INTEGRATION OF FERTILIZERS, PREDACIOUS MITES AND ACARICIDES IN THE MANAGEMENT OF CASSAVA GREEN MITE *MONONYCHELLUS* SPECIES (ACARI: TETRANYCHIDAE) IN KENYA

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DECLARATION

I hereby declare that this thesis is my original work and has not been presented for a degree award in any other University.

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DEDICATION

I dedicate this work to my children George and Abigail and wife Zipporah in appreciation of their sacrifice, patience and support while I was away from home for long durations conducting field research. This support gave me strength and greatly boosted my morale that contributed towards the successful completion of this work.

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TABLE OF ABBREVIATIONS

AEZ: Agro-ecological zone(s)

ANOVA: Analysis of variance

A+ T: Adenine -thymine base pair chain structures of nucleotides; Watson-Crick base pair

CGM: Cassava green mite

CBSVD: Cassava brown streak viral disease

CL3:   Coastal lowlands Three

CMVD: Cassava mosaic viral disease

DNA: Deoxyribonucleic acid; encodes all the genetic information of living organisms

G + C: Guanine-cytosine base pair chain structures of nucleotides

EAAPP: East Africa Agricultural Productivity Project

HCN: Hydrogen cyanide glycosides

ICIPE: International Centre of Insect Physiology and Ecology

IITA: International Institute of Tropical Agriculture

IPM: Integrated Pest Management

KARI: Kenya Agricultural Research Institute

LM4:   Low midlands Four

LM5:   Low midlands Five

RH: Relative humidity

SAS: Statistical software from USA, Cary. Inc. Company; for pure and business sciences

SE: Standard error

SD: Standard deviation

SPSS   Statistical Package for the Social Sciences; developed by IBM Co.

PCR: Polymerase chain reaction

UM3:   Upper midlands Three

ABSTRACT

The cassava mite complex of the genus *Mononychellus* is among the major arthropod pests of the cassava crop in Kenya. The present investigations addressed the integrated pest management (IPM) method as it applies to the protection of field cassava crops from losses due to phytophagous mites. The work employed use of IPM components, namely, predacious mites, soil fertility and acaricides to ensure effective protection. A survey was conducted to determine the taxonomy of the individual species in the complex of the phytophagous and predacious mites of the genus *Mononychellus*, major arthropod pests of cassava crops in Kenya. Standard sampling methods developed by previous workers were employed and yielded both phytophagous and predacious mite specimens for identification. Both morphological and molecular analyses methods were used to sort out the taxonomy of the mites identification based on morphological features and confirmation made by comparison with previously identified specimens by mite taxonomic experts. Confirmation of the species was made by molecular analyses of a fragment of ribosomal DNA (ITS 1& 2) and mitochondrial cytochrome oxidase sub-unit I (COI). The techniques revealed that *Mononychellus progresivus* Doreste as major pest on cassava. Out of the 29 species from all the surveyed agro-ecological zones, two were most abundant, namely, *Euseius fustis* Prichard & Baker and *Typhlodromalus aripo* De Leon. The ecology of *M. progresivus* density growth on different cassava varieties was carefully examined in a screen house study as a baseline for the respective agro-ecological zones. Increased cyanide content of >10<30mg / kg cyanogen glycoside content on the varieties attracted the highest density of *M. progresivus*. Mite economic threshold density for control of *M. progresivus* on cassava was deduced to be ≥27 mites /leaf. Further, in an experiment to determine the most effective mite predator for use as a biological control agent in the IPM study against the phytophagous mite *M. progresivus* was carried out in the laboratory. Of the two mite predators, namely, *T. aripo* (predominant from survey sites) and another newly acquired species from South America, *Phytoseiulus longipes* Evans, the former was chosen on the basis of prey consumption and fast development rate on CGM. Field evaluations were conducted using the selected IPM components stated above. The zones included the eastern dry low-midlands of LM4 at Katumani and LM5 at Kiboko, the cool upper midlands of UM3 at Embu and the warm coastal lowlands of CL3 at Mtwapa. The results revealed that the phytoseiid mite *T. aripo* effectively suppressed population densities of *M. progresivus* in the warm-humid coastal zone. The acaricide (abamectin) spray in the manure integrated treatment gave the best control option for complete suppression of CGM densities at all sites. Soil fertility input led to higher yield in the sandy soils of coastal and eastern lowlands in the *T. aripo* introduced plots. The performance results by the integrated components give choice of implementation of integration of biological (predacious mites), chemical (acaricide) and soil fertilizer inputs on management of the *M. progresivus* on cassava in the different agro-ecological zones of Kenya.

CHAPTER 1

1.0 GENERAL INTRODUCTION

1.1 Background Information

Arthropod pests are a great hindrance to production of cassava (Euphobiaceae: *Manihot Esculenta* Crantz) cultivated by over 800 million people and is the seventh most important staple food crop worldwide (CIAT, 1993; Nweke, 1996). The African continent is an important producer of the crop where it is a staple food crop in the central and western states (Alves, 2002). In view of its perennial status, cassava is a leading poverty alleviation crop in the Southern American countries that produce the highest quantities in the world (Hillocks, 2002). The major pests of cassava in Africa are; cassava mealybug *Phenacoccus manihoti* Mat-Ferr, several whitefly species and cassava green mite (CGM) complex (Herren & Bernett, 1984; Megevand *et al*., 1987; Yaninek *et al*., 1987; Gutierrez, 1987; Yaninek, 1994). While the mealybug menace has been brought under control in most regions of the continent by release of a parasitoid wasp in the early 1990s, the CGM continue to cause yield loss in dry low tropics and subtropics of Africa (Herren & Neuenschwander, 1991; Kariuki *et al.*, 2005). Various whitefly species reportedly transmit viral diseases like cassava brown streak viral disease (CBSVD) and cassava mosaic viral diseases (CMVD) (Alicai *et al.*, 2007). Cassava green mite of the *Mononychellus* species was reported to have spread from east African to the rest of western and central Africa regions in the 1970s (Nyiira, 1972; Byrne *et al*., 1982; Yaninek & Herren, 1988). The species identification was obscure, some workers referring to it as *M. tanajoa* Bondar while others reported it as *M. progresivus* (Yaninek *et al*., 1987; Gutierrez, 1987; Bonato *et al,* 1994). Throughout the African continent

CGM was reported to cause losses ranging from 30 to 80 % depending on the crop variety, cultural practices and local agro-ecological conditions (Yaninek *et al*., 1989; Hanna *et al.*, 2005). The spider mite feeds by sucking the underside leaf tissue of cassava causing chlorosis type of damage (Fig.1.1). As the CGM is reported to cause the highest yield loss in Africa there was need to develop an environmentally safe integrated pest management strategy of the exotic pest after determination of its species.

Figure 1.1: Cassava green mite pest and leaf chlorosis damage (Insert Mag. X 40). Courtesy A. Onzo (International Institute of Tropical Agriculture)

As reported by earlier workers, cassava crop requires fairly warm climate for its development and tuberization in most production regions of the tropical world (Cocke and Rosa, 1975; Connor *et al.*, 1981; Alves, 2002; Leihner, 2002). Similarly, CGM densities increase with temperature as the mite feeds on the leaf photosynthetic area in

the dry lowlands (Yaninek *et al.,* 1989; Yaninek & Schulthess, 1993). Yaninek & Schulthess (1993) suggested soil fertility input as one of the options to increase plant health and yield during the seasonal high densities of green mite (Yaninek *et al.,* 1987; Howeler, 2002; Amanullah *et al.,* 2007).

Whether higher cyanogens content on cassava leaf leads to less damage by CGM is an important question in the present study. The cassava cyanogen compounds are mainly the linamarin (85%) with lesser amounts of lotaustralin free HCN glycosides found in both foliage and root content (Alves, 2002; Iglesias *et al.*, 2002;). No report has detailed how leaf cyanide levels contribute to density growth of CGM in Africa.

The performance of a phytoseiid predator as an effective biological control agent of mite pest depends mainly on the initial predator-prey ratio (Kindlmann & Dixon, 1999; Nakazawa *et al.*, 2011). Sabelis and Rijn (1997) demonstrated that actual control impact could only be measured when yield and other attributes of economic importance are assessed. Other arthropod species and environmental factors play important roles to the final performance score of the predator in suppressing target pest (Lima, 1998; Osekre *et al.,* 2008). Although predacious mites usually achieve excellent control of most phytophagous mites and some insects there are factors limiting their efficiency like initial predator-prey ratio and species diet preference (Grout & Richards, 1992; Bakker *et al.,* 1993). The cassava crop is mainly grown in the western and coastal regions of Kenya (Mohammad *et al*., 1998). The eastern marginal areas are turning to more cassava cultivation as cereal and legumes production indicate a trend of decline as a result of

climate change scenario (FAO, 2010; Maina *et al.,* 2012). Among the number of injurious arthropods reported on cassava in the country, CGM is most prevalent (Kariuki *et al.,* 2002: Mutisya *et al.*, 2011). Hence, within the context of the fact that the phytophagous mite pest continues to cause major damage to cassava in the dry lowland and midland zones of Kenya, there was need to compare biological and chemical control options in addition to manure and mineral fertilizer input applications at specific agro-ecological zones of the country (Ayoola, 2006; Ande *et al.,* 2008).

1.2 Problem Statement and Justification

Integrated pest management (IPM) is an ecosystem-based strategy that focuses on long term prevention of pests or their damage through a combination of techniques such as biological control, habitation manipulation, modification of cultural practices and use of resistant varieties. Pesticides are used only as after monitoring indicates they are needed as according to established guidelines and treatments are made with the goal of removing only the target organism. Pest control materials are selected and applied in a manner that minimizes risks to human health, beneficial and non-target organisms and the environment.

As reported, CGM control in Africa has been credited to predacious mites of the Family Phytoseiidae Berlese (Yaninek & Hanna 2003; Onzo *et al.*, 2003; Kariuki *et al.,* 2005). In Kenya cassava root yield loss of 16 to 40 % has been attributed to high densities of CGM (Ndonga *et al*., 1986). The present research study was meant to carry out a survey to get baseline information of the pest (CGM) occurrence and abundance in the different agro-ecological zones of Kenya. Mite abundance defines injury level while occurrence would

not necessarily result to economic injury to the host crop. The survey would also identify a predacious mite most effective in managing high densities of CGM in the different agro-ecological zones of Kenya with the increased climate variability scenario.

Cassava crop soil fertility requirement has always been disputed in most parts of Africa (Fermont *et al*., 2009). The effect of soil fertility factor on predacious mites or acaricide impact to mites on cassava will give sound data for packaging a holistic approach of combined components of Integrated Pest Management (IPM) of CGM according to the pest dynamics in selected agro-ecological zone conditions. The field validation of the best options or set of options for CGM control in low lands, low midlands and upper midland zones where cassava is grown in Kenya will give an implementable management of CGM and prevent root yield loss—increasing food security and farmer incomes.

1.3 Research Hypothesis

An integrated pest management programme based on sound biological strategies that combine soil fertility, predacious mites and chemical control will effectively control and manage cassava mites that reduce cassava yield in the selected test agro-ecological zones of Kenya.

1.4 General Objective

Establish the taxonomic identity of cassava mites in Kenya and formulate an integrated pest management strategy against the key one.

1.4.1 Specific objectives

1. To conduct a country-wide survey in cassava fields of both predacious and cassava green mite species (CGM) and to identify species occurrence

2. To explore CGM growth and determine density threshold on cassava varieties

3. To determine the most efficient predacious mite for controlling phytophagous mites on cassava that reduce root yield

4. To evaluate efficacy of an integrated pest management (IPM) programme consisting of a combination of fertilizer input, predatory mites and chemical pesticides in selected agro-ecological zones of Kenya.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Taxonomy and origin

The CGM species under the *Mononychellus* genus (Acari: Tetranychidae) was morphologically analyzed by Gutierrez (1987) citing its origin as the South America. Bondar (1938) described the first species of CGM on cassava as *Mononychellus tanajoa*, though the specimen slides sample were lost and work was not traceable for reference according to Gutierrez (1987). Later confirmation of the species was done by Fletchmann and Baker (1970) describing the female dorsum and opisthosoma and position of the dorsal setae. Doreste (1981) described and named two more new species on cassava; *M. manihoti* and *M. progresivus* Doreste. Eight more species were described by Meyer (1974) and Turtle *et al.* (1974) from other host plants. While a total of 27 species of *Mononychellus* genus have been described at least eight (8) are confirmed pests on cassava in South America, these being; *M. bondari* Paschoal*, M. Caribbeanae* McGregor*, M. chemosetosus* Paschoal*, M. estradi* Baker & Pritchard*, M. manihoti* Doreste*, M. Mcgregori* Fletchmann & Baker*, M. progresivus* Doreste *and M. tanajoa* (Gutierrez, 1987). The main feature of morphological identification is the male aedeagus where on *M. progresivus* the tip knob of the organ is slender while that of *M. tanajoa* has globular shape (Gutierrez, 1987). Recent work on the complex species in Africa has involved sex crosses of country populations with Murega (1989) demonstrating that the Kenya and Uganda populations were compatible. A few years later Navajas *et al* (1994) determined that while Brazil populations were not compatible with Benin (West Africa) and Congo (Central Africa) the Columbia mite populations were similar. A nucleotide sequence of

M. progresivus as the species in Africa has been documented and deposited on National Center for Biotechnology Information (NCBI, USA) data base (Navajas *et al.,* 1994). There still exists confusion on what reference should be installed on the species identity in Kenya as most workers on cassava refer to it as *M. tanajoa* (Githunguri *et al.,* 1984; Kariuki *et al.,* 2005; Mutisya *et al*., 2011). Much of the work on CGM control in Africa has been on the assumption that *M. tanajoa* (Megevand *et al*., 1987; Yaninek & Hanna, 2003; Onzo *et al.,* 2009). The importance of knowing the correct species identity would bring both scientific merit and end to the existing uncertainty.

2.2 Biology, ecology and distribution

The biology of CGM has been described by Yaninek *et al.* (1989). The egg stage takes 2-3 days to hatch (in 70% RH and 27 ºC), and the larval stage also takes a day to next protonymph stage. The protonymph takes 1-2 days to deutonymph while the latter takes about 3 days to adult egg laying stage, under the same conditions. The egg to adult duration is about 13 days while life span per female is 24 days and female fecundity is about 60 eggs (Yaninek *et al.,* 1989). Yaninek and Gnanvossou (1993) demonstrated in a laboratory study that the spider mite body biomass accumulation increased with life stage development to adulthood as the pest feeds on cassava leaf tissue. The ecological optimum conditions for CGM present a mite which is temperature dependent where temperature increase from 15-30 ºC gives a gradient of exponential population growth of high reproduction rate of three times at maximum peak temperature (Bonato *et al.,* 1994). Kariuki *et al* (2000) reported that while CGM was distributed in all cassava fields of the eastern, western and low coastal regions of Kenya. Mite density occurrence and

abundance in specific agro-ecological zones has not been studied and the subsequent yield loss reported.

2.3 Pest status and control

Infestation levels of CGM on cassava have reportedly been influenced by environmental factors rather than variety as demonstrated from field data by Yaninek *et al.* (1987). Depending on environmental factors like temperature CGM densities were scored as a few tens to several hundred per leaf (Bonato *et al*., 1994: Kariuki *et al.,* 2002). Early (1980s) control measures for CGM in Africa were projected to include both biological and cultural control strategies (Yaninek, 1994). Some hindrances were encountered of socio-economic nature where resource-poor farmers were not keen to adopt new agronomic practices of cultural control technologies (Dorosh, 1988). Biological control agents were introduced from 1980s to 1990s without the knowledge of the farmer and reports show reduced root loss in some areas of Africa (Bellotti, 2002). Chemical control has not been advocated due to the fact that cassava is grown by resource-poor farmers in the whole of the African continent (Smaling *et al.*, 2002; Fermont *et al.*, 2008). Another explanation is that in some communities the upper cassava leaves are used as vegetable in various cuisines preferred by some communities in coastal eastern Africa region (Sauti, 1984).

2.4 Predacious mites for phytophagous mite pest control

McMurtry and Croft (1997) have defined phytoseiids of Type I group of the genus *Phytoseiulus* as the best performers with highest prey consumption and intrinsic rate of

increase greater than 0.4. In the same review the *Euseiu*s genus (Type IV) which has over 200 species is described as plant pollen feeders group and of least impact on phytophagous mites of most cultivated crops. The genus *Typhlodromalus* of phytoseiid species *T. manihoti* and *T. aripo* have emerged as most closely related on *Mononychellus* species on cassava in Latin America and Africa (Gerson & Weintraub., 2002; Onzo *et al.,* 2012). Amusa and Ojo (2002) found that the presence of *T. aripo* where whiteflies were present as vectors of cassava diseases led to reduced disease incidence. El- Banhawy *et al.*(2000) explored development and reproduction of the predacious mite, *Amblyseius cydnodactylon* on different prey species and found that increase of prey density led to less feeding rate of predator. Likewise, there is usually an indirect ecological-factor effect as an important role to the performance of a phytoseiid as an effective biological control agent (Waage, 2001). The predator population dynamics as influenced by environmental factors and effective predator-prey ratio at introduction time is important when selecting biological control candidates (Zhang, 1993). Of the various control options, Bellotti *et al.* (1999) reviewed CGM pest status in Africa and the impact of biological control and conclusively and reported a 35-60% density reduction by *T. aripo* presence on cassava and root yield increase by 30-37%. Knowledge on how fertilizer input would suit in the integration of predacious mites on CGM control in different agro-ecological zones of Kenya will be an important milestone towards effective management of the pest in the country. Use of chemical control in a selective manner to conserve the beneficial predators at specific agro-eco-zone sites needs to be evaluated.

CHAPTER 3

3.0 GENERAL MATERIALS AND METHODS

3.1 Survey and collection of mite specimens

The cassava phytophagous and predacious mites were sampled from 166 farms in the different geographical zones of low midlands, upper midlands and coastal lowlands during the 2011-2013 periods of production (Table 3.1 & Fig. 3.1). A total of fifteen plants were sampled from five random points of each farm in a square transect (10 x 10m). In each of the sample points randomly selected plants from each of the field positions were collected by beating the same plants with a stick (60 cm length) over a circular plastic board (32 cm diameter) for a constant period of two minutes. The dislodged mites were collected with aid of head loop lenses (Mag.X4) and a hair camel brush (size ooo). The sampled mites were pooled together and preserved in 70% alcohol contained in two different vials; one for the phytophagous and the other for predacious mites. For the phytoseiid *T. aripo* plant apices found with the predator were de-touched and put in separate vials with 70% alcohol. The sampled mites were used both for morphological and molecular identification. Other pests and diseases found on the sample cassava plants were recorded for general assessment of field infestations or incidence from the different agro-ecological zones of Kenya.

