# RESISTANCE OF PREDACIOUS MITE, *EUSEIUS KENYAE* (ACARI: PHYTOSEIIDAE) TO CHLORPYRIFOS (DURSBAN®) IN KENYAN COFFEE FARMS

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**Abstract**
This study was carried out to assess whether the predacious phytoseiid mite, *Euseius kenyae* (Swirski and Ragusa), commonly found in major coffee growing regions in Kenya has developed resistance to Chlorpyrifos. Mite populations were collected from coffee farms harbouring *E. kenyae* and where Chlorpyrifos or other organophosphates were sprayed to manage the primary coffee insect pests. The mites collected were reared in mass in the laboratory for bioassays. The findings showed that under coffee agro-ecosystems, levels of resistance existed among the populations of *E. kenyae* after their exposure to Chlorpyrifos or other organophosphates. The population of *E. kenyae* from a coffee farm (C44) was most susceptible to Chlorpyrifos with $LC_{50}$ = 0.044 that was below the lowest concentration of 0.1875 ml per litre of water which was tested. The *E. kenyae* from coffee farms (C1, C4, C7, C37, C25 and C119) had resistance ratios more than ten times that of C44. The coffee farms (C2, C12, C19, C116, C31, C50 and C72) had populations of *E. kenyae* susceptible to Chlorpyrifos at concentration of 0.75 ml per litre of water which is the field recommended rate for control of insect pests in coffee. The population of *E. kenyae* from C7 was resistant to the highest field rate of 200% (1.5 ml per litre of water) with $LC_{50}$ of 1.716 and resistance ratio of 39 times. The existence of resistance populations of *E. kenyae* is an aspect that needs to be considered in the integrated pest control strategies against coffee insect pests.

**Key words**: Resistance, predacious mite, chlorpyrites, coffee

## 1.0 Introduction

Regular use of pesticides leads to insects developing resistance through selective breeding from resistant survivors. The succeeding generations of the pest following each pesticide application tend to comprise of higher proportions of resistant individuals. Consequently, resistance in pest populations persists for many generations in the field. Resistance is a decreased response of a population of animals or plant species to a pesticide or control agent as a result of their application. In insects, the factors that cause resistance are genetically controlled, with the progeny of the resistant parents tending also to be resistant.

Pesticides spray such as the fungicides, insecticides and acaricides affects the natural enemies for instance the predacious mites that control insect pests. Prior to widespread use of synthetic organic pesticides, spider mites were insignificant pests on crops. Heavy toxicity of most pesticides to predacious phytoseiid mites and subsequently their elimination after prolonged application in the field led to outbreak of spider mites (Readshow, 1975). The majority of commonly used pesticides have a broad spectrum activity that adversely affects the predacious mites either through direct mortality or elimination of their main prey (El-Banhawy, 1976). As a result of these, there has been search for selective pesticides with low toxicity towards phytoseiids though these have been rare since most pesticides are designed and marketed on the basis of their wide spectrum action (Croft, 1972). However, despite the adverse effects of pesticides to the phytoseiids, studies have shown that some strains of phytoseiids are likely to develop resistance particularly to organophosphorous compounds (Croft and Meyer, 1973; Croft and Stewart, 1973; Grande and Ingrassia, 1988).

The use of selective insecticides may improve conservation of natural enemies and therefore contribute to the success of integrated pest management (IPM) programmes (Galvan *et al.,* 2006). Galvan *et al.,* (2006) showed that Ladybird beetle, *Harmonia axyridis* (Pallas), was tolerant to Spinosad (Tracer®). Although insecticides such as chlorinated aryl hydrocarbons and DDT are generally known to be highly toxic to many predatory mites, some of them have limited direct effects on certain species. Tolerance to DDT has been observed in larvae of *Chrysopa* spp and *Anthocoris musculus* (Say) and several species of phytoseiids. Phytoseiids such as *Amblyseius fallacis* Garman and *Typholodromus caudiglans* Shuster are known to have acquired resistance to these compounds (Huffaker *et al.,* 1969). El-Banhawy (1976) indicated that several insecticides commonly applied for pest control in fruit trees were not detrimental to the predacious mites where the population had acquired resistance to these products after many years of application. These strains of predacious mites resistant to some common insecticides are desirable.

