition.

Appendix III: Commonly used insecticides in the study farms at Kibwezi

Chemical name	Chemical group
Lambda-cyhalothrin*	Pyrethroid
Cypermethrin*	"
Alpha-cypermethrin*	"
Carbosulfan	Carbamate
Bifenthrin	Pyrethroid
Diazinon	Organophosphate
Dimethoate	Organophosphate
Methomyl	Carbamate
Thiocyclam-hydrogen oxalate	Botanical?
Cyromazine	Triazine
Thiometon	Pyrethroid

Names followed by an asterisk (*) were the three most commonly used chemicals.

Appendix IV: Commonly used insecticides in the study farms at Kihara

Chemical name	Chemical group
Lambda-cyhalothrin*	Pyrethroid
Alpha-cypermethrin	"
Cypermethrin	"
Dipel (<i>B. thuringiensis</i>)	Biopesticide
Others 1&2 (see append. 1)	Homemade botanicals?

The name followed by an asterisk (*) was the most commonly used chemical.

Appendix V: Commonly used insecticides in the study farms at Mwea

Chemical name	Chemical group
Lambda-cyhalothrin*	Pyrethroid
Cypermethrin*	"
Alpha-cypermethrin*	"
Endosulfan	Organochlorine
Bifenthrin	Pyrethroid
Diazinon	Organophosphate
Dimethoate	Organophosphate
Methomyl	Carbamate
Fenthion	Organophosphate
Malathion	Organophosphate
Fenitrothion	Organophosphate
(Celecron?)	?
Others 1&2 (See append. II)	Homemade botanicals?

Names followed by an asterisk (*) were the three most commonly used chemicals.

Appendix VI: Commonly used insecticides in the study farms at Nguruman

Chemical name	Chemical group
Cypermethrin *	Pyrethroid
Lambda-cyhalothrin*	"
Alpha-cypermethrin*	"
Endosulfan	"
Dimethoate	Pyrethroid
Carbosulfan	Carbamate
Methomyl	Carbamate
Omethoate	organophosphate
Fenitrothion	organophosphate
Others 1 (See apend. II)	Homemade botanicals?

Names followed by an asterisk (*) were the three most commonly used chemicals.

Appendix VII: Commonly used insecticides in the study farms at Kitui

Chemical name	Chemical group
Lambda-cyhalothrin*	Pyrethroid
Dimethoate *	Organaphosphate
Cypermethrin*	Pyrethroid
Endosulfan	Organochlorine
Malathion	Organaphosphate
Diazinon	Organaphosphate
Carbosulfan	Carbamate
Omethoate	Organaphosphate
Amitraz	Amidine
Others 1 and II (See apend. II)	Homemade botanicals?

Names followed by an asterisk (*) were the three most commonly used chemicals.