Table 3.1: Cassava production sampling zones and the respective climatic conditions

Zone	No. farms	Locality (farm areas)	Altitude (m)	Annual temp (°C)	Annual rainfall (mm)
LM 2, 3	20	Busia, Kakamega	1200–1500	≤ 16	1600–2700
LM 4, 5	54	Kitui, *Machakos, Makindu	900–1600	16–20	400–800
UM 2,3	20	*Meru, *Embu	1300–1400	20–24	1400–1800
LM 1,2, 3	22	Kisumu, Migori	1200–1400	24–30	1200–2000
CL 2, 3	50	Mombasa; north, south coastal strip	0–250	25–28	1800–2500

*Sample sites where the wild cassava host *Manihot glaziovii* Muller was found growing in the wild or at town centres as ornamental as well as shed trees.
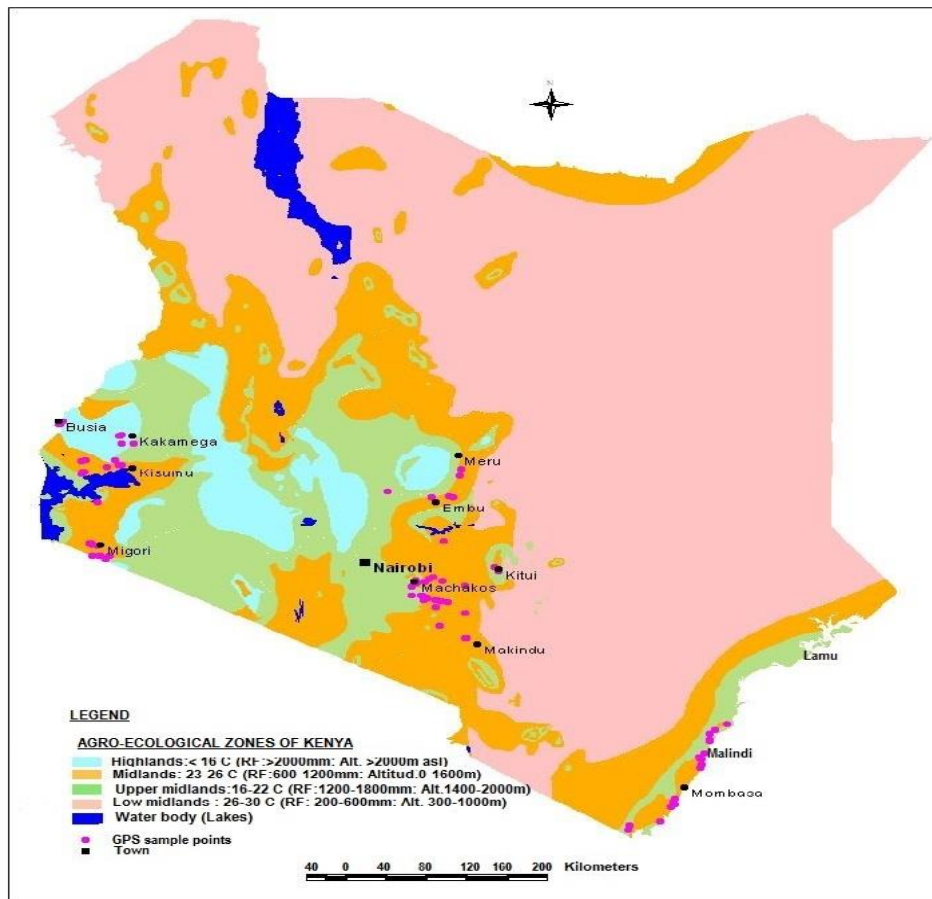
Figure 3.1: Map of Kenya showing survey areas of cassava production regions in the different agro-ecological zones

3.2 DNA extraction from mite specimens

Genomic DNA was extracted from individual specimens collected from 15 farm samples representing three different regions with distinct climatic zones i.e. eastern, coastal and western areas of the country. Each zone had five farm samples of about 100 individuals. Later the five farm samples were pooled into one and about 500 adult mite specimens from each of the three zones put in one vial where individual specimens were extracted. Extraction samples of green mite motiles were ground in epidle tubes for adequate maceration of individual specimens. Extraction was carried out by using tissue kit (Qiagen, GHBB, German) according to the manufacturer's instructions. The DNA samples were eluted in 30 μl of buffer AE and stored at 4C. Polymerase chain reaction (PCR) was performed in a total volume of 30 μl containing 1X PCR buffer, 2.5 mM $MgCl_2$, 10mM of a dNTP mix, 20 uM of each primer, 2.5 units Taq and 2 μl (approx 5 ng) of purified template DNA and the mixture was incubated in an Applied Biosystems 9700 thermal cycler. Base pair bands of 300Bp were observed on radar of 1000bp. The ITS1&2 regions were amplified using the primers for insect arthropod specimens as 5' AGAGGAAGTAAAAGTCGTAACAAG-3' for the 3' end of the 18SrDNA and 5'-ATATGCTTAAATTCAGGGGG-3' for the 5' end of the 28S. The mitochondrial COI primers used were as described by Navajas *et al* (1994) as 5'-TGATTTTTTGGTCACCCAGAAG-3' and 5'-TACAGCTCCTATAGATAAAAC-3' (Navajas *et al*., 1999). Amplification was conducted for both ITS1&2 and COI PCR with an initial denaturing step at 95^0C for 4 min, followed by 35 cycles of 92^0C for 1min, 51^0C for 2min, 72^0C for 1min, and a final extension at 72^0C for 9 minutes. Amplified PCR products of 25 ul were subsequently visualized following 1% agar rose gel

electrophoresis and staining with ethidium bromide, then later purified using the QIAquick® PCR purification kit (QIAGEN, Germany), according to the manufacturer's instructions.

3.3 Cassava variety establishment for pest mite study

Nine cassava varieties; three from coast (Kalezo, Karibuni and Tajirika), eastern (MM990183, MM99005 and x-Mariakani) and western (MM97/3567,MM96/2480 and MM96/9380) cassava production regions were planted on plastic pots of 18cm-length x 18cm-width and 15cm-height. The varieties were planted in sandy loam soil in the pots. Each variety planted in 12 pots cassava plants making a cluster block (4rows x 3plants) in a screen house. The cuttings were watered after every two days to maintain saturated moisture on the soil. Leaf characteristics of the varieties were 5-7 lobes in most seedlings. One month after planting when plant height was approximately 32cm, some 10 motile life stages of cassava green mite (CGM) collected at Mtwapa-KARI-Research field (03° 16.024 S, 040° 02.930 E) of coastal area were introduced on each variety. Five days later monitoring of CGM numbers per leaf was carried out by estimating adult stage by aid of magnifying (X 4) lenses. The damage scored was taken from severity level of 1-5 (1=No damage score, 2: ≤25% leaf damage, 3: ≤50% damage, 4: ≤75% damage and 5:=100% wilted leaf) (Yaninek *et al*., 1989). The estimate number of spider mites per leaf was scored to determine injury level in relation to *M. progresivus* numbers. The screen house (5m-lenth x 3m-width) conditions were temperature 20.0 ± 2 °C and relative humidity of $63 \pm 4\%$, with clear glass cover on the top. The experiment was repeated thrice in space and time.

3.4 Phytoseiid mass production for prey feed rate test

Cassava green mite was cultured on a local cultivar, x-Mariakani planted in a plot 50m x 30m at KARI-Katumani Research Station (01° 34.949 S, 037 ° 14.426E, 1609 masl). CGM infestations occurred from the fourth month and reached peak levels of 200-350 mites per leaf during the dry spell on the top plant canopy. The mite infested plant apices (Fig.3.2) were collected each morning for mass rearing of the phytoseiid, *T. aripo* in the laboratory. *Tetranychus evansi* (Baker & Pritchard) was reared on potted tomato seedlings of variety CAL J at the International Centre of Insect Physiology and Ecology (ICIPE), Nairobi. *Phytoseiulus longipes* Evans was reared on the pest *T. evansi* and later to be evaluated on its performance as biological control of CGM. Regular watering and additions of fresh plantlets was done to give the phytophagous mites young plants for egg-laying and subsequent healthy individual for the experiment. The predatory mite *T. aripo* was collected from a cassava field at Kenya Agricultural Research Institute-Kiboko Station (02° 12.872 S, 037° 42.960 E, 934m asl) of the eastern region.

Fifty cassava apices infested with *T. aripo* were picked and transferred in a cool box and delivered to the laboratory at KARI-Katumani for mass rearing. Four plastic containers of 23cm-height x 24cm-diameter were half-filled with water. In the water some 8-10 apices of the x-Mariakani cultivar of 30 to 35cm stem lengths were stood in the containers with the shoot part above the water. The cultivar x-Mariakani plant shoot is usually tri-branched resulting to three apices per shoot. Each shoot contained about 200 to 350 CGM actives enough to feed 30 *T. aripo* actives for two days. Five *T. aripo* infested apices were placed next to the x-Mariakani apices in the containers above water to prevent

phytoseiids from drowning. Some ten similar such containers were maintained in 27 ± 1 ℃ and 75 ± 5% RH in a temperature-humidity controlled room for the mass production of the predator. *T. aripo* life stage cohorts were collected from the apices after three to four days. The photo-period conditions were mainly timed at 12L:12D hr of light and darkness.

To get same age life stage cohorts, 20-50 *T. aripo* females were placed on two leaf disks placed on top of each other (abaxial part up) and infested with CGM actives on a Petri-dish ringed with wet cotton. High numbers of eggs were collected two days later for the experiment. Similarly, *P. longipes* gravid females were isolated from the mass culture and left for 48hrs to oviposit. After hatching, the larvae were transferred singly to the upper surface of leaf discs of cassava plant (2 cm in diameter), placed on cotton wool saturated with water in petri dishes.

Figure 3.2: *Typhlodromalus aripo* De Leon within cassava apex (Mag.X 10). Courtesy of Alexis Onzo (Internatinal Institute of Tropical Agriculture).

3.5 Study sites at different agro-ecological zones

Four study sites were established to evaluate the effects of predacious mite *T. aripo*, the acaricide, abamectin and insecticide, chlorpyrifos for managing of CGM pest densities for three years covering 2011-2013. The site locations were at Kiboko for zone LM5 of the lowlands located (02° 12.872 S, 037° 42.960 E), Katumani zone LM4 of the midlands at 01° 34. 858 S, 037° 14.580 E, Embu zone UM3 of upper midlands (00° 31.642 S, 037° 28.971 E), Mtwapa zone CL3 coastal lowlands (03° 16.024 S, 040° 02.930 E). Soil types were varied; Kiboko, Feralsols; Katumani, Cambisols; Embu, Nitisols and Mtwapa, Luvisols. Climatic data (2011-2013) from the nearest meteorological stations of Embu, Katumani, Kiboko and Mtwapa was collected for analysis of the weather site conditions. Plot preparation involved land clearing, planting and weeding of plot treatments. Soil fertility levels at the sites were taken at 20cm depth with soil auger to analyze for mineral contents at start of evaluation.

Data generated was analyzed using ANOVA-GLM of Fishers Least Significant Difference (LSD). Significant means were separated using Student Neumann Keuls (SNK). Data was log- transformation (x + 1) for normality test and later presented in actual mean levels. Regression analysis was carried to compare independent and dependent variables. SAS V8 software was used in the different analyses.

CHAPTER 4

4.0 IDENTIFICATION AND DISTRIBUTION OF PHYTOPHAGOUS AND PREDACIUOS MITES IN DIFFERENT AGRO-ECOLOGICAL ZONES OF KENYA

4.1 Introduction

The cassava green mite species complex, a serious pest of field cassava crops, is distributed all over the eastern African region since its accidental introduction in Uganda in the 1970s (Nyiira, 1972; Megevand *et al.*, 1987; Gutiérrez, 1987). It is important to determine the phytophagous mites' distribution and abundance, as well as their natural enemies; the predacious mites' occurrence in the different agro-ecological zones of Kenya (Yaninek & Schulthess, 1993; Yaninek *et al.*, 1989). Cassava being highly susceptible to CGM, high levels of infestation has been reported to influence yield output particularly in the dry lowlands of Sub-Sahara Africa (SSA) (Yaninek, 1994; Onzo *et al*., 2005, Kariuki *et al*., 2005, Zundal *et al.*, 2007). The wild cassava variety, *Manihot glaziovii* Mueller was commonly found infested by the same CGM species in eastern region of Africa (Jennings, 1957; Jennings & Iglesias, 2002; Allem, 2002).

Navajas *et al.* (1994) showed a close similarity to the genomic characteristics of different African populations of CGM to those of Colombia (South America) whereas the populations from Brazil (South America) were found to be different. Gutierrez (1987) disputed presence of *M. tanajoa* in Africa after a careful analysis of the species occurrence of the phytophagous from different countries of central, eastern and western Africa and confirmed *M. progresivus* as the common species.

The present study was therefore carried out to determine species identity of both phytophagous and predacious mites in Kenya and their distribution and abundance.

4.2 Materials and Methods

4.2.1 Morphological identification

One hundred mites' specimens of phytophagous and predacious mites from different farmer plots were cleared in lactic acid for 48 hours, mounted in Hoyer's medium and dried in an oven at 40°C for seven successive days (Yaninek *et al.*, 1989). Identification of specimens was carried out following El-Banhawy and Knapp (2011) and Chant and McMurtry (2005) respectively, for predacious and phytophgous species. Confirmations of the identifications of the species were based on comparison with formerly identified specimens by experts (M. Knapp for *Tetranychus evansi* (Baker & Pritchard); E.Ueckermann for *Mononychellus* species and El-banhawy for phytoseiid species.

4.2.2 Nucleotide sequence and analysis

The purified PCR products (4-5ul) were directly sequenced on both strands using an ABI 3100 series automated sequencer (Applied Biosystems Inc) and the nucleotide sequences were aligned using MEGA (Windows Version 5.2.2) for the *Mononychellus* species specimens. Analyses on the sequenced nucleotide enabled delimiting the base divergence specific sites following earlier works and procedures by Stevens (1991) and Hall (2013). The predacious mites' sequences were analyzed using the software BioEdit (Window Version 5.0) for nucleotide pairwise alignment procedure of base compositions to determine phytoseiid species phylogenetic divergence base compositions with other

known species sourced from the National Center for Biotechnology Information (NCBI, U.S.A) Gene Bank data (Hall, 2013). The nucleotide sequences were BLAST on the National Center for Biotechnology Information (NCBI) date base to determine identity match with other species in the gene bank. CLUSTALX2 and MEGA 5.2.2 was used for multiple alignment and phylogenetic analyses, respectively.

4.3 Results

4.3.1 Distribution of *Mononychellus* species

Results of the studies on the distribution of *Mononychellus* species showed an increasing infestation trend particularly in dry lowlands (LM 3, 4, 5) sampling sites of Machakos, Makindu and Kitui (Fig.4.1). The *Mononychellus* species population abundance fluctuated between 10 and 400 individuals per farm sample. In the coastal strip (CL 2, 3) and the western midlands (LM 2, 3) the population was found to be 10-200 mites per sample. However, mite infestation was lowest in the upper eastern midlands (Embu and Meru) (UM 2, 3). Out of 166 farms sampled, 50% had over 100 CGM/sample and there was clear evidence of chlorotic leaf damage.
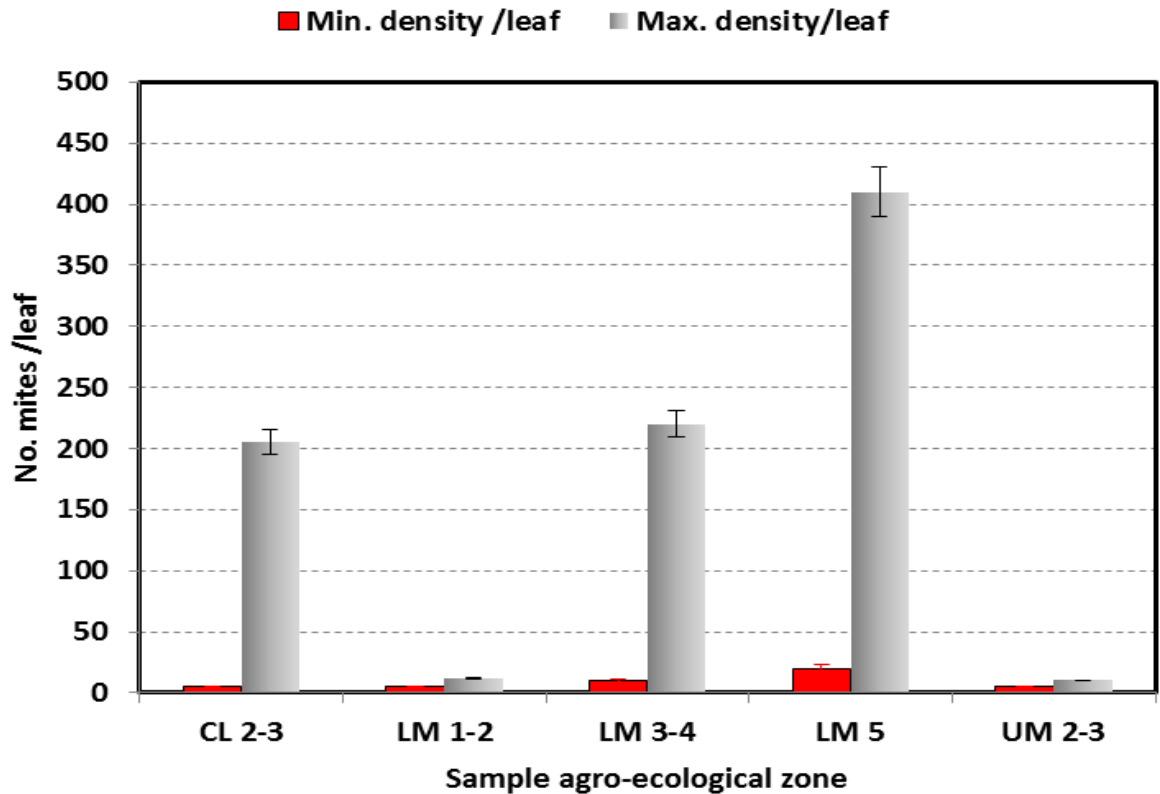
Figure 4.1*: Distribution range (minimum and maximum peaks) of the cassava green mite *Mononychellus* species in different agro-ecological zones of Kenya

4.3.2 Farmer practice and plant health biotic constraints

Only 1.4% manure use was reported in the western Kenya region and no inorganic fertilizer use for cassava was recorded in any of the sampled farms (Table 2.1). The mean CGM per leaf was highest in western upper midlands, approximately 116.0 ± 16.2, followed by eastern low midlands with 32.0 ± 5.2 mites per leaf. A wild cassava species, *M. glaziovii* was found in eastern areas of Kenya, in Machakos, Meru and Embu with low infestation of CGM (<20 mites/leaf). The highest disease incidence was cassava mosaic viral disease (CMVD) and it was in Nyanza, coast and eastern of 87.3, 61.1 and 56.4 % farm incidence, respectively. Field cassava brown streak viral disease (CBSVD) was

highest in Nyanza at 22.3%. Cassava mealybugs, *P. manihoti* were scored in the sample farms. There was no pest control measure that was practiced by farmers, chemical or cultural. Likewise farmers did not use any inorganic fertilizer on cassava production.

Table 4.1: Kenya agro-ecological zones (AEZs), mean (*SE*) manure / inorganic fertilizer use, pest spider mites and disease incidence in Kenya in 2011

Region	AEZs	Manures (%)	Pest mites per leaf	CMVD (%)	CBSVD (%)
Eastern	UM3, LM 4-5	0a	32.0 (5.2)b	56.4 (4.3)b	1.5 (0.5)d
Coast	CL 2-3	0a	28.0 (2.8)b	61.1(5.8)b	41.2 (4.4)a
Western	LM 2-3	1.4 (0.4)b	116.0 (16.2)a	52.2 (11.2)b	18.4 (6.3)c
Nyanza	LM 1-2	0a	26.4 (8.5)b	87.3 (11.1)a	22.3 (2.5)b
F		5.6	26.8	22.1	1.2
P.		0.0002	< 0.0001	< 0.0001	< 0.0001

Different letters denote significant ($P < 0.05$) at 5% level, (LSD-GLM) of abiotic and biotic parameters

4.3.3 Abundance and identity of phytophagous mites

Morphologically the female *M. progresivus* Doreste is characterized by the dorso-central setae (c_1, d_1, e_1) progressively being longer towards the posterior, and c_1, measured about half the distance to basis of second pair (d_1). The male aedeagus unlike that of *M. tanajoa* is globular and without terminal angulation (Gutierrez, 1987). Identification results showed that three species of tetranychid mites were found infesting cassava in the fields of the different agro-eco-zones (Table 4.2). The *M. progresivus* (Fig. 4.2) was the most common species of spider mite identified and represented 99% of the total number of mites in the sampled fields (165). The tomato spider mite *T. evansi* (0.4%) (Fig.4.3) and the brown spider mite *Eutetranychus orientalis* Klein (0.6%) (Fig.4.4) together constituted only 1% of the mite samples. While *E. orientalis* was found in some four

farms in coastal farms, *T. evansi* was in the eastern Kenya farms in isolated six fields,

where tomato production was prevalent.