Most integrated pest control programmes depend on certain insecticides to control a variety of insect pests, for instance, the Codling moth in apples (Croft, 1982) and the Mediterranean fruit fly in citrus (El-Banhawy, 1997). In such programmes, predacious mites with developed resistance have been shown to persist and biologically control insect pests and mites (Croft and Meyer, 1973; El-Banhawy, 1997). Such resistance may also be present in phytoseiid mites on coffee farms where organophosphate compounds like Chlorpyrifos

(Dursban® 480EC) (o,o-diethyl o-(3,5,6-trichloro-2-pyridinyl) ester) have been used over a long period of time.
In an ecosystem like coffee farms, populations of predacious phytoseiid mites are likely to develop resistant strains when exposed to regular sprays of the commonly used insecticide, Chlorpyrifos, for the management of the key insect pest such as Coffee Berry Borer, *Hypothenemus hampei* (Ferrari). These resistant strains have the potential of controlling secondary pests such as red spider mites, thrips and scales, thereby containing them below their economical injury levels.

This study reports the sensitivity of *E. kenyae,* the most common and widely distributed predacious mite on coffee to Chlorpyrifos 480EC.

## 2.0 Materials and Methods

### 2.1 Coffee Farms for Collection of *Euseius kenyae* (Swirski and Ragusa)

A survey was initially carried out to identify farms with *E. kenyae* and the history of pesticides use to control major insect pests of coffee. The mites were collected from 14 coffee farms distributed among the three coffee growing agroecozones (UM1- coffee/tea zone, UM2- main coffee zone, and UM3- marginal coffee zone) of Kenya. Seven of the selected farms had history of Chlorpyrifos use for at least five years preceding this study, four had none for last five years prior to this work whereas three of the farms applied either Fenitrothion (Sumithion 500EC) (Dimethyl 3-methyl-4-nitrphenyl phosphorothioate), Omethoate (Folimat 500EC) (Dimethyl S-(N-methylcarbomonylmethyl) phosphorothioate) or a combination of the two (Table 1).

### 2.2 Laboratory Mass Rearing of *Euseius kenyae* (Swirski and Ragusa)

Young coffee seedlings aged between six and eight months potted in polyethylene bags (size 5" x 9˝ gauge 200) with perforations to allow water drainage and aeration were used to carry and transport the mites to the laboratory. The mites were collected by dislodging them from coffee branches using a beating stick. Dislodged mites were collected on a blue coloured rigid plastic collecting board or beating tray of 8 inches radius placed underneath the coffee branches. The mites on a beating tray were transferred to the leaves of coffee seedlings using a fine camel's hair brush. Fifty to a hundred individuals per coffee farm were obtained. Both the underside and upper side of the leaves were carefully dusted with coffee pollen grains as a source of food for the mites. The seedlings were labeled with the collection site, date and the farm owner. Each labeled seedling was placed in a plastic bucket measuring 14˝ x 14.5˝ and filled quarter way with synthetic sand granules (size particles of 0.3 x 0.3 mm). The granules provided stable anchorage for seedlings during transportation to the laboratory.

Coffee seedlings containing the predacious mites from the field were removed from the buckets on arrival at the CRF laboratory. The seedlings were each transferred into labeled small plastic bucket measuring 9˝x 9˝ with holes at the bottom. The buckets were filled with well fertilised soils to provide the environment suitable for the growth of the seedlings. Seedlings from each collection site were placed in separate rearing rooms in the laboratory

where fresh coffee pollen grains were dusted after every three days to feed the mites. The mites were given a period of two to three months to multiply and establish themselves. The rearing was carried out under normal laboratory conditions with mean temperature of 25± $2^0$C and relative humidity of 75%.

### 2.3 Toxicological Assessment of Chlorpyrifos (Dursban 480EC) against *Euseius kenyae* (Swirski and Ragusa)

After the collected mites had multiplied and established themselves in the laboratory, fresh colonies approximately of the same age (two weeks old) from each of the collection sites were raised for toxicological assessment. Eggs estimating two hundred (200) from the reared colonies for each site were harvested and transferred to fresh coffee seedlings with the aid of camel's hair brush. Four seedlings each introduced with 50 eggs were used as the rearing units. The eggs were incubated under laboratory conditions for a period of two weeks. The newly hatched mites, aged two weeks, were harvested for the toxicological study. The females being the biased sex in *E. kenyae* and more available in each colony were used for the study. The harvested females were placed in a plate of 6˝ diameter and internally surrounded with a thin layer of wet cotton wool. This was followed by putting them in a refrigerator at $4^0$C for several minutes (10-15 minutes) in order to reduce their mobility before transferring them to Petri dishes with leaf discs (2 cm in diameter) treated with 1.5, 0.75, 0.375 and 0.1875 ml of Chlorpyrifos per litre of water as concentrations.

Young fresh coffee leaves from a farm with no history of insecticides use were plucked and leaf discs of 2 cm in diameter cut. Four batches of 20 mites from the same population and age were exposed to treated leaf discs. Concentrations of Chlorpyrifos (1.5, 0.75, 0.375 and 0.1875 ml per litre of water equivalent to 200, 100, 50, and 25% field rates) were used. Distilled water was used in as the control treatment. Petri dishes with cotton wool soaked in water were prepared as the arena for bioassay.

The discs were immersed separately in the different concentrations of Chlorpyrifos or distilled water for ten seconds, after which they were removed and placed in different clean Petri dishes to dry. Each of the dried leaf discs was placed upside-down in the Petri dish and replicated four times for each concentration. A strip of cotton wool was put around the edges of the disc to prevent the mites from escaping. Pollen grains were dusted on each of the discs as a source of food for the mites. The mites were exposed to the five concentrations for 48 hours after which mortality was recorded. The experiment was repeated twice. Percentage mortality was corrected using Abbott's formula (1925) and plotted on a log-dosage probit paper according to Finny (1952).

## 3.0 Results

The populations of *E. kenyae* from coffee farms showed variation in their responses to Chlorpyrifos (Table 1). Individuals from coffee farm C44 had mites most susceptible to Chlorpyrifos ($LC_{50}$ = 0.044) and were used as standard reference. Different populations of *E. kenyae* varied in their susceptibility or response to Chlorpyrifos (Table 1).

In the coffee farms C1 and C4 where Chlorpyrifos was regularly applied as foliar spray, had strains of *E. kenyae* less susceptible to various field rates (200, 100, 50, and 25%) were analysed (Table 1). Other farms where Chlorpyrifos was either foliar sprayed or banded, the populatios of *E. kenyae* were susceptible to Chlorpyrifos even at the lowest field rate. For instance, C2, C12, C19 and C116 had $LC_{50}$ of 0.172, 0.068, 0.102 and 0.116, respectively, that were lower than 25% field rate.

The coffee farms with no history of Chlorpyrifos use for over five years prior to this work showed varied responses to Chlorpyrifos. Some had populations of *E. kenyae* with high level of resistance to Chlorpyrifos at field rates of 100 and 200%. For example, coffee farms, C7 and C119 had $LC_{50}$ of 1.716 and 1.008, respectively. Their respective resistance ratios were 39.0 and 22.9 (Table 1). The coffee farm C31 under similar treatment had $LC_{50}$ of 0.436 that was moderately susceptible to Chlorpyrifos.