Table 4.2: Phytophagous species occurrence and distribution in the agro-ecological zones

No.	Species	% presence	Zones
	Subfamily Tetranychinae		
1	*Mononychellus progresivus* Doreste	99	UM 2-3, LM 1-5, CL 2-3
2	*Tetranychus evansi* Baker & Pritchard	0.4	LM 4 -5
3	*Eutetranychus orientalis* Klein	0.6	CL 2, LM 2
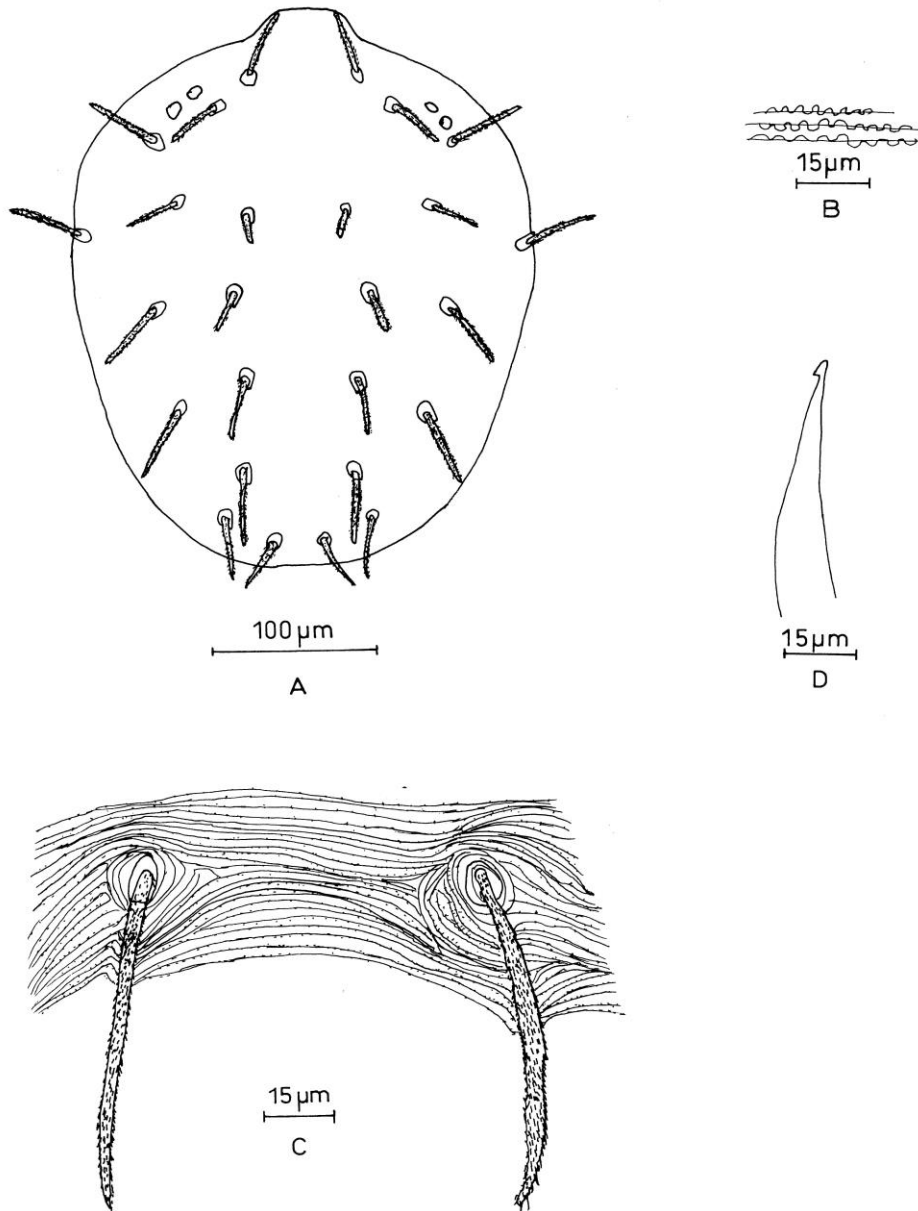
Figure 4.2: *Mononychellus progresivus* Doreste (Female). A, Dorsal view showing arrangement of setae; B. Lobes of striae; C, Striae of dorsal hysterosoma; D, Aedeagus of male.
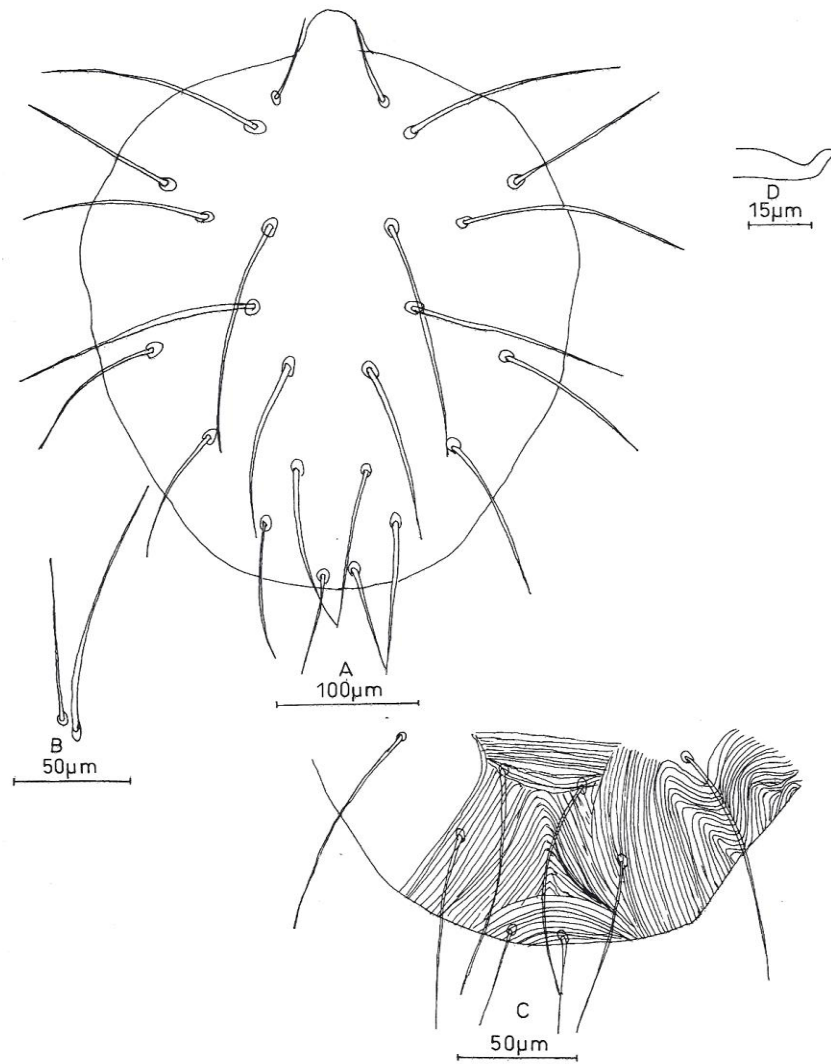
Figure 4.3: *Tetranychus evansi* Baker & Pritchard (Female). A. dorsal view showing arrangement of setae, B. Duplex setae on tarsus I; C. Striae of dorsal hysterosoma; D. Aedeagus of male.
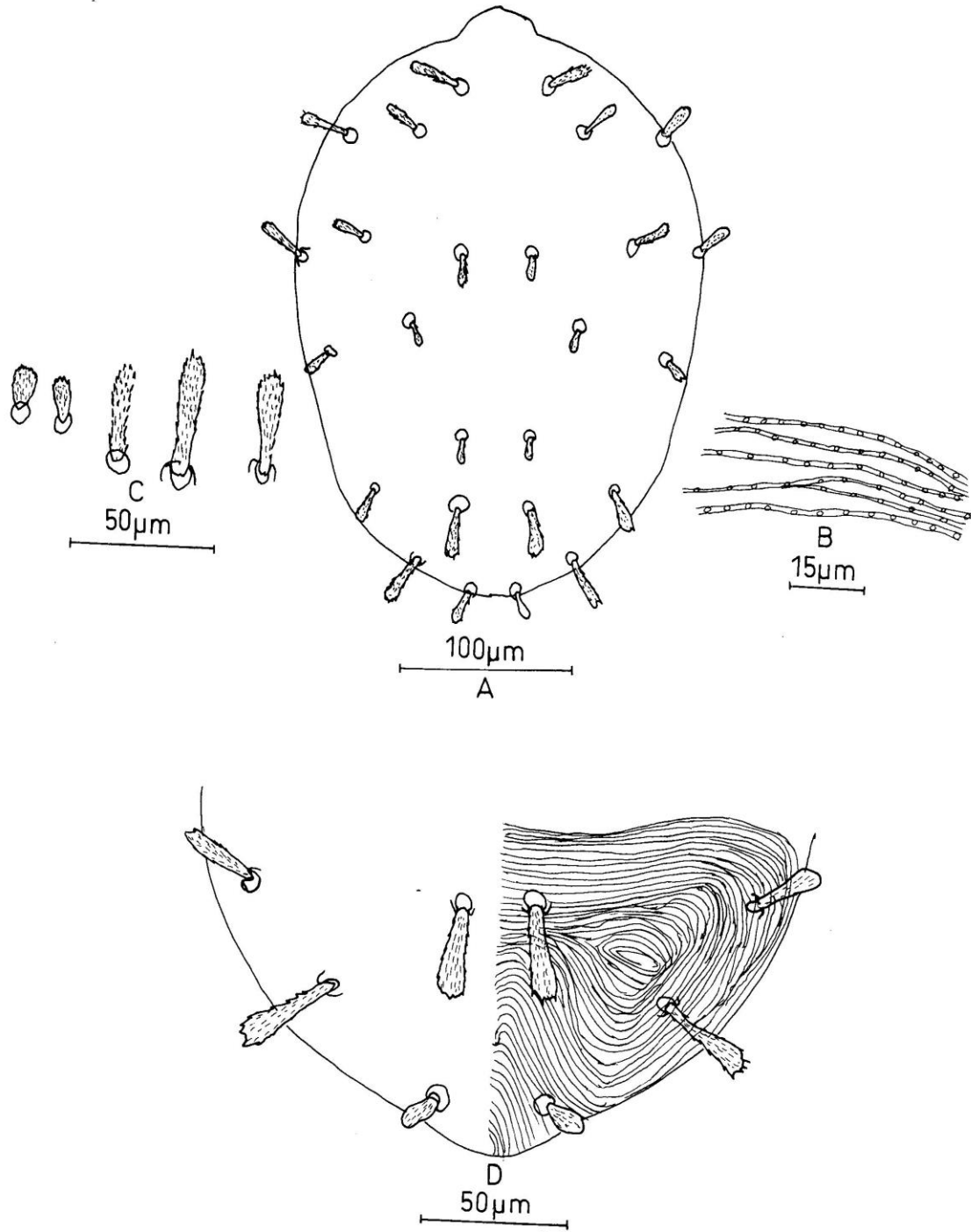
Figure 4.4: *Eutetranychus orientalis* (Klein) :Female. A. dorsal view showing arrangement of setae; B. lobes of striae: C, variations of dorsal setae; D, Striae of dorsal hysterosoma.

4.3.4 Genetic similarity of Kenyan *M. progresivus* to populations in Africa

 Results of sequencing a fragment of the ribosomal DNA (ITS2) of the Kenyan samples

of *M. progresivus* showed a close similarity to that of the other populations of the

Democratic Republic of Congo and Benin on running the sequenced nucleotide on the

National Center for Biotechnology Information (NCBI) bank data base. Out of the 787

nucleotides, there were three variable positions at 223, 316 and 328 indicating 99%

similarity (of ID: emb/X79902.1) as shown on Figure 4.5A.   Further, the sequences of

the mitochondrial fragment (C0I) (bp 372) were conserved and showed 100% (ID:

X77902.1) similarity (Fig.4.5B) (cf Table 4.3).