The coffee farms where Fenitrothion, Omethoate or their combinations were applied, the populations of *E. kenyae* were susceptible to Chlorpyrifos at various field rates evaluated. Only populations from C25, with $LC_{50}$ = 0.491 was moderately tolerant to the field rates of Chlorpyrifos (Table 1).

Irrespective of the source, different populations of *E. kenyae* varied in their responses to Chlorpyrifos. The resistance ratios for populations from C1, C4, C7, C37, C25 and C119 were ten times more than that of the susceptible population (C44). Their $LC_{50}$ were equivalent to 100% (0.75ml of Chlorpyrifos in one litre of water) field rate (Table 1).

The concentrations of Chlorpyrifos assessed against the populations of *E. kenyae* collected from various coffee farms under different treatments were toxic but at different levels. The populations from C7 and C44 had the lowest and the highest susceptibility respectively to the concentrations tested. The mortality increased with increase in concentrations at varying rates. For instance, populations from C7 and C119 had gradual increase in mortality as the concentrations increased, unlike in C44 where mortality was high and almost constant irrespective of varying concentrations (Figure 1).

The *E. kenyae* populations from farms either exposed to Chlorpyrifos or not for the last five years had higher resistance to the concentrations tested compared to the most susceptible (C44) and almost equivalent to that of most resistance populations (C7) (Figure 2). The coffee farms treated with either Fenithrothion, Omethoate or their combinations had most of *E. kenyae* populations susceptible to Chlorpyrifos concentrations tested. For instance, the population of C72 was susceptible to Chlorpyrifos and with almost the same level as that of C44 (Figure 3).

The cumulative mean percentage mortalities from various concentrations of Chlorpyrifos on different populations of *E. kenyae* statistically varied from each other [F=33.72, df = (3, 262), n=280, P= 0.05] (Table 2). The mean mortality rate from mite's population collected from C7 was significantly lower than in all the other coffee farms except C119 [F=33.72, df = (3, 262),

n=280, P= 0.05]. The C44 population had the highest mean percentage (%) mortality that was statistically significant [F=33.72 df = (3, 262), n=280, P= 0.05] from that of C1, C2, C4, C7, C25, C31, C37, C50 and C119.(Table 2)

Table 1: *Response of different populations of Euseius kenyae (Swirski and Ragusa) from coffee farms in Kenya to different concentrations (1.5, 0.75, 0.375 and 0.1875 ml per litre of water) of Chlorpyrifos*

| Treatment | Population source | Location /Agroecozone | $LC_{50}$ | Slope | Resistance ratio |
|---|---|---|---|---|---|
| Chlorpyrifos * | C1 | Kiambu UM2 | 0.653 | 0.653 | 14.84 |
| Chlorpyrifos ** | C2 | ,, ,, | 0.172 | 2. 055 | 3.91 |
| Chlorpyrifos * | C4 | ,, ,, | 0.623 | 0. 311 | 14.16 |
| Chlorpyrifos ** | C12 | ,, UM1 | 0.068 | 2.028 | 1.54 |
| Chlorpyrifos * | C19 | Muranga UM2 | 0.102 | 3.040 | 2.32 |
| Chlorpyrifos * | C116 | Nyeri UM2 | 0.166 | 2. 351 | 3.77 |
| No Chlorpyrifos | C7 | Kiambu UM3 | 1.716 | -0.550 | 39.00 |
| ,, | C31 | Meru UM2 | 0.436 | 1.446 | 9.91 |
| ,, | C37 | Embu UM1 | 0.684 | 0.624 | 15.54 |
| ,, | C119 | Nyeri UM3 | 1.008 | -0.009 | 22.91 |
| Fenitrothion / Omethoate * | C25 | Meru UM1 | 0.491 | 1.071 | 11.16 |
| ,, | C50 | Kirinyaga UM2 | 0.224 | 2.089 | 5.09 |
| ,, | C72 | Machakos UM2 | 0.088 | 4.163 | 2.00 |
| Chlorpyrifos** | C44 | Embu UM2 | 0.044 | 3.472 | - |

*Key: *Foliar spraying;*
*** Banding;*
*CI=Rukera Farm*
*C2= Mburu Farm*
*C4= Gitonga Farm*
*C7= Mongalia Estate*
*C12= Kibubuti Estate*
*C19= Gichore Farm*
*C25= Kithuu Farm*
*C31= Kirai Farm*
*C37= Ndwiga Farm*
*C44=Kariuki Farm*
*C50= Kamau Farm*
*C72= Kitavi Farm*
*C116=Mbuthia Farm*
*C119=Muringato Estate*

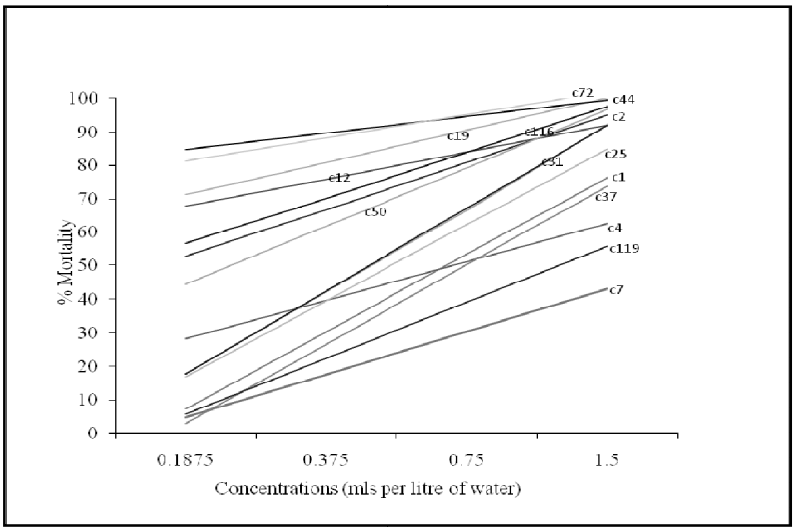


*Figure 1: Susceptibility of Euseuis kenyae (Swirski and Ragusa) populations to chlorpyrifos from coffee farms under different insecticide(s) treatments*

***Key***

*CI = Rukera Farm*
*C2 = Mburu Farm*
*C4 = Gitonga Farm*
*C7 = Mongalia Estate*
*C12 = Kibubuti Estate*
*C19 = Gichore Farm*
*C25 = Kithuu Farm*
*C31 = Kirai Farm*
*C37 = Ndwiga Farm*
*C44 = Kariuki Farm*
*C50 = Kamau Farm*
*C72 = Kitavi Farm*
*C116 = Mbuthia Farm*
*C119 = Muringato Estate*

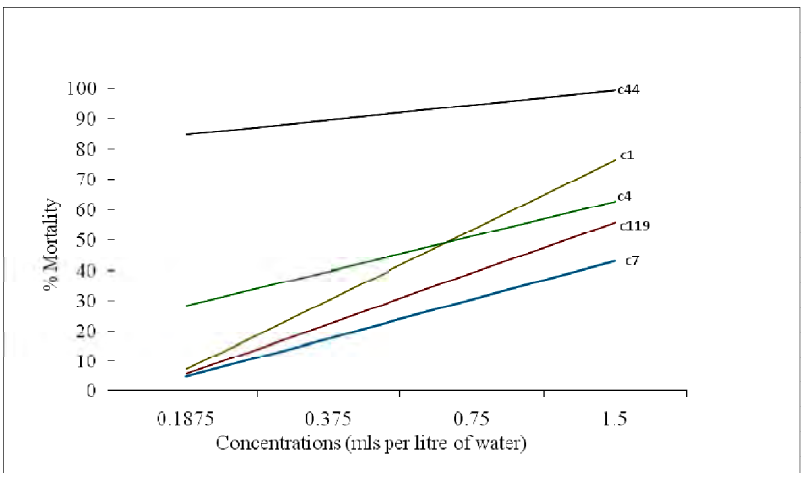


*Figure 2: Susceptibility of Euseuis kenyae (Swirski and Ragusa) populations to Chlorpyrifos from coffee farms either exposed or not exposed to Chlorpyrifos treatment*