A)
```
  1    ATACCAACTA   TACATTGAGT   AGATACAGGG   CCTCTGTCTA   CCATGATAG    AGATCATGTG   60
 61    TATGTGTGTA   TATCTGTGCA   TGGTATAACA   CATACTATGT   GTACATGTAT   GTATCTTGCA   120
121    GGATCGTAGC   GCTTTAACTG   GAAACGGTTA   AGGTTAGCTA   CACGCCTGCA   TATAATTTAC   180
181    AGAAAGTATG   TACCTTGCCT   ATGACCGGAA   ACAGTTATAT   CGAAATCTAC   ATATTAGTAT   240
241    GGATATTATA   TTGTTCCTTG   CGTGTTATGT   GGTAAAACAC   ATATCATTGC   CGGTCAGAGA   300
301    TATATATGTA   TGTTAAATAG   TATGGTTAAT   CAATTTTTGT   ACTTAGTTTT   ACACACATTT   360
361    CCCATGATCT   AGATATTCTA   TTCCTTTTCG   TGGAAGTATG   TATATACAAC   CTCGTAAGGA   420
421    GATGCATCAA   TGATGTGATA   TCTTGATATC   TGTATGCTAC   TACAGCTAGT   AAGCGGCAGA   480
481    GCAGCAGTTG   ATCTGACTGT   CAAGCAAATC   ACTGGCAGGG   ACCCTGAGAG   AACCCGTCAA   540
541    TCTGCCGACG   TTAAAGTCGT   ACAGCAGATT   AGTAAGACGC   GACATGACCT   GTCGAAAAGG   600
601    TTCGTCTCCT   TGAAAGGGGT   CTCGTTTGCA   CTCTAAGGTG   ATTTGTCATC   TGTTAGCGAT   660
661    GCTTCTGTAT   TGCAGACACA   GACAAGTATT   ACGGGGCAAT   CATTGATTAG   CAAATATGTT   720
721    TGAGTCTCTT   GACTGTGTAT   TATGTGAACA   CACACACACA   CCAAGTGTAC   TCTTTCCATA   780
781     TACCTACA                                                                   787
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B)
```
  1    TTAATTCTAC   CAGGATTGG    TATAATTTCT   CATATTATTA   GTTATAATTT   AGGTAAAAAA   60
 61    GAAGTATTTG   GTAAATTAGG   AATAATATTT   TATTTACTAT   C.AATTGGTT   TATTAGGATT   120
121    TGTTGTATGA   GCTCATCATA   ACAATAATTA   TGGTATAGAT   GTTGATACTC   GAGCTTATTT   180
181    TACAGCAGCT   TACTATCATT   TTGCTATCCC   TACAGGTATT   AAAATTTTTA   GATGATTTAC   240
241    TACTATCATT   AATTCTTCAT   TTAATTTTAA   TATTTCTGTC   TATTGATCAA   TAGGATTTTT   300
301    AATTATATTT   TCTATTGGAG   GTTTTACAGG   AATCATTGCT   TCAAATTCTT   GTTTAGATAT   360
361    CTCTTTACAT       GA                                                          372
```

Figure 4.5A and B: (A) the nucleotide sequence of ribosomal DNA (ITS2) and (B) mitochondrial cytochrome oxidase subunit I (COI) of the cassava green mite, *Mononychellus progresivus* Doreste sampled from Kenya. Base coloured positions shows the variable regions with Congo- Benin sequences.

TABLE 4.3: Three variable positions among the 787 nucleotides of Kenyan *Mononychellus progresivus* sequence aligned with Congo population (from NCBI data base)

	COI	ITS2		
Nucleotide position		223	316	328
Kenya	-	A	A	A
Congo-Benin (NBI-data)	-	T	C	T
Similarity (%)	100.00	99.99		

Figure 4.6 (A and B) compares *M. progresivus* nucleotide phylogenetic differences from Kiboko, Katumani and Mtwapa. The Figure 4.6 (A and B) shows constructed phylogenetic trees of ITS2 and COI regions of the mite. The difference on the nucleotide divergences could only be attributed to the biogeographic sites, as overall they are same species to M. progresivus found in Congo and Benin.

A   B

Figure 4.6: Neighbour-Joining phylogenetic tree based on (A) internal transcribed spacer 2 (ITS2) and (B) cytochrome oxidase subunit I (COI) nucleotide divergences of *Mononychellus progresivus* showing phylogeny positions among related taxa from NCBI. Bootstrap values (>50%) based on 1000 replications are shown at branch nodes.

4.3.5 Distribution of predacious mites and morphological identification

A total of 462 phytoseiid individuals were sampled from 165 farms. Examinations indicated a total of 10 genera and 29 species (Table 4.4). The species occurrences under each genus greatly varied according to the geographical zones. The highest occurrence was *Euseius fustis* Pritchard & Baker where a field presence of 37% (162 farms) in comparison to 34% (in 145 farms) presence of the introduced *T. aripo*. Other sampled species showed a lower degree of occurrence. For example the predatory mite, *Neoseiulus onzoi* Zannou, Moraes & Oliveira had 3.6% presence, *Euseius neofustis* Moraes & McMurtry 12% and other species scored less than 1% presence. The predatory mite *E. fustis* was found at different levels in all agro-climatic zones (LM2-3, LM4-5 UM2-3 and CL2-3), while *T. aripo* though present in most of the same regions, it missed in the cool-wet zone UM2-3. At genus level, the highest observed group was *Euseius* Wanstein with 55.1% field presence, followed by *Typhlodromalus* Muma with 34% presence, yet the genera *Neoseuilus* Hughes and *Amblyseius* Berlese each was about 4% in the same fields. The other genera *Iphiseius* Berlese, *Propriociopsis* Muma, *Typhlodromips* De Leon, *Uckermannseius* Chant & McMurtry and *Kuzinellus* Wainstein had less than 5% field presence.

Table 4.4: Bio-geographical distribution of phytoseiid mites in separate cassava zones of Kenya

No.	Species	No. individuals	No. farms	Zone
	Sub-family *Amblyseiinae*			
	Genus *Neoseiulus* Hughes			
1	*N. asperisetatus* Zannou, Moraes & Oliveira	1	1	LM 2
2	*N. onzoi* Zannou, Moraes & Oliveira	19	6	LM 2, 3, 4
3	*N. scapilatus* Van der Merwe	1	1	CL 2, 3
4	*N. teke* (Pritchard and Baker)	1	1	CL 2
5	*N. tekeleius* El-Banhawy & Knapp	1	1	LM 2, 3
	Total Genus presence	*23*	10	
	Genus *Typhlodromips* De Leon			
6	*Typhlodromips bonyorei* El-Banhawy& Knapp	4	2	LM 2
	Total Genus presence	*4*	2	
	Genus *Amblyseius* Berlese			
7	*A. sundi* Pritchard & Baker	2	1	LM 2, 3
8	*A. largoensis* Muma	14	5	CL 2, 3
9	*A. herbicolus* (Chant)	1	1	CL 2, 3
10	*A. ankaratrae* Blommer	1	1	LM 2
11	*A. swirskii* Athias-Hanriot	2	1	CL 2, 3
	Total Genus presence	*20*	9	
	Genus *Typhlodromalus* Muma			
12	*T. aripo* De Leon	177	145	LM 1-5, CL 2, 3
	Total Genus presence	*177*	145	
	Genus *Uckermannseius* Chant & McMurtry			
13	*Ueck. mangrovei* El-Banhawy & Knapp	1	1	LM 2
	Total Genus presence	*1*	1	LM 3
	Genus *Transeius* Chant & McMurtry			
14	*T. mesabahaensis* (Moraes and McMurtry)	1	1	CL 2
	Total genus presence	1	1	
	Genus *Euseius* Wainstein			
15	*E. papayana* (Van der Merwe)	5	3	LM4, UM2, 3
16	*E. Africanus* (Evans)	7	3	UM2, LM2, CL3
17	*E. albizzae* (Swirski&Ragusa)	1	1	LM5
18	*E. bewende* (Pritchard & Baker)	1	1	CL 3
19	*E. fustis* (Pritchard & Baker)	196	162	LM2,3, UM2,CL3
20	*E. baetae* (Meyer &Rodriguez)	1	1	CL2
21	*E. lokele* (Pritchard & Baker)	2	1	UM3
22	*E. natalensis* (Van der Merwe)	2	1	LM 3
23	*E. neofustis* (Moraes&McMurtry)	66	11	LM3-5, CL3
24	*E. rhusi* (Van der Merwe)	1	1	CL3
25	*E. talinga* (Pritchard & Baker)	4	2	LM2, CL3
26	*E. vandenbergae*(Ucckermann & Loots)	8	3	LM 2,3, 5
	Total Genus presence	*228*	20	
	Genus *Iphiseius* Berlese			
27	*I. degenerans* (Berlese)	6	2	LM2,UM3
	Total Genus presence	*6*	*3*	
	Genus *Propriociopsis* Muma			
28	*Propriociopsis atrichos* Moraes, Zannou & oliveira	1	1	LM2
	Sub-family Typhldrominae Chant &McMurtry			
	Genus *Kuzinellus* Wainstein			
29	*K. kariuki* El-Banhawy & Knapp	1	1	LM2-3
	Total specimens species and % sampled	*462*	*166*	

4.3.6 *Typhlodromalus aripo* **and** *Euseius fustis* **nucleotide divergence**

At molecular level, application of Basic Local Alignment Search Tool (BLAST) at the
NCBI site Gene Bank showed that *T. aripo* was closely related to *E. ovalis* Evans (94%),
E. sojaensis Ehara (93%) and *T. limonicus* McGregor (92%) on the internal transcribed
spacer 1(ITS1) region (Table 4.5). On the cytochrome oxidase subunit I (COI) region, *T.
aripo* was phylogenetically related to *N. californicus* McGregor (89%), *E. nicholsi* Ehara
& Lee (84%) and *E. baraki* Wainstein (82%) (Table 4.6).

Similarly, *E. fustis* was found to be phylogenetically related to *I. degenerans* Berlese
(93%), *E. ovalis* (92%) and *E. nicholsi* (91%) on internal transcribed spacer 1 (ITS 1)
gene region (Table 4.6). On the cytochrome oxidase sub unit I (COI) gene region, *E.
fustis* was found closely related to *N. californicus* (88%), *E. nicholsi* (82%) and *E.
stipulates* Athias-Henriot (81%) as shown on Table 4.6.

As shown in Table 4.7, *T. aripo* was found to have higher G+C base pair compositions of
32.4% in comparison to *E. fustis* of 28.9% on the cytochrome oxidase subunit I (COI).
Subsequently *E. fustis* had 71.1% A+C base pairs composition on the same gene region.
On the internal transcribed spacer 1 (ITS1), *E. fustis* had higher G+C bases pairs 45.3%
while *T. aripo* had 43.6%. The A+T composition was 56.4% for *T. aripo,* while *E. fustis*
had 54.7% base pairs on same ITS1 region. On comparison with other similar region
gene nucleotides from NCBI Gene Bank of *N. califonicus* and *E. stipulatus*, a
composition of 35.1 and 30.2% base pairs were found for A+T bases on the COI region,
respectively. The internal transcribed spacer 1 (ITS1) base pair composition was
determined as 57.8 and 56.4% for *N. califonicus* and *E. fustis* respectively.

Table 4.5: Internal transcribed spacer 1 (ITS1) BLAST results of *Typhlodromalus aripo* and *Euseius fustis* nucleotide match (%) of related species from NCBI data base

Morphological identification	bp (letters)	% Match	NCBI Accession	Gene region	Species
Typhlodromalus aripo De Leon	596	94	JN020166.1	18S rRNA	*Eseius ovalis* Evans
		93	AB618065.1	18S rRNA	*Euseius sojaensis* Ehara
		92	HM18927.1	18S rRNA	*Typhlodromalus limonicus* McGregor
Euseiu fustis Pritchard & Baker	138	93	AY121984.1	18S rRNA	*Iphiseius digenerans* Berlese
		92	FJ515687.1	18S rRNA	*Euseius ovalis* Evans
		91	JQ864578.1	18S rRNA	*Euseius nicholsi* Ehara & Lee

Table 4.6: Cytochrome oxidase subunit I (mtCOI) BLAST results of *Typhlodromalus aripo* and *Euseius fustis* of related species from NCBI data

Morphological identification	Bp (letters)	% Match	NCBI Accession	Gene Region	Species
Typhlodromalus aripo De Leon	411	89	AB500128.1	mtCO1	*Neoseiulus californicus* McGregor
		84	KF308628.1	mtCO1	*Euseius nicholsi* Ehara & Lee
		82	JQ60904.1	mtCO1	*Euseius baraki* Wainstein
Euseius fustis Pritchard & Baker	407	88	AB5001281.1	mtCO1	*Neoseiulus californicus* McGregor
		82	KF308644.1	mtCO1	*Euseilus nicholsi* Ehara & Lee
		81	FJ404587.1	mtCO1	*Euseius stipulates* Athias-Henriot

Table 4.7: Species molecular weight (Daltons) comparison between *Typhlodromalus aripo* and *Euseius fustis* on similar gene region on pair wise alignment

Species molecule composition	cytochrome oxidase subunit 1 (CO1)				internal transcribed spacer 1 (ITS1)			
	Bp	Mol. Wt	G+C (%)	A+T (%)	bp	Mol. Wt	G+C (%)	A+T (%)
Typhlodromalus aripo	411	122,685	32.4	67.6	596	180,254	43.6	56.4
Euseius fustis	407	124,836	28.9	71.1	138	41,982	45.3	54.7
Neoseiulus californicus	729	218,185	35.1	64.9	635	193,126	42.2	57.8
Euseius stipulates	453	135,227	30.2	69.8	651	195,104	43.6	56.4

A phylogenetic tree of both ITS1 and COI of *T. aripo* and *E. fustis* yielded more information on how the two species were different (Fig. 2.7). Comparably *T. aripo* was found closely related to species *E. nicholsi* than *E. fustis* at both ITS1 and COI regions.

The pairwise alignment of *T. aripo* and *E. fustis* indicated a large phylogenetic divergence with identity similarity of 0.187 and alignment score of 184 base pairs on ITS1 region. Likewise the COI region indicated a nucleotide divergence of 0.436 identities on alignment score of 118. The highest number of variants of *T. aripo* to *E. fustis* on COI region was from 301 to 400 base pairs with a score of 79 (19%) variants of *T. aripo* (411bp) to *E. fustis* (407bp). The least nucleotide difference was found on the tail part of the alignment from 400 to 411 base pairs of 6 (1.5%) variants of *T. aripo* to *E. fustis* COI region. Total calculated nucleotide divergence was 34% of *T. aripo* to *E. fustis* on the COI region. Similarly, on the ITS1 region *T. aripo* (596bp) had total divergence of 14% to *E. fustis* (139 bp). Figure 4.8 gives base pairs compositions for *T. aripo* and *E. fustis* where the former led with high number of Ts (pyrimidine) at 44% and the latter with high As (purine) content at 47% on COI region.
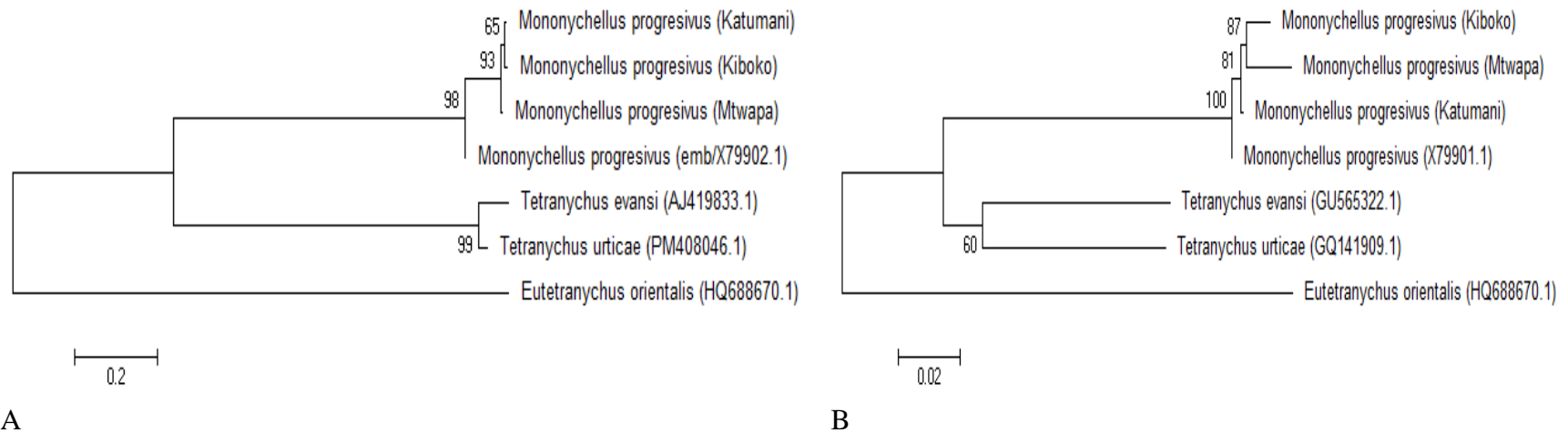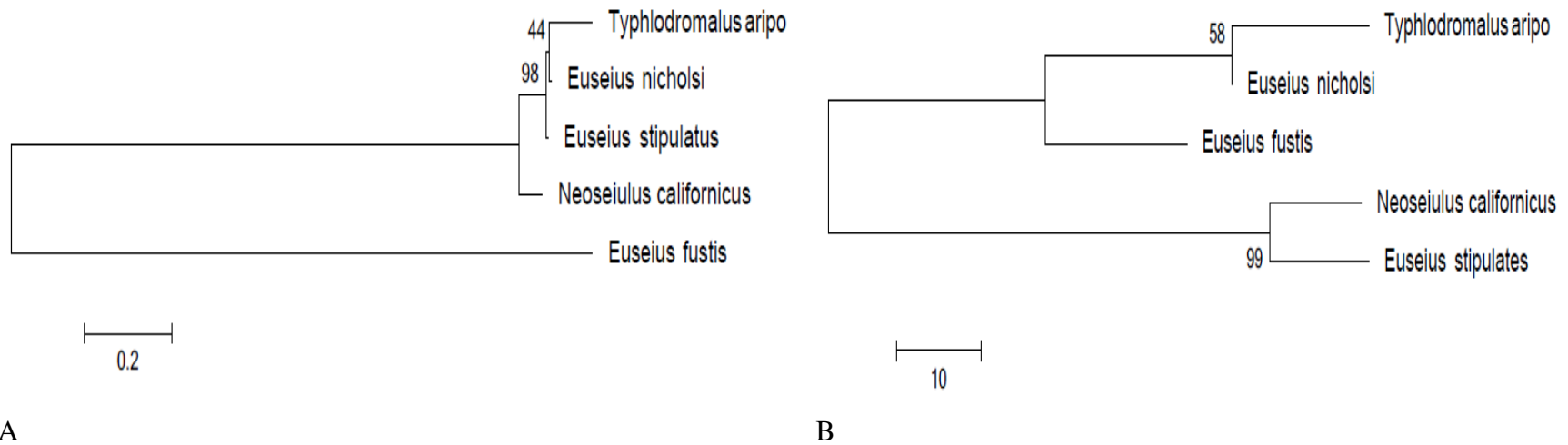
Figure 4.7: Neighbour-Joining phylogenetic tree based on (A) internal transcribed spacer 1 (ITS1) and (B) cytochrome oxidase subunit I (COI) nucleotide divergences between *Typhlodromalus aripo Euseius* and *fustis* showing phylogeny positions among related taxa from NCBI. Bootstrap values (>50%) based on 1000 replications are shown at branch nodes.

(A)

(B)
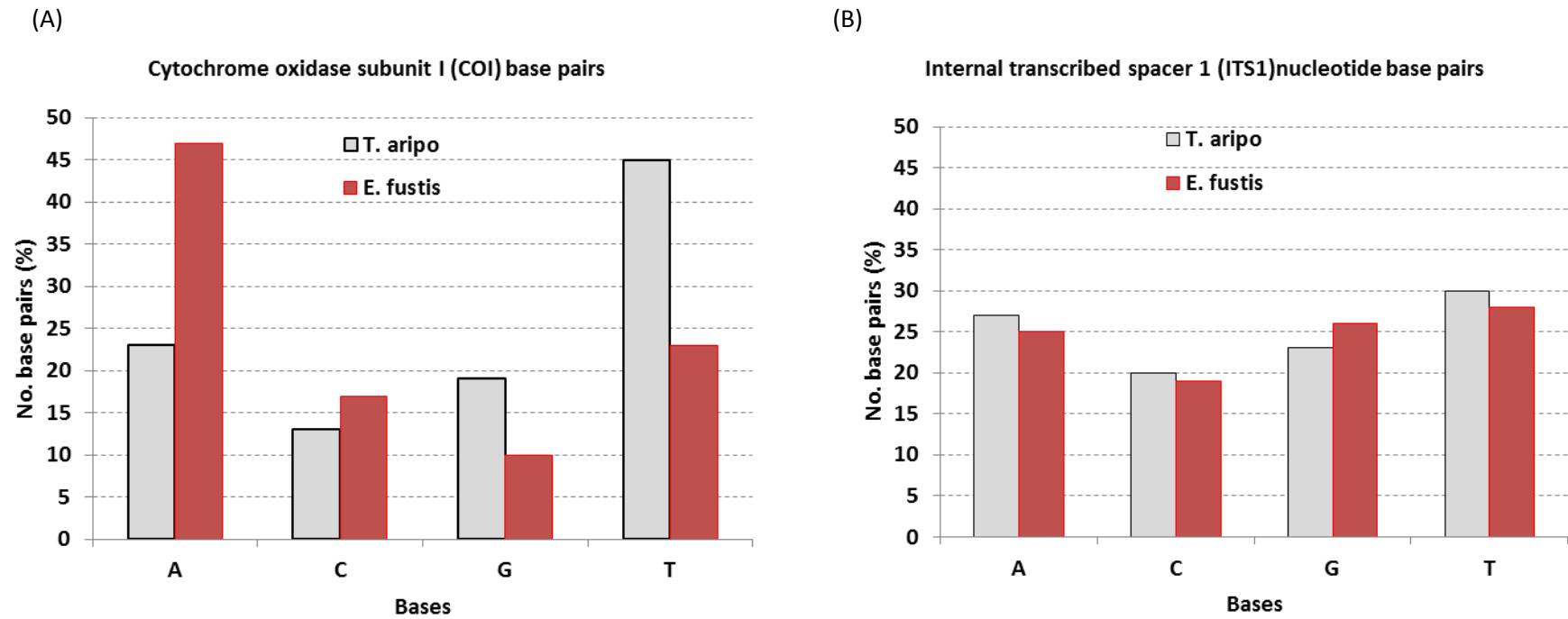
Figure 4.8: Comparison between nucleotide base composition percentages of *Typhlodromalus aripo* and *Euseius fustis* on (A) cytochrome oxidase subunit I (COI) and (B) internal transcribed spacer 1 (ITS1) gene regions showing the four bases.

4.4 Discussion

The dry lowlands of Kenya disclosed the highest incidence of *M. progresivus* thereby supporting earlier reports of the pest distribution (Bonato *et al*., 1994; Hillock, 2002; Kariuki *et al*, 2005). Yaninek *et al.* (1987) and Bonato *et al* (1994) reported on the biological basis of the mite pest causing high leaf damage of cassava photosynthate leaf area and subsequently root yield loss in Africa (Yaninek *et al.,* 1989). In Kenya, Kariuki *et al* (2005) confirmed that higher densities of the mite lead to higher root yield loss.

Although cassava crop was introduced to Africa in the 16th Century, the cassava green mite (CGM) has become a serious pest of cassava plants only from the late 1970s lowering the root yield on most farms (Hillocks, 2002; Kariuki *et al*., 2005). Since 1970s it has been believed that CGM was accidentally introduced when cassava cuttings were imported to East Africa from South America, Brazil (Nyiira, 1972; Yaninek & Herren, 1986; Bellotti, 2002; Hillocks, 2002). Gutierrez (1987) reviewed some samples collected from different regions of Africa in early 1980s and concluded that most likely the species in East Africa is *M. progresivus.* Murega (1989) through cross-breeding studies found that CGM populations of Kenya and Uganda were compatible. During the course of this study the morphological examination of several samples from Kenya, as well as Tanzania and Uganda borders and found that all the samples agreed with the definition of *M. progresivus*. The earlier results of the sequence of a fragment of ribosomal DNA (ITS2) and mitochondrial cytochrome oxidase subunit I (COI) of CGM from Benin (West Africa) and DRC (Central Africa) showed that the two populations were similar and different from that of Brazil (Navajas *et al*., 1994).

The present results on the individual mite sequencing of the populations from the 15 farms (of the three zones) in Kenya of the same two markers indicated close relationships of populations with that of Benin and DRC fields. Further, based on the microscopic examinations of the diagnostic characters of the specimens and the molecular analysis of individual pooled mites, the common cassava green mite in Kenya, *M. progresivus* appeared different from that of Brazil; possibly identified as *M. tanajoa* (Navajas *et al*., 1994). The multiple alignment and the subsequent construction of phylogenetic trees on ITS2 and COI regions of Kenya species alongside NCBI data showed that the species in Kenya was similar to populations of Colombia, Benin and Congo.

It was also observed in the survey that the wild cassava species, *M. glaziovii* is abundant in the East African region and is reported to be used in cross breeding experiments against cassava diseases (Jennings and Iglesias, 2002). As Iglesias (2002) pointed out, several cross-breeding programs in East Africa have previously collaboration with International Institute of Tropical Agriculture (IITA, Ibadan) to improve the domesticate cassava, *M. esculenta* against the cassava mosaic viral disease (CMVD). This wild cassava was also found infested by the same *M. progresivus* throughout the survey though at lower levels than the domesticate species. The *M. progresivus* was also recorded on different species of *Manihot* in South Africa and on other plant species in Democratic republic of Congo (Meyer, 1987). Therefore, two possible conclusions can be drawn based mainly on results of morphological and molecular analyses carried out during the present study in light of the earlier work by Navajas *et al* (1994); (1) the common cassava green mite in Kenya, *M. progresivus* shows close similarity to the species described in Venezuela where it was first observed (Meyer, 1974; Doreste, 1981)

and (2) the similar genetic characteristics of the populations in other parts of the continent indicate the possibility of wide-spread species that has not been well studied. The latter assumption needs to be investigated further both through appropriate molecular and morphological studies if most of the CGM populations in Africa constitute a mixture of *M. progresivus* and *M. tanajoa* or a single species.

Phytoseiid occurrence from the survey results indicated diverse species from the agro-ecological zones. The phytoseiid *T. aripo* was not found in cooler upper midlands (UM2, 3) most probably due to its sensitivity to the low temperature (≤ 16 °C) (Mutisya *et al*. in press). Recent work by Zannou *et al* (2007) showed that the genus *Euseius* had wide number of species on cassava from the southern region of African continent. In the present study the *Euseius* genus has yielded some 12 species from the different diverse bio-geographical zones of Kenya. *Neoseiulus* and *Amblyseius* genera had five each. Tixier *et al* (2010) demonstrated that it was possible to extract DNA for sequencing from single female mite specimens and later use the carcass to carry out a morphological observation. This is important since there are many phytoseiid species in Africa whose molecular identification must be guided by the expertise on morphology before reliability of molecular data is acceptable (Stevens, 1991; Navajas *et al.*, 1996, 1997).

In conclusion, it was determined that the procedures for mite sequencing and nucleotide analyses on the cytochrome oxidase subunit I (COI) nucleotide compositions could be deduced to identify phytoseiids superiority in terms of adaptation to long persistence in diverse climatic conditions (Konakandla *et al.*, 2007). In our case *T. aripo* was found to be rich with Ts (pyrimidine) at 44% content while *E. fustis* had high As (purine) content at 47% on COI region. The COI region is found in the mitochondrial region of the cell

organelles and is responsible for oxygen acquisition for the cell and subsequent aerobic respiration tissue (Hebert *et al.*, 2003; Davis *et al.*, 2012). The pyrimidine rings make up tissue structure for body regulation functions (Nelson and Michael, 2008). Purines function as neurotransmitters in body tissue, hence dependent on the pyrimidine functions (Davies *et al.*, 2012). The question arises: which phytoseiid species has been persistence on cassava plants and with highest level of performance as bio-control agent of cassava green mite? *T. aripo* fits the description here. The phytoseiid species feeds on both plant and herbivore diets hence can survive and breed for some time in absence of preferred prey (Bakker *et al.*, 1993; Magalhães and Bakker, 2002; Gnanvossou *et al.*, 2003, 2005). *T. aripo* has been reported as one of the most efficient suppressors of *M. progresivus* densities in comparison with indigenous phytoseiid in Africa (Yaninek & Hanna, 2003; Onzo *et al.*, 2003, 2008; Kariuki *et al.*, 2005; Zannou *et al.*, 2007). While *E. fustis* was found in all agro-ecological zones, its population densities were usually low probably due its diet preference of plant pollens least found on cassava. In comparison *T. aripo* was found to persist in most warm-humid (LM 5, CL 2-3) environments and absent in the cold zones (UM 2, 3) of Kenya. Likewise the pairwise alignment enabled analysis of the base pair variance between *T. aripo* and *E. fustis* where the second last codon positions indicated the highest base pair variants on COI region. A total of 34% divergence was deduced from the alignment of *T. aripo* to *E. fustis* nucleotide on COI gene region. If one of the base pairs nucleotide is by far of short length in comparison to the case of *E. fustis* (ITS1) it was found that only limited actual pairwise alignment from the two nucleotides would be used for measure of divergence of the species. As found out from the analyses it is evident that by use of basic analysis software like BioEdit it was possible to carry out

nucleotide analyses and delimit phytoseiid species speciation of both exotic and indigenous species from diverse environment and describe phylogeny characteristics at molecular level. Hence, the construction phylogenetic trees by use of MEGA 5.2.2 enabled inclusion of other phytoseiid species from NCBI data base giving phylogenetic position of *T. aripo* and *E. fustis* among similar phytoseiids. These molecular tools continue to influence detailed studies on pests and beneficial organisms with new information as it has been found with spider mite species (Navajas *et al*, 1994, 1996, 1997; Hall, 2013).

CHAPTER 5

5.0 PHYTOPHAGOUS MITE *MONONYCHELLUS PROGRESIVUS* GROWTH AND DAMAGE ON CASSAVA VARIETIES

5.1 Introduction

Mite pest abundance on plant host depends on presence or absence of deterrent chemical compounds, or antibiosis on the organ most vulnerable to pest attack (Kawano & Bellotti, 1980; Bellotti *et al.*, 1999). In the same context *M. progresivus* population growth on cassava plant and the subsequent economic injury level (EIL) would be as a result of deterrence level of variety chemical compound compositions besides other plant characteristics (Komkiewtcz *et al.,* 1993; Higley and Pedigo, 1993). On plant host resistance, cassava mealybug *P. manihoti* resistance was found to be correlated to leaf pubescence and unexpanded leaves (Hahn, 1984). Similarly spider mite pest resistance or tolerance has been attributed to plant vigor, leaf pubescence and antibiosis mechanism (Bellotti and Byrne, 1979; Kawano and Bellotti, 1980; Bellotti *et al.,* 1999). Other invertebrate deterrents reported on cassava are the hydrocyanic acid (HCN) which repels some grasshoppers (Bellotti, 2002). The cassava cyanogen compounds are mainly the linamarin (85%) with lesser amounts of lotaustralin free HCN glycosides found in both foliage and root content (Iglesias *et al*, 2002; Alves, 2002). No report has detailed how leaf cyanide levels contribute to density growth of *M. progresivus* pest. Recent studies have indicated high mortalities of the predatory mite *Phytoseiulus longipes* Evans when fed *M. progresivus* which fed on cassava leaf tissue (Mutisya *et al.,* 2012).

Whether higher cyanogens content on cassava leaf leads to less damage by *M. progresivus* is an important question in the present study. The study explored how HCN

content (mg/kg) levels of different cassava varieties influence *M. progresivus* density growth and subsequent leaf tissue damage.

5.2 Materials and Methods

5.2.1 Mite population dynamics over time

The population growth of CGM to the peak at each estimated damage score level was recorded for varietal comparison from the nine varieties, of Kalezo, Karibuni, Tajirika, x-Mariakni, MM990183, MM99005, MM97/3567, MM96/2480 and MM96/9380. The durations (days) to particular damage score of a variety in comparison with other varieties would indicate susceptibility /tolerance to *M. progresivus* attack. Likewise mite density growth rate was determined at specific time durations of days. The period for experimental monitoring was 55 days since introduction of 10 individuals of CGM adult motiles on each variety. On day five, after mite introduction, the number of CGM /leaf on each cassava variety was scored as the start point. Follow up of mite densities on the whole leaf lobes were estimated after every three days. This was by randomly picking (without detaching) the third mature leaf from the apex and estimating the number of the mites/leaf and visual damage score (Yaninek et al., 1989). This was continued up to 55[th] day of the experiment.

5. 2. 2 Determination of cassava leaf biomass loss

During the determination of leaf biomass loss on cassava varieties, five leaves (replicated four times) were picked from each variety representing damage (D) scores of D.1, D.2, D.3, D.4 and D.5. The choice of the sample leaf position was important because the

highest numbers of CGM are usually found at the apex of cassava plant. The leaf samples

for D:1-5 were collected in Khaki paper (No 25) bags and taken to the laboratory for

weighing. An electronic weighing balance (Santorius Basic-BA 310s) of three decimal

units (000.000 g) was used to weigh the leaves after brittle drying them in oven at 60 °C.

To get single leaf mean weight (mg) of the sample leaves the total weight was divided by

four (replicates). To arrive at the dry weight (DW) loss or biomass loss at each damage

score, D.1 was taken as the leaf DW with no CGM damage score. Subsequent weights of

D.2, D.3, D.4 and D.5 were taken and weight loss calculated as:

$$DWloss\% = (D.1 - (D.1\times)100/D.1$$

Where, D.1 is the weight of non-damaged leaf, x representing values 1-5 as damage

scores of a particular cassava variety.

5. 2. 3 Cyanide content in relationship to biomass loss

Cassava leaf cyanide (HCN mg/kg) content was determined by Picric Acid analysis. The

procedure involved cutting fresh cassava leaf into disks of specific diameter to the size of

test-tube to be used for the test using a cock borer. The cassava leaf disks (of 1.0cm-

diameters) were inserted to the bottom of 1.5-cm diameter tube and the prepared 400-liter

Picric acid solution poured into standard size Petri-dish. Five drops of Tuluone volatile

liquid was added to leaf disk in the test-tube to vaporize the gaseous cyanide (HCN).

Whatman No 1 (6cm x 1cm) strip paper was soaked with the Picric acid solution and

placed onto opening of each test-tube to avoid touching the Tuluone-wet leaf and left for

24 hr period for colour change. A control test-tube treatment (with leaf disc and similar 5

drops of water) was set along all the tubes with three replicated variety leaf disk samples. The colour chart was from the range of 1-9 starting with yellow (1= no cyanide) to dark brown (5 = of highest cyanide level) according to Natural Research Institute (1996) procedure. The reading ranges where calculated for mean values. Later comparison of cassava variety biomass loss (DW loss %) and cyanide content was compared to find out if there was relationship between spider mite damage to cyanide (HCN) level. The procedure was carried out on day 10, 20, 33 and 54 for each experimental repeat assessment.

Data collected were log-transformed (x +1) before ANOVA GLM (LSD) was carried on the variety score of each variety. Mite density on each variety was treated as above before analysis (ANOVA-GLM) at 5% level. Similarly, ANOVA (GLM) was carried out to determine significance difference of leaf biomass loss (%) and cyanide content of the different cassava varieties, using SAS software V8 (1999-2001), where means were separated with Student Neumann Keuls (S.N.K) Post Hoc Test at 5% level. Pearson correlation was used to determine leaf cyanide content correlation (r) level and slope (b) to CGM density growth and the subsequent biomass loss on the cassava varieties. Microsoft Excel (2010) was used to graph the relationship between time durations (days) and mite density growth.

5. 3 Results

5.3.1 Mite densities and visual damage scores

The nine potted cassava varieties sampled for 55 days presented different density growth rates and damage levels of *M. progresivus* at specific time durations (days). The damage

score of D.3 and D.4 were critical since they represented visual leaf damage of 50 and 75% respectively. There was significance difference ($F_{8,\ 26}$ = 75.1; $P < 0.0001$) among spider mite densities on the varieties at D.2 (Table 5.1). Similarly the number of days to achieve damage level (D.2) was significantly ($F_{8,\ 26}$ = 53.0; $P < 0.0001$) different among the cassava varieties. Visual damage among the varieties were significantly different at D.3 ($F_{8,\ 26}$ = 866; $P < 0.0001$), D.4 ($F_{8,\ 26}$ = 722; $P < 0.0001$) and D5 ($F_{8,\ 26}$ = 25.7; $P < 0.0001$). On similar trend number of days to reach such damage were significant where D.3 ($F_{8,\ 26}$ = 25.2; $P < 0.0001$), D.4 ($F_{8,\ 26}$ = 118; $P < 0.0001$) and D.5 ($F_{8,\ 26}$ = 58.5; $P < 0.0001$) difference durations (days) were scored among varieties.

Variety Kalezo of the coastal lowlands region had the shortest period of attaining D.3 and D.4 damage levels in 12.7 and 30.3 days of spider mite populations of 320 and 870 CGM/leaf, respectively. Variety MM990183 of the eastern lowlands was the second in attaining D.3 and D.4 in 14.3 and 33 days of pest populations of 560 and 980 mites per leaf. The X-Mariakani variety (eastern lowlands) was the third with the shortest period of 18.0 and 30.1 days of attaining damage scores of D.3 and D.4 with pest populations of 310 and 950 mites /leaf. The most tolerant- varieties were from the western midland region of the country; varieties MM97/3567 and MM96/9308 which had the least number of mites throughout the experimental period. It was also observed that variety MM97/3567 damage score D.5 was not attainable due to the low mite numbers leading to a maximum of D.4 visual score on the leaves. The susceptible varieties had leaf wilt by 54[th] day. The peak density levels of *M. progresivus* in durations of days are shown on Figure 5.1.