***Key***
*CI = Rukera Farm*
*C4 = Gitonga Farm*
*C7 = Mongalia Estate*
*C44 = Kariuki Farm*
*C119 = Muringato Estate*

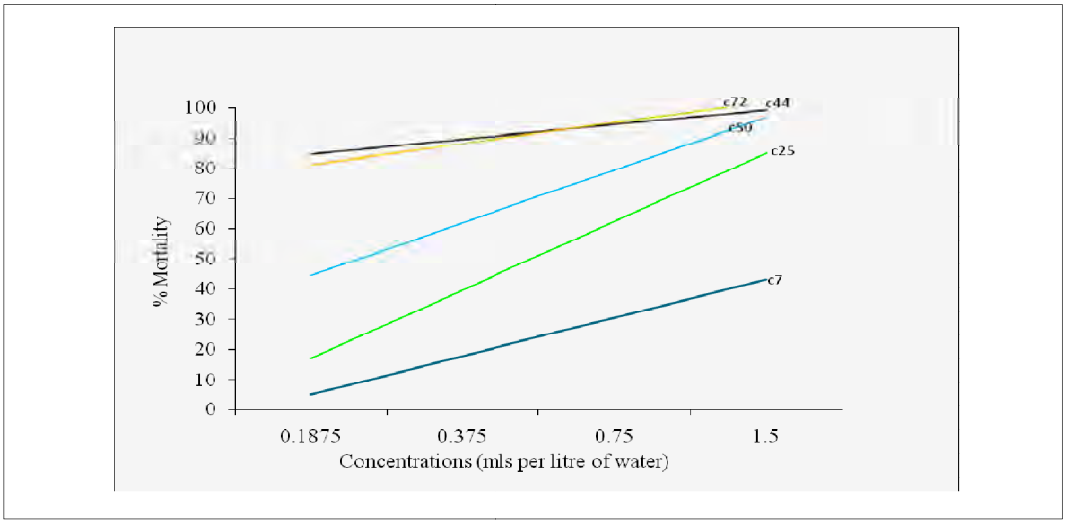


*Figure 3: Susceptibility of Euseuis kenyae (Swirski and Ragusa) populations to Chlorpyrifos from coffee farms exposed to Fenitrothion or Dimethoate treatments*

***Key:***
*C =Mongalia Estate*
*C2 =Kithuu Farm*
*C44=Kariuki Farm*
*C50= Kamau Farm*
*C72= Kitavi Farm*

*Table 2: Mean Mortality (%) of different populations of Euseuis kenyae (Swirski and Ragusa) after exposure to different concentrations (1.5, 0.75, 0.375 and 0.1875 ml per litre of water) of Chlorpyrifos*

| Treatment | Population source (Farm) | Mortality (%) ± S.D |
|---|---|---|
| Chlorpyrifos | C44 | 74.0 ± 37.8 [a] |
| Fenitrothion /Omethoate | C72 | 74.0± 37.5 [a] |
| Chlorpyrifos | C19 | 69.8± 35.4 [ab] |
| Chlorpyrifos | C12 | 65.8± 32.6 [ab] |
| Chlorpyrifos | C116 | 62.3± 35.1 [ab] |
| | | |
| Chlorpyrifos | C2 | 61.3± 32.4 [b] |
| Fenitrothion /Omethoate | C50 | 58.8± 33.0 [b] |
| No Chlorpyrifos | C31 | 45.3± 33.5 [c] |
| Fenitrothion /Omethoate | C25 | 42.3± 31.2 [c] |

| | | |
|---|---|---|
| Chlorpyrifos | C4 | 38.8± 24.4 $^{cd}$ |
| Cholopyriphos | C1 | 36.0± 33.8 $^{cd}$ |
| No Chlorpyrifos | C37 | 35.0± 29.3 $^{cd}$ |
| No Chlorpyrifos | C119 | 29.0± 21.4 $^{de}$ |
| No Chlorpyrifos | C7 | 21.3± 17.3 $^{e}$ |