Table 5.1: Mean (±SE) cassava green mite motiles density score in relation to visual damage score (D) and duration (days) in screen house (days) in 20.0 ± 2 °C, RH $63 \pm 4\%$

Mites / days	No CGM	Duration	No. CGM	Duration	No. CGM	Duration	No. CGM	Duration	No. CGM	duration
Variety	**(D.1)**	**(Days)**	**(D.2)**	**(Days)**	**(D.3)**	**(Days)**	**(D.4)**	**(Days)**	**(D.5)**	**(Days)**
Kalezo	9	5	75 ± 5b	8.4 ± 3d	320 ± 20c	12.7 ± 2c	870 ± 30e	30.3 ± 2bc	1370 ± 100a	33.0 ± 2d
Karibuni	8	5	65 ± 6c	9.0 ± 2d	300 ± 30de	21.0 ± 1b	820 ± 23f	33.0 ± 3b	1170 ± 70b	43.0 ± 4b
Tajirika	11	5	55 ± 4d	13.0±2c	280 ± 20f	28.0 ± 2a	740 ± 41h	33.0 ± 1b	860 ± 43c	35.7 ± 0cd
MM990183	8	5	75 ± 10d	8.5 ± 4d	560 ± 20b	14.3 ± 2c	980 ± 32a	33.0 ± 3b	1050 ± 50b	36.7 ± 2g
MM99005	10	5	90 ± 11a	8.6 ± 3d	325 ± 15c	20.6 ± 7b	930 ± 30c	33.0 ± 2b	1020 ± 60b	36.0 ± 0c
X-Mariakani	9	5	77 ± 7b	9.3 ± 2d	310 ± 10cd	18.0 ± 6b	950 ± 22b	30.1 ± 4b	1190 ± 30b	36.7 ± cd
MM97/3567	5	5	42 ± 2e	21.3 ± 5a	250 ± 10g	29.3 ± 5a	770 ± 30g	55.3 ± 2a	530 ± 20d	55.3 ± 2a
MM96/2480	6	5	59 ± 9d	9.3 ± 3d	625 ± 25a	22.3 ± 3b	890 ± 44d	30.6 ± 3b	1100 ± 95b	33.6 ± 1cd
MM96/9308	8	5	71± 5c	17.0± 5b	290 ± 22f	21.7 ± 8b	580 ± 39i	28.0 ± 4c	770 ± 32c	36 ± 2cd
F			*75.1*	*53.0*	*886*	*25.2*	*722*	*118*	*25.7*	*58.5*
P			*<0.0001*	*< 0.0001*	*<0.0001*	*< 0.0001*	*< 0.0001*	*<0.0001*	*< 0.0001*	*< 0.0001*

Different low case letter denote significant difference (< 0.0001) (Fishers Least Significant Difference) at 5% level (df = 8, 26)
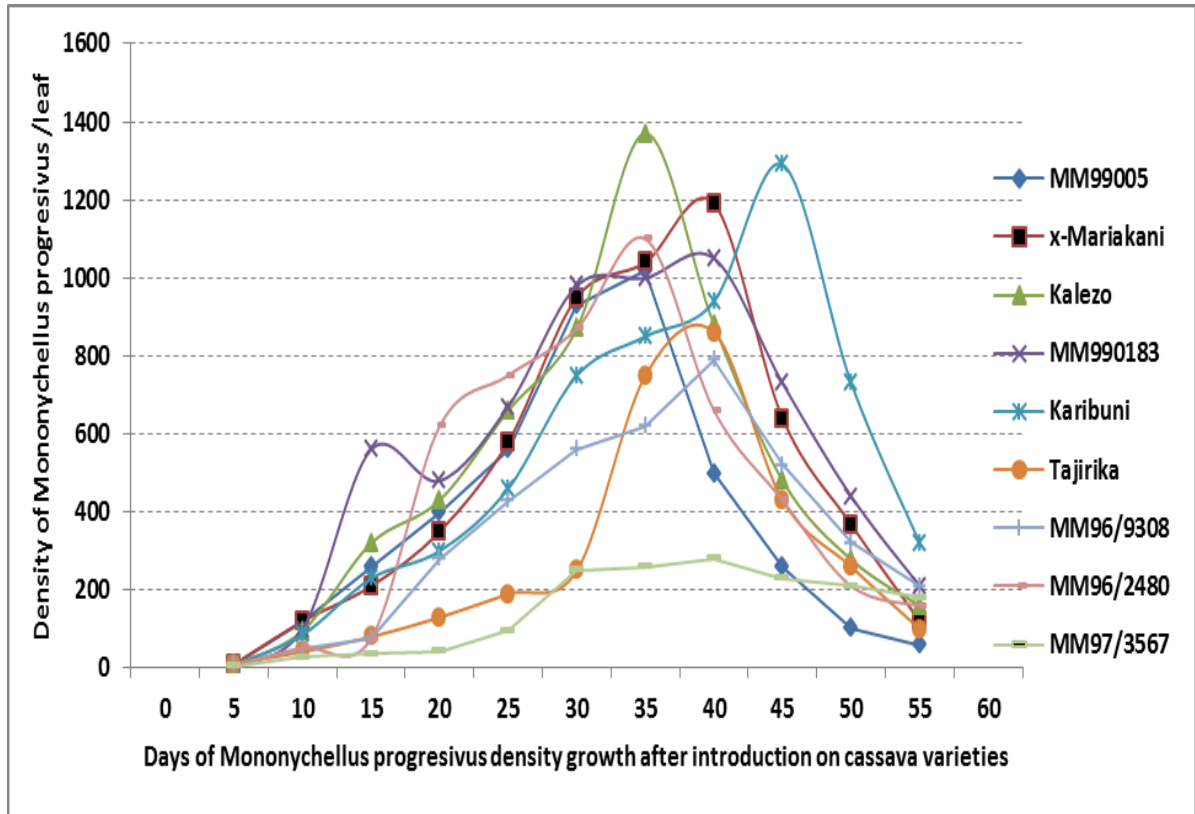
Figure 5.1: Cassava green mite, *Mononychellus progresivus* density growth to peak levels and drop on different varieties at 20.0 ± 2 °C and relative humidity $63 \pm 4\%$.

5. 3. 2 Leaf biomass losses

The highest significant biomass losses due to spider mite damage was observed on variety Kalezo of 15% ($F_{8, 26} = 12.1$; $P < 0.0001$), 22% ($F_{8, 26} = 5.7$; $P < 0.05$), 54% ($F_{8, 26} = 8.8$; $P < 0.0001$) and 67% ($F_{8, 26} = 727.5$; $P < 0.0001$) and leading the other varieties at damage scores D.2, D.3, D.4 and D.5, respectively (Table 5.2). The least biomass losses were on variety MM97/3567 at visual damage D.2, D.4 and D.5 at 4.0, 14.0 and 14.3%. The most susceptible varieties were Kalezo and MM96/2480 from coastal and western regions of Kenya as indicated by overall biomass losses.

Table 5.2: Mean (±SE) leaf biomass loss due to cassava green mite *Mononychellus progresivus* damage at 20.0 ± 2 °C, RH $63 \pm 4\%$

	Leaf biomass loss (%) on the cassava varieties			
Variety	D.2	D.3	D.4	D.5
Kalezo	$15 \pm 0.2a$	$22 \pm 0.6a$	$54 \pm 1.1a$	$67.2 \pm 5.7a$
Karibuni	$12 \pm 0.2ab$	$18 \pm 0.4b$	$51 \pm 0.5ab$	$64.1 \pm 5.1b$
Tajirika	$9 \pm 0.4b$	$14 \pm 0.7bc$	$23 \pm 0.9c$	$29.4 \pm 4.9e$
MM990183	$12 \pm 0.5ab$	$18 \pm 0.2b$	$51 \pm 0.9ab$	$54.5 \pm 3.3c$
MM99005	$9 \pm 0.2b$	$13 \pm 0.7c$	$44 \pm 0.4ab$	$55.6 \pm 4.1c$
X-Mariakani	$12 \pm 0.5ab$	$18 \pm 1.1b$	$43 \pm 0.2ab$	$54.4 \pm 6.5c$
MM97/3567	$4 \pm 1.2c$	$15 \pm 1.4bc$	$14 \pm 1.2c$	$14.3 \pm 2.5 f$
MM96/2480	$15 \pm 0.8a$	$17 \pm 0.7bc$	$42 \pm 0.3ab$	$66.3 \pm 2.4a$
MM96/9308	$8 \pm 0.9b$	$16 \pm 0.6bc$	$36 \pm 0.8b$	$46.2 \pm 3.0d$
F	12.1	5.7	8.8	727.5
P	< 0.0001	0.0018	<0.0001	< 0.0001

Similar letters within columns denote no significant difference ($P >0.05$) at 5% level (df = 8, 26)

It was observed that second level damage (D.2) had biomass loss >10% for Kalezo, Karibuni, MM990183, X-Mariakani and MM96/2480 varieties. The variety with the least loss of leaf biomass which could be considered as being relatively tolerant to *M. progresivus* was MM97/3567 of western midlands region with 4.0, 14.0 and 14.2 %, for D.2, D.3, and D.4 for CGM damage levels. Leaf visual damage score D.5 was not observed on Variety MM97/3567.

5. 3. 3 Effect of leaf cyanide on mite infestations

Kalezo, Karibuni, x-Mariakani and MM96/2480 varieties had most significant ($F_{8, 26} = 18.8$; $P < 0.0001$) cyanide content (mg/kg) of the nine varieties. These same varieties (Kalezo, Karibuni, x-Mariakani) showed highest CGM densities ($F_{8, 26} = 34.1$; $P < 0.0001$) among the varieties. Net biomass loss subsequently was significantly ($F_{8, 26} = $

36.5; $P < 0.0001$) highest on the same four varieties. The varieties with cyanide (HCN)

content levels >10<30 mg/kg had the highest spider mite population peaks than those of

<10 cyanide levels (Table 5.3). Variety Kalezo had cyanide content of 20 mg/kg with *M.*

progresivus peak score of 1370 mites/leaf. Karibuni was third with mite density peak of

1170 mites /leaf and similarly with HCN of 20 mg/kg. The X-Mariakani variety led in

second position with the highest peak mite infestations of 1190 *M. progresivus* /leaf with

same HCN content of 20.0mg/kg. Varieties MM99005 and MM990183 had HCN content

level of 12.5 mg/kg and exhibited mid infestation levels of 1020 and 1050 mites/leaf with

biomass loss of 55 and 54%.

Table 5.3: Mean (± S.D) highest number of cassava green mite *Mononychellus progresivus* per leaf and biomass losses (%) in relation to cyanide (HCN) level on cassava

Cassava variety	HCN (mg/kg)	Peak No. CGM	Net biomass (%) loss
Kalezo	20.0 ± 7.1a	1370 ± 100a	67.2 ± 5.7a
Karibuni	20.0 ± 7.1a	1170 ± 70b	64.1 ± 5.1ab
Tajirika	12.5 ± 3.2b	860 ± 43c	29.4 ± 4.9d
MM990183	12.5 ± 3.5b	1050 ± 50b	54.5 ± 3.3bc
MM99005	12.3 ± 2.5b	1020 ± 60b	55.6 ± 4.1bc
X-Mariakani	20.0 ± 7.1[a]	1190 ± 30b	54.4 ± 6.5bc
MM97/3567	8.5 ± 4.9 c	530 ± 20d	14.3 ± 2.5e
MM96/2480	20.0 ± 7.1a	1100 ± 95b	66.3 ± 2.4a
MM96/9308	14.0 ± 5.7b	770 ± 32c	46.2 ± 3.0c
F	18.8	34.1	36.5
P	< 0.0001	< 0.0001	< 0.0001

Means with different letters within columns are significantly different (P<0.0001), at 5% level (Fishers Least Significant Difference Test).
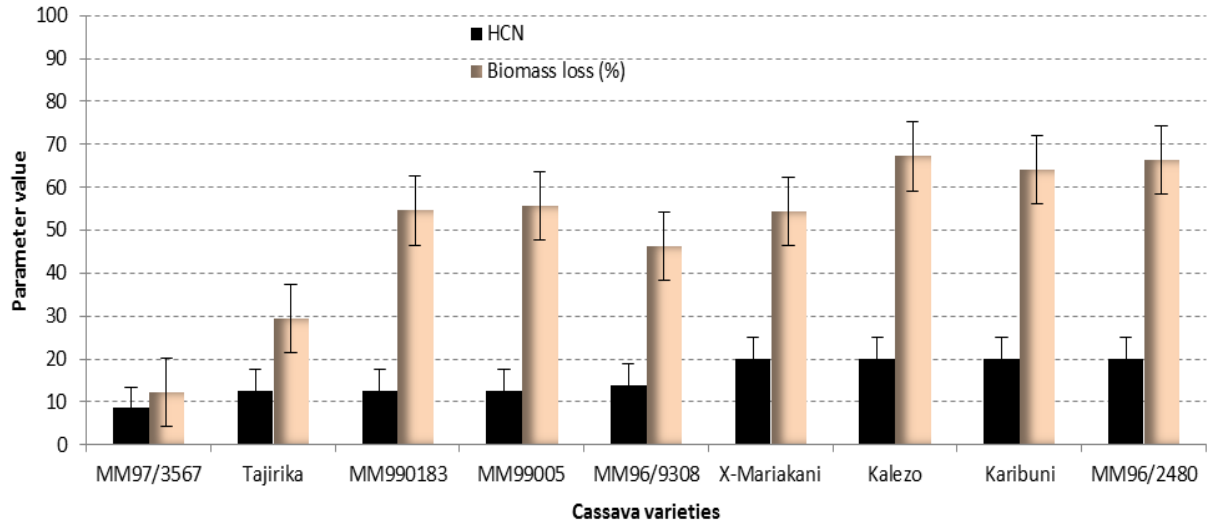
Figure 5.2: Cassava variety mean (±SD) leaf cyanide content (HCN.mg/kg) and subsequent leaf biomass loss (%) by cassava green mite, *Mononychellus progresivus*

5.3.4 Relationship between leaf cyanide content and biomass loss

Eight of the nine varieties showed a positive correlation of increase of cyanide content (mg/kg) leading to higher biomass loss due to higher number of *M. progresivus* feeding on the leaf tissue (Table 5.4 and Fig. 5.3). Increase of CGM infestation days led to correlated higher biomass loss. Correlation values indicated in Table 5.4 show increasing slope (b) values with increasing days on *M. progresivus* densities on the cassava varieties. Similar trend was observed for biomass loss slope. Correlation relationship (r) of HCN leaf content (mg/kg) increased with mite density increase in time and space on the varieties. Were it possible to calculate the area under each variety triangle it would be observed that varieties like Tajirika, Kalezo and x-Mariakani were most susceptible to CGM. Arranging the varieties in order of least HCN (mg/kg) to the highest content showed an increase on leaf biomass loss (%) as Figure 5.2 shows.

Table 5.4: Relationship between cassava cyanogens content (HCN mg/kg) and cassava green mite *Mononychellus progresivus* densities and the subsequent leaf biomass loss in 54 days

Factor		Time period (days) of mite population growth			
		10	20	33	54
CGM density:	Slope (b)	57.3	259.4	829.4	900.4
	t-value	6.621	3.869	13.823	6.182
	r	0.2	0.1	0.4	0.5
	F-value	1.734	2.710	0.771	0.114
Biomass loss:	Slope (b)	6.2	9.4	9.6	10.4
	t-value	3.812	7.051	5.322	4.402
	r	0.6	0.7	0.9	0.8
	F-value	0.026	0.028	0.022	0.026

Linear regression effect of leaf cyanide content of cassava varieties, with Pearson correlation (r) values at 5% significant level.
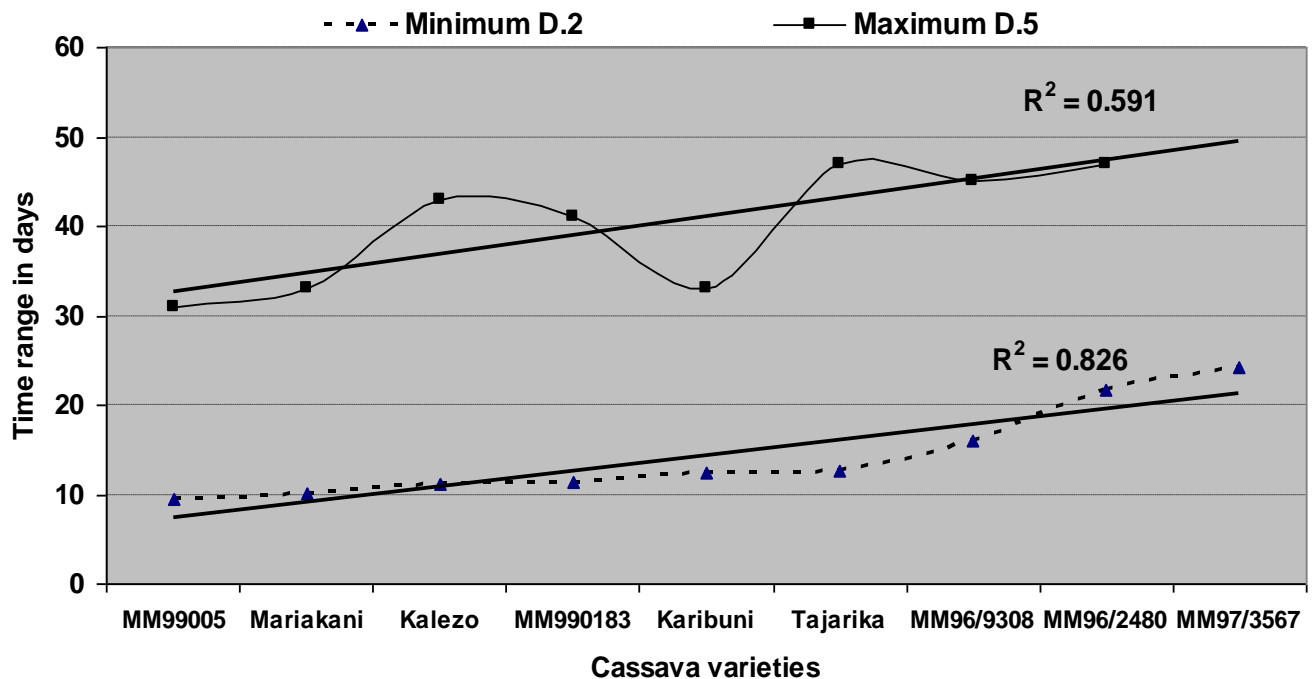
Figure 5.3: Range in days for minimum (D.2) and maximum (D.5) leaf damage score on cassava varieties under cassava green mite *Mononychellus progresivus* infestation.

5.3.5 Density threshold level for mite control

At the afore mentioned environmental conditions (20.0 ± 2 °C, RH 63 ± 4%), a variety like x-Mariakani demonstrated a high threshold density of *M. progresivus* of 77 mites per leaf (D.2) on day 10 as shown on Figure 5.4. The next 30 days (**a** - **c**) had the density grow from 77 to 950 (**c** - **b**) mites /leaf. The red line **d** - **e** shows the level (≤100) at which the farmer could maintain *M. progresivus* density to maintain cassava crop free of mite damage. Between day 43-47 days cassava crop was observed to wilt and drop leaves on the plant apex and *M. progresivus* actives migrate or suffer mortality.
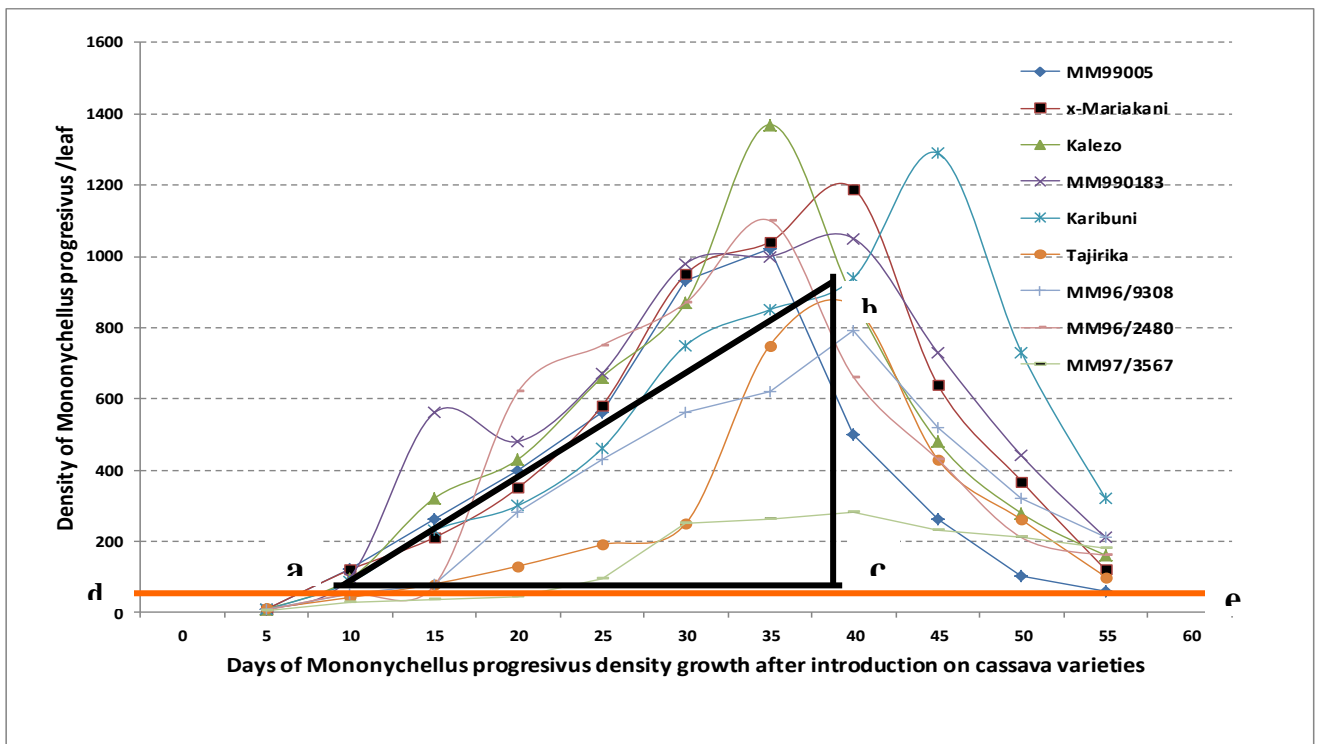
Figure 5.4: Density growth and drop of cassava green mite *Mononychellus progresivus* at 20.0 ± 2 °C, RH 63 ± 4%. Line d-e indicates density threshold level for mite control. The density growth duration of 30days (a-c) of 77 to 950 (c-b) mites per leaf on variety X-Mariakani is shown.

Combining the least visual damage (D.2) from Table 5.1 and biomass loss (%) values (Table 5.3) for each variety (without consideration of HCN mg/kg), an equation $y = 0.04x + 7.55$ ($R^2 = 0.155$) gives more accurate density threshold for control *M. progresivus*, of ≥ 27 mites per leaf of $<10\%$ biomass loss (Fig. 5.5).

Relationship between number of mites to cassava leaf biomass loss (%)
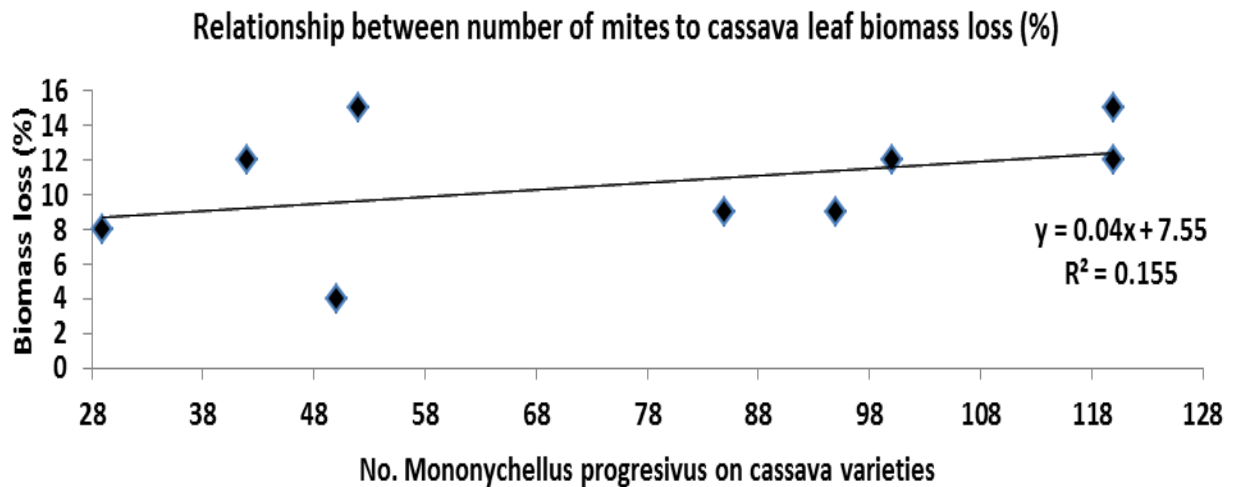
Figure 5.5: Relationship between cassava green mite *Mononychellus progresivus* density and leaf biomass percentage loss

5.4 Discussion

In the present study, the visual damage scoring has been equated to level of leaf biomass losses (%) which allowed indicative loss of the leaf photosynthetic area on the plant. The triangular area under the curve of density comparably gives each variety susceptibility level of CGM. A positive correlation was evident on leaf cyanide content increase to increased biomass loss (%). Basing density threshold to ultimate biomass loss (%) it was found that ≥ 27 mites/leaf was the threshold level of CGM density for implementation of immediate control measure for the pest mite. Ezulike and Egwuatu (1990) estimated

CGM density threshold for control to be 20 mites /leaf. Though the value achieved is fairly close to the value achieved in the present study the procedures used are different and demonstrate the value of using biomass loss to determine threshold level of mites on cassava. The present approach has included nine varieties while Ezulike and Egwuatu (1990) used only two varieties, hence enhancing accuracy of the results. Most Tetranychid mite species feed on the underside of cassava leaf tissue sucking cell-sap and reducing photosynthetic leaf area (Jeppson et al. 1975; Bellotti and Byrne, 1979; Capinera 2008). Yaninek and Gnanvossou (1993) compared dry matter increase of the spider mite life history and concluded that cumulative dry matter increased as CGM cohorts development progressed upward from juvenile to adult life stages of the mite. The present study indicates at what density level CGM would cause leaf injury level translatable actuated to root carbohydrate loss. Environmental conditions dictate the subsequent damage level of the mite on each cassava variety depending on susceptibility to CGM (Yaninek et al. 1989; Ayanru and Sharma, 1984; Byrne et al. 1982). Yaninek et al (1989) reported that cassava crop attacked by *M. tanajoa* Bondar lost 10 to 30% dry matter in the dry season where leaves wilted and recovered 25 to 45% in the subsequent wet season. What was missed in that study was CGM density threshold determined on specific cassava varieties from specific regions as is the case with the present study of the pest mite, CGM.

Inclusion, the determined threshold (≥ 27 mites/leaf) levels for CGM control is the benchmark on which CGM control / management would be based in an integrated pest management programme as envisioned in the present work.

CHAPTER 6

6.0 CHOICE OF PREDATORY MITE FOR MANAGEMENT OF THE PHYTOPHAGOUS MITE, *MONONYCHELLUS PROGRESIVUS* ON CASSAVA IN AN INTEGRATED PEST MANAGEMENT

6.1 Introduction

The performance of a phytoseiid predator as an effective biological control agent of mite pest depends mainly on the initial predator-prey ratio (Kindlmann & Dixon, 1999; Nakazawa *et al.*, 2011). Osekre *et al.* (2008) found that environmental factors play some influence on the predator's ability to suppress prey densities below economic injury levels (EILs). Sabelis and Rijn (1997) demonstrated that actual control impact could only be measured when yield and other attributes of economic importance are assessed. Other arthropod species and environmental factors play important roles to the final performance score of the predator in suppressing target pest (Lima, 1998; Osekre *et al.*, 2008). An evaluation of the choice predator for management of *M. progresivus* on cassava crops for sound control strategy in different environmental conditions where it is cultivated in Kenya was deemed paramount.

The predatory mite, *T. aripo* was first described from Trinidad (Tobago) and reported later from Brazil (Denmark & Muma, 1973). This predator, among others, was introduced to East Africa during the 1990s to control the cassava green mite (CGM), *M. progresivus* (then referred to as *M. tanajoa*), a pest that constrains production of the cassava (Gutierrez, 1987; Hanna *et al.*, 1998; Yaninek & Hanna, 2003). In Kenya, *T. aripo* was released in 1995/6 and its establishment and subsequent persistence led to