*Means with the same letter are not significantly different [F=33.72 (df, 13, 262), n=280, P >0.05, Student-Newman-Keuls Test] from Standard deviation (S.D.), of the mean*

## 4.0 Discussion and Conclusions

Predacious phytoseiid mites regulate the populations of phytophagous mites and other insect pests. In their absence, phytophagous mites and insect pests such as thrips may upsurge and cause outbreaks (El-Banhawy, 1976). Past studies have indicated that as a result of regular use of insecticides, species of predacious phytoseiid mites could develop resistance to insecticides. Such resistance has been reported from phytoseiid mites such as *Neoseilus fallacis* (German), *Metaseilus occidentalis* (Nesbitt), *Phytoseiulus persimilis* A. - H., *Amblyseius cydnodactylon* (Shehata and Zaher) (El- Banhawy *et al.,* 2000).

The present investigations indicate that under coffee agro-ecosystems, resistance occurred among different populations of the common predacious phytoseiid mite *E. kenyae* after their exposure to Chlorpyrifos, which is commonly used to control key insect pests of coffee. The population of predacious mites from C44 was susceptible to Chlorpyrifos despite the regular use of this insecticide in the farm. Normally Chlorpyrifos is applied either through foliar spraying or banding of coffee stems at the base for the control coffee scale insect pests. Foliar spraying exposes the insect pests or the biological control agents such as predacious mites to Chlorpyrifos residues, thus causing high chances of resistance to develop through selective breeding from resistant survivors. However, the population of predacious mites from C44 in this case when assessed against Chlorpyrifos was found to be more susceptible to the product than any other farm. This indicated that the farming system practiced in C44 that involved banding of coffee stems at the base to manage pests such as the Green scales, *Coccus alpinus*, is a practice that makes the predacious mites or any other biocontrol agent less exposed to Chlorpyrifos residues, hence less opportunity of developing resistance. The observation made during the predacious mite's collection in C44 showed that there was no foliar spraying in the farm. Instead banding was the major and common practice exercised in this farm as it experienced frequent infestation by the Green scales.

Although the coffee farms C7 and C119 had not used Chlorpyrifos for over five years prior to this study, the farms were neglected with no weeding, fertiliser application, pruning or spraying of any insecticides being carried out. Despite this, the two farms were large coffee plantations meaning that in the past, coffee was intensively farmed with the possibility of the two farms heavily applying Chlorpyrifos to manage various primary coffee insect pests. It is therefore possible that the resistance established from the populations collected from these farms probably had developed by then and still exists to date.

Predacious mites normally develop resistance to different groups of insecticides. To ascertain this under coffee agro-ecosystems, populations of *E. kenyae* collected from farms

where the insecticides Fenitrothion and Omethoate were used, resulted into population with low resistance to Chlorpyrifos.

It was evident from this study that populations of *E. kenyae* with resistance or low susceptibility to Chlorpyrifos exist under coffee agro-ecosystems. This can therefore lead to selection of predacious phytoseiid mite strains resistant to Chlorpyrifos. According to Schulten *et al.,* (1976) and Golorkina and Akssyutova (1990), modern integrated pest management on crops, employs use of resistant strains of predacious mites. The present strains of *E. kenyae* with resistance to Chlorpyrifos can effectively be employed in a biocontrol strategy to manage minor coffee insect pests while Chlorpyrifos still manage the key target pests with less effect on biocontrol agents such as *E. kenyae*. At present no incidence of resistance has been reported on key insect pests such as *Hypothenemus hampei* against Chlorpyrifos application thus making the resistant strains of *E. kenyae* suitable for use in an integrated pest management of coffee in Kenya.

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