reduction of severity of *M. progresivus* on cassava as has been reported (Kariuki *et al*., 2000; Jones, 2002; Yaninek & Hanna, 2003). Phytoseiid *T. aripo* reportedly develops and reproduces on the different stages of *M. progresivus* and survives on some plant material like pollen grains of maize, castor oil and cassava plant exudates (Cuellar *et al*., 2001; Gnanvossou *et al.*, 2005). The predatory mite, *Phytoseiulus longipes* Evans, was described from Zimbabwe (Evans, 1958). Later, it was reported from other localities including Cape Province, South Africa; Rio Grande do Soul, Brazil and Los Andes, Chile (Kanouh *et al.*, 2010). This predatory mite is able to develop and reproduce on different species of spider mites such as *Tetranychus urticae* Koch, *T. evansi* (Baker & Pritchard), *T. marianae* McGregor, *T. pacificus* McGregor, *Oligonychus punicae* (Hirst), *P. citri* (McGregor) (Badii & McMurtry, 1984; Furtado *et al.,* 2007). Individuals of the predator developed, survived and reproduced on *T. evansi* under a range of temperatures 15–30°C (Ferrero & De Moraes, 2007). The phytoseiid was imported to Kenya 2005 through International Centre of Insect Physiology and Ecology hence the need to try its efficacy on *M. progresivus* control (Mutisya *et al.*, 2012). Although predacious mites usually achieve excellent control of most phytophagous mites and some insects there are factors limiting their efficiency like initial predator-prey ratio and species diet preference (Grout & Richards, 1992; Bakker *et al.,* 1993). The present study was to evaluate *T. aripo* and *P. longipes* consumption rate, immature development and reproduction on *M. progresivus* in three different temperatures and humidity conditions.

6.2 Materials and Methods

6.2.1 Predator prey consumption rate

Prey daily feed rate on the two predators was observed and compared to determine effective control performance of each phytoseiid, *T. aripo* and *P. longipes*. Each phytoseiid was given daily ration according to daily requirement for each species. *T. aripo* was given 40-60 individual motile stages and 100 eggs of *M. progresivus* while *P. longipes* was supplied with 25 and 40 respective same individual cohorts on the feed disks described on general materials and methods (Chapter 1). Feed rate for each predator was observed for 28 days. The cumulative prey consumption would be reflected on the predator prey consumption rate of Lotka-Volterra model:

$$dN/dt = rN - aPN$$

where, dN is prey density change in time (dt) and rate of prey increase (rN) affected by predator feed rate (aPN) as revised by Beretta and Kuang (1998). Observations were carried out every 12 hours and diet rations replenishments undertaken for each predator test disk as required.

6.2.2 Life stage development

Freshly emerged individual larvae, protonymphs, deutonymphs and females of each phytoseiid were each placed on leaf disks (abaxial part up) inside a plastic disk (3.5cm-width x 8cm-length) of 0.4cm-depth x 1.5cm-diameter (Plate 6.1). The bottom part of the disk was covered with 22mm glass slide fastened with rubber bands. Life stage prey

consumption rate was recorded twice a day and rations replenished each observation time until individual predators reached maturity.
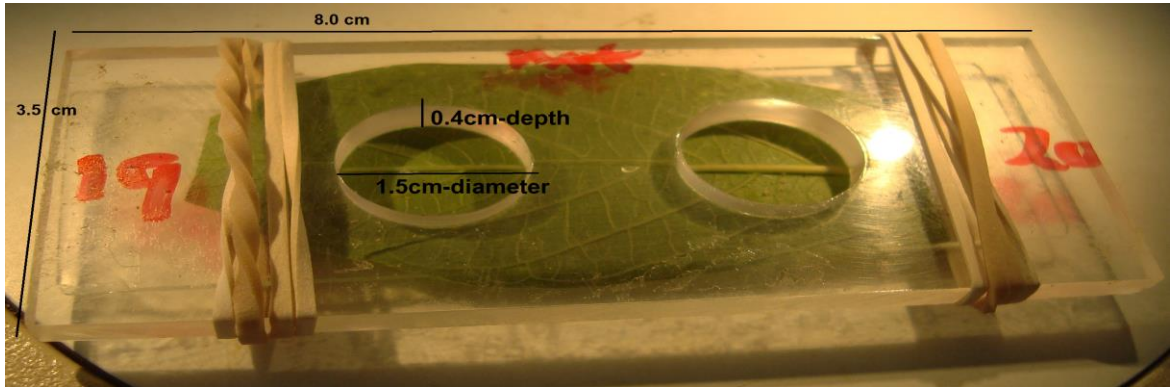
Figure 6.1: Leaf disk unit showing feed area of *Typhlodromalus aripo* life stage cohorts on cassava green mite (eggs and active), *Bemisia tabaci* and *Phenacoccus manihoti*.

A fresh cassava leaf placed on wet Whitman. No.1 paper slip was inserted between the plastic disk and the glass slide to keep it moist. The opening hole of plastic disk was the area of placing the predator and the prey for observation. Another 22mm glass slide was used to cover the rearing hole to prevent escape of both prey and predator. Individual life stages of larva, protonymph, deutonymph and female (mated) were given daily rations of prey as per treatment. Life stages of larva, protonymph, deutonymph and adult female were given daily rations of 100 *M. progresivus* eggs and another unit fed 60 CGM motiles for both cohort stages of *T. aripo* and *P. longipes*. The test was on relative humidity regimes of 40, 75 and 92% and under temperatures of 12, 27 and 33 ºC in the described disks. Humidity containers of 8cm-diameter x 9cm height with tight covers were used to hold RH regimes to constant as modification of Winston and Bates (1960) and Friese *et al.* (1987) for the main mass rearing unit. A battery powered instant recording thermo-hygrometer (Model CE) was used to monitor humidity in the salt

chambers. Photo-period conditions of 12L:12D were achieved through 24 hr automatic timer in the room chambers where 40 watts fluorescent light tubes (Phillips model) were installed for lighting. Prey rations replenishment was carried out for each unit after observation and recording of fed quantity. Similar data was collected on *P. longipes* as in *T. aripo.* Experiments were run for 28 days on adult female observation while on immatures it was until all life stage had reached adulthood. The experiment was repeated three times. Immatures development and survival to adult stage, as well as female fecundity were recorded.

6.2.3 Egg drought tolerance test

Some 50 freshly laid predator eggs were observed at 6 hr interval in the described chambers of 40, 75 and 92% RH and at temperatures of 12, 27 and 33 ºC. Three replicates were mounted for each predator set on cassava leaf disk and repeated. The recorded score on number of egg hatch and live larvae were used to determine *T. aripo* egg hatch (%) in optimum and adverse climatic conditions. The eggs were observed until they hatched into larvae or not on the leaf surface.

Data for mean developmental periods (days) of different *T. aripo* and *P. longipes* life stages of larva, protonymph and deutonymph, female longevity and fecundity, and daily prey consumption were subjected to analysis of variance (ANOVA, GLM) at 99% confidence level (P=0.01). Log (x+1) transformation was carried out on data entries before subjecting the data to ANOVA for test of significances. SAS Version 8 (2001) was used for means separation with Student Neumann Keuls (S.N.K) Post Hoc Test. Predator egg drought tolerance was graphed using Microsoft Excel 2010.

6.3 Results

6.3.1 Predator-prey consumption rate

The larval stage did not feed on predators, *T. aripo* and *P. longipes* (Table 6.1). The most significant ($F_{11, 263} = 729.6$; $P < 0.0001$) prey consumption rate by females of *T. aripo* on CGM egg and motiles were at 27 °C. Similarly, protonymph significantly ($F_{11, 263} = 304.1$; $P < 0.0001$) consumed highest prey of CGM as well as deutonymph ($F_{11, 263} = 118.9$; $P < 0.0001$) at 27 °C. *Mononychellus progresivus* motiles consumption dropped to low levels of 2.1 (44%), 2.3 (21%) and 2.7 (10%) for protonymph, deutonymph and adult stages in the cold condition (12° C). Maintaining the same humidity (75%) and raising temperature to 33 ℃ resulted into 31, 41 and 64% reduction of *M. progresivus* egg consumption for protonymph, deutonymph and adult females respectively. Similarly, subsequent reduction was recorded for CGM actives in the same temperature (33 ℃).

The predatory mite, *P. longipes* consumption rate of *M. progresivus* showed less than 2 eggs or motiles of the prey at 12 ℃, shown in Table 4.1. At 27°C the majority of the individuals lasted about 10 days to reach the deutonymph stage, yet failed to complete the development to maturity irrespective of the stage of the tested two preys. At 27°C protonymph, deutonymph and female (adult) consumed 2.5, 5.0 and 9.2 eggs, respectively. At 33°C, protonymph, deutonymph and females consumed 4.3, 7.5 and 10.2, respectively. Consumption of motile stages of *M. progresivus* was lower at each temperature than the egg feed rate.

Table 6.1: Mean (± SD) consumption rate per day of *Typhlodromalus aripo* and *Phytoseiulus longipes* when fed on the cassava green mite (CGM) *Mononychellus progresivus* at three different temperatures in 75% RH

Phytoseiid species	No. Observed		Consumption rate / day		
		Prey	Protonymph	Deutonymph	Adult (female)
			12 ºC		
T. aripo	22	CGM eggs	2.3 ± 1.6g	2.2 ± 1.0f	4.3 ± 1.1g
	24	CGM motiles	2.1 ± 0.9h	2.3 ± 0.7f	2.7 ± 1.3gh
			27 ºC		
	23	CGM eggs	5.2 ± 0.4a	16.5 ± 3.2a	61.6 ± 13.2a
	22	CGM motiles	4.8 ± 0.2b	11.2 ± 2.6b	28.3 ± 2.3b
			33 ºC		
	20	CGM eggs	3.6 ± 1.6e	9.8 ± 1.5c	22.4 ± 4.2c
	21	CGM motiles	4.2 ± 1.2d	10.4 ± 2.0bc	10.2 ± 2.5d
			12 ºC		
P. longipes	20	CGM eggs	1.0 ± 1.0i	1.5 ± 1.3f	1.2 ± 0.3h
	20	CGM motiles	1.0 ± 1.0i	1.0 ± 2.2f	0.6 ± 0.2h
			27 ºC		
	20	CGM eggs	2.5 ± 1.3g	5.0 ± 1.4e	9.2 ± 3.5e
	20	CGM motiles	3.0 ± 2.1f	4.3 ± 2.2e	6.3 ± 2.3f
			33 ºC		
	20	CGM eggs	4.3 ± 1.6cd	7.5 ± 2.1d	10.2 ± 2.5e
	20	CGM motiles	4.5 ± 0.7c	5.5 ± 2.2e	7.1 ± 3.2f
		F	304.1	118.9	729.6
		P	< 0.0001	< 0.0001	< 0.0001

Means in the same column of predator cohort stage followed by a different letter denote significant difference ($P < 0.0001$, LSD-GLM) at 5% level (df = 11, 263)

At 40%RH, a significant ($F_{3, 23}$ = 244.1; P < 0.0001) consumption rate of *T. aripo* females of 52.3 and 26.2 CGM eggs and actives against *P. longipes* feed rate of 1.1 and 1.2 (Table 6.2) was scored. At 92%RH, a significant ($F_{3, 23}$ = 456.2; P < 0.0001) consumption rate of *T. aripo* of 42.3 and 26.5 prey numbers was scored against *P. longipes* 1.5 and 1.4 on CGM eggs and actives respectively.

Table 6.2: Mean daily number (± SE) of prey consumption rate by *Typhlodromalus aripo* immature and adult life stages (females) and egg reproduction at three temperatures and different humidities

Predator	Prey	12 °C			27 °C			33 °C		
		Immatures	Females	Eggs	Immatures	Females	Eggs	Immatures	Females	Eggs
					40%RH					
T. aripo	CGM eggs	1.8 ± 2.3a	4.1 ± 2.2a	0	11.6 ± 3.9a	52.3 ± 8.4a	2.1 ± 4.1a	6.4 ± 5.2a	33.6 ± 7.6a	0.8 ± 4.2ab
	CGM actives	1.5 ± 0.9b	2.3 ± 1.2b	0	8.1 ± 6.1b	26.2 ± 6.2b	1.8 ± 3.2a	5.6 ± 2.9a	24.1 ± 6.5b	1.1 ± 1.2a
P. longipes	CGM eggs	0.7 ± 2.1c	0.4 ± 0.8c	0	0.7 ± 1.3c	1.1 ± 3.2c	0.2 ± 0.8b	0.6 ± 0.2b	1.1 ± 4.2c	0.7 ± 0.8b
	CGM actives	0.5 ± 0.9d	0.6 ± 0.6c	0	0.8 ± 1.8c	1.2 ± 5.2c	0.3 ± 0.7b	0.5 ± 0.1b	1.2 ± 2.3c	0.7 ± 0.5b
	F	17.3	23.4		112.5	244.1	8.9	54.2	316.7	7.8
	P	< 0.0001	< 0.0001	-	< 0.0001	< 0.0001	0.0013	< 0.0001	< 0.0001	0.0510
					75%RH					
T. aripo	CGM eggs	1.4 ± 0.8b	3.2 ± 0.2a	0	10.2 ± 4.5a	61.6 ± 4.2a	2.2 ± 5.5a	7.2 ± 4.3a	39.3 ± 9.8a	1.9 ± 3.5a
	CGM actives	1.6 ± 0.4a	2.4 ± 0.6b	0	7.6 ± 3.7b	28.3 ± 3.6b	1.9 ± 6.2a	6.9 ± 2.6a	22.0± 5.2b	1.6 ± 0.8b
P. longipes	CGM eggs	0.5 ± 0.3c	1.4± 0.5c	0	1.3 ± 1.3c	1.9 ± 2.2c	0.3 ± 0.4b	0.9 ± 0.5b	1.3 ± 1.2c	0.6 ± 0.9c
	CGM actives	0.3 ± 0.2d	1.2 ± 0.3c	0	1.2 ± 1.8c	1.2 ± 0.8c	0.2 ± 0.8b	0.7 ± 0.3b	1.2 ± 0.8c	0.4 ± 0.4c
	F	16.6	14.2		105.6	882.4	19.7	15.5	57.9	6.8
	P	< 0.0001	< 0.0001	-	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
					92%RH					
T. aripo	CGM eggs	1.2 ± 0.1b	2.4 ± 4.6a	0	9.4 ± 5.2a	42.3 ± 8.7a	2.0 ± 2.2a	7.3 ± 4.3a	25.4 ± 4.6a	1.4 ± 2.2a
	CGM actives	1.5 ± 0.3a	2.5 ± 8.2a	0	7.2 ± 3.8a	26.5 ± 9.4b	2.1 ± 2.2a	6.8 ± 5.5a	22.4 ± 6.3a	1.5 ± 2.2a
P. longipes	CGM eggs	0.4 ± 0.4c	0.8 ± 0.4b	0	1.1 ± 4.2b	1.5 ± 2.1c	0.2 ± 2.2b	0.9 ± 0.6b	1.3 ± 4.9b	0.1 ± 2.2b
	CGM actives	0.3 ± 0.2c	0.9 ± 0.6b	0	1.0 ± 2.2b	1.4 ± 4.2c	0.3 ± 2.2b	1.0 ± 0.8b	0.9 ± 0.9b	0.1 ± 2.2b
	F	9.3	11.3		64.5	456.2	21.7	11.8	188.1	4.1
	P	< 0.0001	0.0016	-	0.0006	< 0.0001	< 0.0001	0.0004	< 0.0001	0.0001

Different lower case letters denote significant difference (*P* < 0.05) at 5% level at the bottom of each column indicates value of Fisher's Least Significance Difference (LSD-GLM) for each life stage under different temperatures (df=3, 23) under 10 days observation

The predator-prey ratio of *T. aripo* on *M. progresivus* motile densities was found to be linear and could be deduced from the regression equation: y = 2.424 x -7.333 (R^2 = 0.9807) shown on Figure 6.2A. The effective predator numbers for specific prey density of 100, 450, 1000 for visual damage scores of D.2 ($\leq$ 25%), D.3 (50%) and D.4 (75%), were deduced as 4, 19 and 42 predators respectively. These were the *T. aripo* individual numbers required to feed and eradicate in one day the indicated *M. progresivus* densities of motile stage. Comparable predictions on number of *T. aripo* required for one day consumption rate of *M. progresivus* eggs and motile stages were projected on Figure 6.2A & B where it was shown that double number of eggs would preferably be fed by the phytoseiid predator than the indicated density of *M. progresivus* actives (motiles), where, y = 2.4667x-0.8889 (R^2 = 0.9755), as the estimate equation of the ratio.

A   B
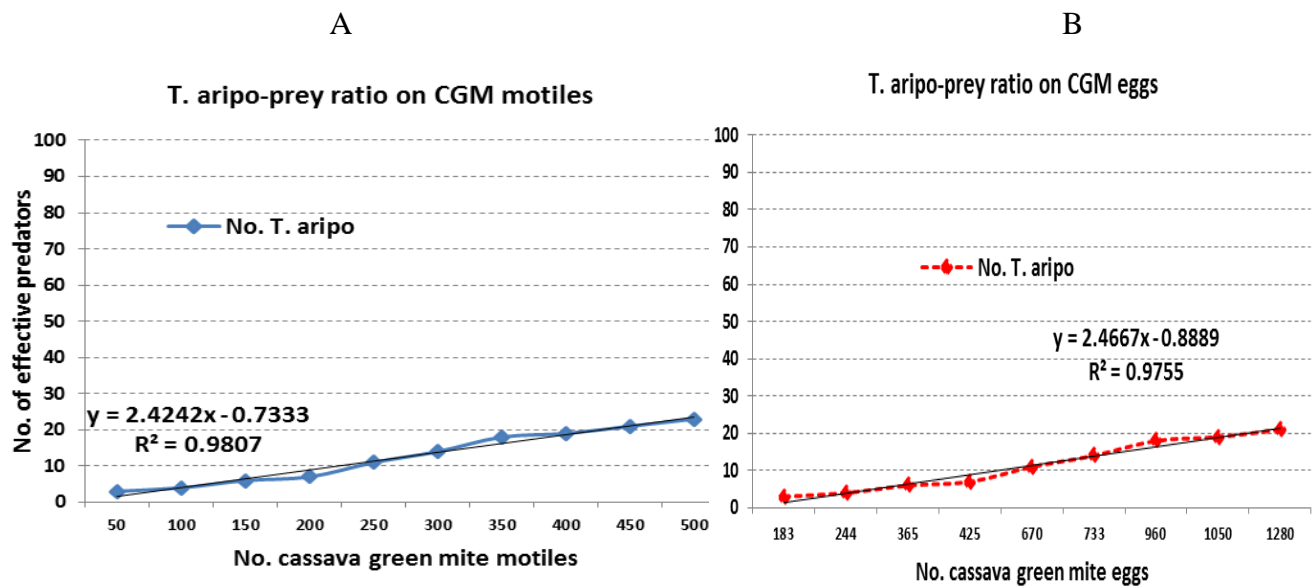
Figure 6.2A and B: Comparable consumption rate prediction of *Typhlodromalus aripo* on motiles and eggs of *Mononychellus progresivus* at daily feed rate of 1:28 (motiles) and 1:61(eggs)

6.3.2 Predator life stage development and reproduction

The shortest developmental period of immatures was significant ($F_{11, \ 35} = 668.0$; $P <$ 0.0001) at 33 °C (75%RH) of 3.4 and 3.0 of *T. aripo* against *P. longipes* at 4.3 and 4.8 on CGM eggs and actives prey respectively, albeit low survival (Table 6.3). At the lowest temperature of 12 °C less than 30% reached maturity after about 30 days. At 33 °C *T. aripo* life stage durations were significantly shorter and survival was less than 10% on diets of eggs and motile stages of *M. progresivus*. The maximum survival was recorded at 27 °C (96%) and individual cohorts took about 4 days to reach maturity.

The predatory mite, *P. longipes* developed and survived on *M. progresivus* at the different three test temperatures, although the durations and percentage of survival and mortality was greatly varied according to the prey species and the tested temperatures. At 12 °C the majority of the individuals lasted about 10 days to reach the deutonymphal stage, yet failed to complete the development to maturity irrespective of the stage of the test prey as shown on Table 4.3. At 27 °C and 33 °C, the percentage of individuals reaching maturity was about 40% on the two stages of *M. progresivus*. The *M. progresivus* prey species greatly affected the development of *P. longipes.* Although immatures fed on some eggs and motile stages of *M. progresivus* many individuals of similar cohorts suffered high mortality. None of the matured mated females which were maintained on life stages of *M. progresivus* were able to reproduce any eggs.

Overall, no major effect was observed predator development days and survival at 40%RH and 92%RH (Table 6.4).

Table 6.3: Life stage development durations (days ± SD) and fecundity of the predatory mites *Typhlodromalus aripo* and *Phytoseiulus longipes* when fed on the cassava green mite (CGM) *Mononychellus progresivus* at three different temperatures in 75% RH

Predator species	No. Observed	Prey	Durations (days)			Total Days	% Maturity	Female Longevity (days)	Eggs / female / day
			Larva	Protonymph	Deutonymph				
						12 ºC			
T. aripo	24	CGM eggs	6.4 ± 1.8b	9.5 ± 2.5b	9.2 ± 2.1a	25.1 ± 1.7b	29d	10.2 ± 4.3c	0c
	22	CGM motiles	7.7 ± 1.5a	11.2 ± 1.9a	9.1 ± 2.7a	28.0 ± 1.8a	25d	8.3 ± 2.6d	0c
						27 ºC			
	24	CGM eggs	1.4 ± 0.5e	1.8 ± 0.7de	1.3 ± 0.8c	4.4 ± 0.3ef	96a	27.9 ± 2.6b	2.4 ± 1.3a
	24	CGM motiles	1.2 ± 0.3e	2.0 ± 0.6d	1.1 ± 0.2c	4.3 ± 0.5ef	92a	28.4 ± 2.1a	1.9 ± 0.9ab
						33ºC			
	22	CGM eggs	1.2 ± 0.2e	0.9 ± 0.2f	1.3 ± 0.6c	3.4 ± 0.2gh	12e	7.4 ± 1.6e	1.8 ± 0.6ab
	24	CGM motiles	1.0 ± 0.1e	1.1 ± 0.1ef	0.9 ± 0.5c	3.0 ± 0.1h	8e	7.1 ± 0.9ef	1.6 ± 0.2ab
						12 ºC			
P. longipes	20	CGM eggs	4.9 ± 0.4e	5.4 ± 0.5c	-	-	0e	4.0 ± 2.8h	0c
	20	CGM motiles	5.0 ± 1.2c	5.5 ± 0.5c	5.5 ± 0.7bc	25.5 ± .7b	10e	3.0 ± 1.7i	0c
						27 ºC			
	20	CGM eggs	0.9 ± 0.5c	1.2 ± 0.3ef	1.2 ± 0.5b	4.6 ± 0.7ef	35c	7.3 ± 4.2e	0c
	20	CGM motiles	2.3 ± 0.4e	2.5 ± 0.5d	2.2 ± 0.6c	6.5 ± 0.5c	25d	6.8 ± 1.9f	0c
						33ºC			
	20	CGM eggs	0.8 ± 0.3d	1.8 ± 0.5de	1.8 ± 0.5c	4.3 ± 0.8e	8e	5.2 ± 2.3g	0c
	20	CGM motiles	1.4 ± 0.3e	1.2 ± 0.4ef	2.2 ± 0.3c	4.8 ± 0.7d	4e	4.7 ± 2.4g	0c
		F	69.7	143.1	15.7	668.0	416.5	2776.6	4.1
		P	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0017

Different letters within entire column of predatory cohorts denote significant ($P < 0.05$) difference at 5% level (Fisher Least Significant Difference, GLM) (df = 11, 35).

Table 6.4: *Typhlodromalus aripo* immature developmental period (days ± SE) and female (*F.*) longevity on different prey types at specific climatic conditions

		Immature development period (days) and female longevity								
Predator	**Prey**	**12 °C**			**27 °C**			**33 °C**		
					40%RH					
		Dev. days	% maturity	F. longevity	Dev. Days	% maturity	F. longevity	Dev. Days	% maturity	F. longevity
T. aripo	CGM eggs	12.3 ± 6.4d	10a	6.2 ± 7.2a	5.1 ± 3.2d	94a	24.6 ± 4.3a	4.6 ± 2.6d	12a	4.8 ± 1.8a
	CGM actives	15.8 ± 9.3c	7b	5.2 ± 1.2b	5.5 ± 2.6c	92a	22.3 ± 3.7b	4.8 ± 6.2c	7b	5.2 ± 0.8a
P. longipes	CGM eggs	17.8 ± 7.2a	3c	2.1 ± 2.4d	7.4 ± 3.5b	12b	6.2 ± 4.0c	5.2 ± 2.4b	4c	1.8 ± 2.2b
	CGM actives	16.4 ± 4.8a	2d	3.3 ± 1.8c	8.5 ± 2.7a	8c	6.3 ± 6.2c	5.8 ± 2.5a	2d	1.9 ± 0.4b
	F	213.2		164.1	89.3		112.4	94.7		23.8
	P	< 0.0001		< 0.0001	< 0.0001		< 0.0001	< 0.0001		0.0140
					75%RH					
T. aripo	CGM eggs	17.2 ± 4.3b	8a	9.3 ± 4.2a	4.3 ± 0.9c	96a	27.6 ± 2.8c	3.8 ± 2.8c	14a	7.6 ± 2.9a
	CGM actives	18.6 ± 3.6b	5b	6.4 ± 2.1b	4.2 ± 0.6c	92a	28.6 ± 5.6c	3.6 ± 1.8c	8b	7.1 ± 2.1b
P. longipes	CGM eggs	23.4 ± 5.2a	3c	4.6 ± 1.5c	7.4 ± 2.2b	14b	4.6 ± 3.2b	6.2 ± 3.2a	3c	2.7 ± 3.8c
	CGM actives	22.6 ± 4.2a	2d	4.2 ± 0.8d	8.3 ± 1.8a	11b	3.8 ± 2.5a	5.8 ± 2.6b	0d	1.9 ± 1.9d
	F	165.5		324.2	64.1		33.9	55.1		103.9
	P	0.0156		< 0.0001	< 0.0001		< 0.0001	< 0.0001		< 0.0001
					92%RH					
T. aripo	CGM eggs	16.5 ± 8.2c	6b	9.6 ± 5.2b	3.8 ± 0.8c	92a	25.2 ± 4.4b	4.2 ± 3.2d	14a	6.4 ± 4.2a
	CGM actives	15.9 ± 6.8c	4b	9.9 ± 4.5a	3.6 ± 0.2c	92a	27.5 ± 5.6a	4.6 ± 2.7c	10b	5.6 ± 2.0b
P. longipes	CGM eggs	20.3 ± 4.7b	2d	4.8 ± 3.6c	6.2 ± 1.4b	8c	3.2 ± 4.7c	6.5 ± 2.4a	6b	1.2 ± 1.0c
	CGM actives	22.1 ± 5.5a	2d	2.3 ± 4.1d	8.4 ± 1.2a	5d	3.1 ± 7.2c	6.3 ± 1.2b	4c	0.9 ± 0.1c
	F	523.2		416.8	19.5		403.9	43.4		12.8
	P	< 0.0001		< 0.0001	< 0.0001		< 0.0001	< 0.0001		< 0.0001

Different lower case letters denote significant difference ($P < 0.05$) at 5% level at the bottom of each column indicates value of Fisher's Least Significance Difference (LSD) (GLM) for each life stage under different temperatures (df = 3, 23) in 10 days observation period.

6.3.3 Egg drought tolerance

Egg hatch (%) was varied at the different humidities and temperature conditions (Fig.6.3). Regression of humidity regimes to egg hatch (%) of the predatory mite, *T. aripo* indicated high drought tolerance level, where 100% egg hatch was determined at 60% RH, while 50% egg hatch (rh_{50}) was demonstrated at less than 40% RH as shown on the regression equation ($y = 2.53x + 82.5$, $R^2 = 0.547$) in Figure 6.2. A lower relative humidity of 55% enabled 100% egg hatch of *P. longipes* with a 50% hatch occurring at <40% RH. At 50% RH *T. aripo* and *P. longipes* had 90 and 80% egg hatch, respectively.
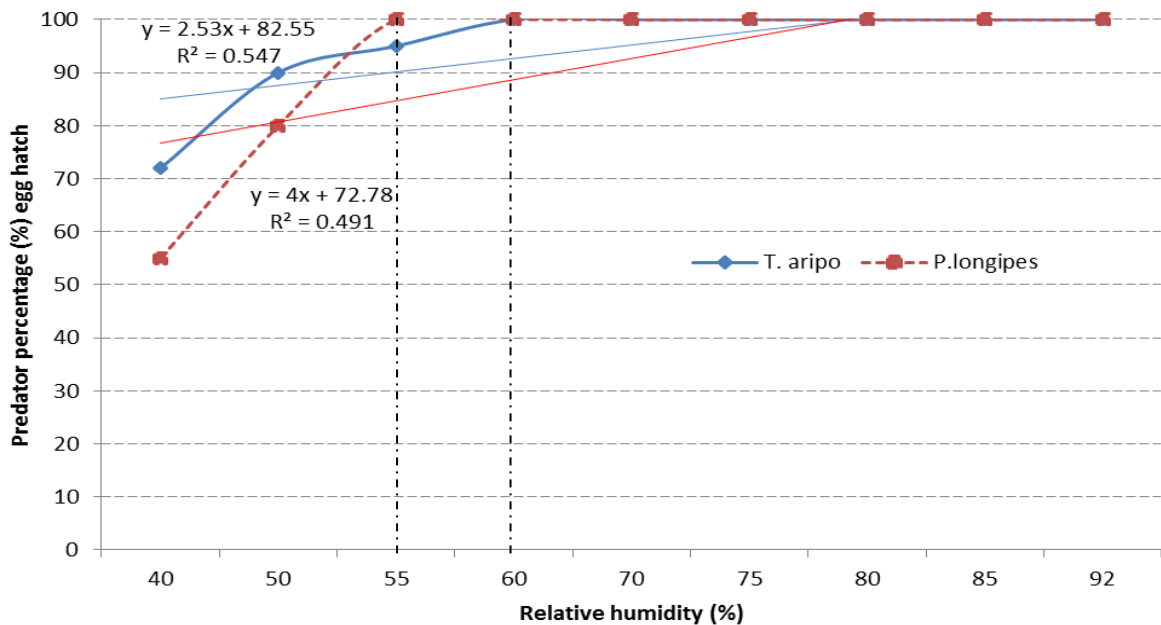
Figure 6.3: Comparable egg hatch (%) of *Typhlodromalus aripo* and *Phytoseiulus longipes* on different humidity regimes at 27 ℃.

6.4 Discussion

Majority of the phytoseiid mites are facultative natural enemies and feed on different prey and non-animal diets like pollen grains (El-Banhawy *et al*., 2000, 2001; Van Baalen *et al.,* 2001). Knapp *et al* (2013) reported effective biological control of most cassava

invertebrate herbivores by introduced *Amblydromalus limonicus* Garman and McGregor indicating economic value of such a phytoseiid predator.

The present study has shown that the phytoseiid *T. aripo* consumed 28.3 actives or 61.6 eggs / day of *M. progresivus* prey in comparison to *P. longipes* which consumed 6.3 and 9.2 on similar prey cohorts at the optimum temperature and humidity regimes. No effect of developmental period and survival was observed from the results. The high mortality of *P. longipes* disqualifies it as reliable candidate for *M. progresivus* control on cassava. In a study on diet range of *T. aripo* it was reported that the phytoseiid was able to develop and reproduce on the *M. progresivus* under laboratory conditions (Gnanvossou *et al*., 2003, 2005). In the same comparative study *T. aripo* survived for a limited period of time on pollen grains of castor species (Magalhães & Bakker, 2002; Gnanvossou *et al.*, 2005). In the present study the number of *M. progresivus* consumed daily increased by average of 28.3 *M. progresivus* motiles /female predator per day. Chant (1961) reported negative effects of large populations of prey to the phytoseiid mite *Typhlodromus occidentalis* Nesbitt. The best results are obtained when predatory mites are released at lower prey population (Bravenboer & Dosse, 1962; Chant, 1961). Onzo *et al.* (2009) studied *T. aripo* feeding behavior on cassava canopy, in the study the phytoseiid was demonstrated to avoid prey populations from the lower canopy of cassava plant by moving to the plant apex where it was more suitable to rest at day time.

Magalhães and Bakker (2002) reported that *T. aripo* usually gets supplement feed from cassava apex tissue unlike other phytoseiids like *Neoseilus idaeus* Denmark and Muma and *Phtytoseiulus persimilis* Athias-Henriot. Most eggs of *T. aripo* were found on the

apex than plain leaves in the mass rearing unit in the present study. Further, it has been shown from the present study that *T. aripo* is sensitive to extreme low humidity and temperatures in comparison to other phytoseiids. The most optimum conditions for its survival were recorded as approximately 75% RH and 27°C. Shipp *et al* (1997) reported that abiotic factors such as temperature and humidity limit the impact of phytoseiids on tetranychids and other insect hosts and thus the need for complementally control intervention arises. De Courcy *et al.* (2004) reviewed different phytoseiid species performance on prey populations in extreme low temperature and concluded that strain difference need to be considered as well. In the present study *T. aripo* immatures suffered the highest mortality in both low temperatures (12 °C) and humidities (≤40%). At the higher humidity (≥75%) both immatures and adult had least mortality on the different prey options. The predatory mite *T. aripo* showed preference for egg cohorts than the motiles. A similar observation was reported by other workers on the predacious mite *P. persimilis* Athias-Henriot and *Galendromus occidentalis* Nesbit on prey *Tetranychus urticae* Koch (Blackwood *et al*, 2001; Mohammad *et al*., 2004). On the other hand *P. longipes* immatures partially developed on diet of *M. progresivus* eggs and motiles at the more optimum temperature of 27 °C but survival was low (<40%). Ferrero and De Moraes (2007) reported over 80% immatures survival on preferred diet prey of *T. evansi*. Cassava plant leaf tissue contains the toxic cyanide substances which act as defense agents against generalist herbivores (Gleadow & Eoodrow, 2002; Capinera, 2008). Predacious mites are extremely sensitive to toxic materials (Bartlett, 1964; Herne & Chant, 1965; Hernderson & Tilton, 1995) and adverse effects could arise in different indirect ways. El-Banhawy (1976) showed that the predatory mite, *Amblyseius brazilli*

El-Banhawy suffered high mortalities and irregular reproduction when the females were fed on prey previously contaminated with insecticides. Therefore it is hypothesized in this study that the transmitted cyanide toxicity to the immature and adults of *P. longipes* caused the interruption in the life cycle and the severe deterioration in reproduction. While *P. longipes* had 100% mortality in less than 12 days, *T. aripo* demonstrated an effective predator-prey ratio of 1:28 or 1:62 per day on *M. progresivus* motile and egg stages. On extrapolation it would mean the a few individual *T. aripo* actives would feed on both egg and motile stages of the prey and prevent economic injury on cassava (Lima, 1998; Nakazawa *et al.*, 2006). Osekre *et al.* (2008) cautioned that predator performance would depend on other environmental factors and other herbivores on cassava in the field. Hence, as Beretta and Kuang (1998) reported the projected predator-prey effectiveness is usually more of an estimate than a straight forward eco-mathematics solution of the dynamics at play. With the present results it was then easy to make right choice of the phytoseiid predator for management of *M. progresivus* densities below injury levels. *T. aripo* qualified to be the predator to manage *M. progresivus* on cassava as the results demonstrated high consumption and high predator survival in the different environment conditions. High egg hatchability in the dry environment has indicated a resilient predator in advance extreme condition. The high occurrence and field abundance of *T. aripo* from the survey results obtained from different agro-ecologies confirms the suitability of the phytoseiid as the choice predator in integrated management of *M. progresivus*. The rich literature on the success of *T. aripo,* in suppressing mite pest *M. progresivus* supports the present study results (Yaninek & Hanna, 2003; Onzo *et al*., 2005, 2009).

CHAPTER 7

7.0 **MANAGEMENT OF THE PHYTOPHAGOUS MITE *MONONYCHELLUS PROGRESIVUS* ON CASSAVA CROP IN DIFFERENT AGRO-ECOLOGICAL ZONES BY INTEGRATION OF SOIL FERTILITY, PREDACIOUS MITE *TYPHLODROMALUS ARIPO* AND SELECTED ACARICIDE**

7.1 Introduction

Cassava crop in Kenya attracts and supports a variety of insects and mite populations some of which have been reported as injurious and reduce cassava tuber yields (Kariuki *et al.*, 2000; Hillocks, 2002; Knapp *et al.,* 2013). The crop is mainly grown in the western and coastal regions of Kenya (Mohammad *et al.*, 1998). The eastern marginal areas are turning to more cassava cultivation as cereal and legumes production indicate a trend of decline as a result of climate change scenario (FAO, 2010; Maina *et al.,* 2012). Among the number of injurious arthropods reported on cassava in the country, *M. progresivus* is most prevalent (Kariuki *et al.,* 2002: Mutisya *et al.*, 2011). Hence, within the context of the fact that the *M. progresivus* pest continues to cause major damage to cassava in the dry lowland and midland zones of Kenya, there was need to compare biological and chemical control options in addition to manure and mineral fertilizer input applications at specific agro-ecological zones of the country (Ayoola, 2006; Ande *et al.,* 2008).

An integrated pest management programme consists of a number of specific control tactics each with ability to control pest species on its own, but combined to work together for better results (Bellotti *et al.*, 1994; Sabelis & Rijn; 1997; Gerson & Weintraub, 2007). As to any pest management system, information on the correct identification of the pest concerned is paramount, as is its biology. More information is needed on the

environmental influence of these pests and on their natural control factors of treatments applied against them (Hoogerbrugg *et al*., 2011). It was for these reasons that a comparative integration of four components, namely synthetic fertilizer, compost manure, predacious mite *T. aripo* and acaricide was carried out in selected different agro-ecological zones in Kenya. The aim was to demonstrate their effects on improving cassava yield by reducing the phytophagous mite *M. progresivus* population levels below those causing economic injury levels. An effective predator-prey ratio of 1:28 was determined of *T. aripo* on *M. progresivus,* as baseline study of the evaluation. The estimate ratio is a mean number of motile prey individuals fed on daily by the   predator leading to suppression of the pest on cassava and deterring of economic injury levels. Other workers have shown that a selective acaricide like abamectin had low toxicity to fish and mammalian vertebrates, and conservative of phytoseiid predators in the environment (Lasota & Dybas, 1990; Zhang & Sanderson, 1990; Hoogerbrugg *et al*., 2011). The present investigation was designed to determine the effects of the combined four mite control treatments on which the yield of cassava was evaluated. This is because to develop the necessary understanding of a pest management system it is important to carry out analysis of the pest density dynamics as well as crop production in relation to economic advantage.

7.2 Materials and Methods

7.2.1 Compost manure preparation

The ratio of the compost manure ingredients were 40:3:20 of goat manure, chicken droppings and cassava fodder, ensiled in a compost pit of 12 tons. The pit of 3m x 5m

had the ground floor covered with black polythene paper. After a thorough mix of the compost materials with a shovel, 440 litres of water was added to the 12 tons heap to enhance microbial decomposition. Another polythene paper was used to cover the top to enable heat trap inside for fast decomposition (Fig.7.1). A hand thermometer 30cm-long was used to monitor temperature in each morning hours (9:00 to 10:00hrs) every one week. After about two months the temperature dropped from 36 ºC to level of open environment temperature indicative of complete decomposition of the manure ingredients (FORMAT, 2005). The 12 tons compost manure was enough for each season application at the four agro-ecological zone sites at Katumani (LM4), Kiboko (LM5), Embu (UM3) and Mtwapa (UM3).

A   B

Figure 7.1: Preparation of compost pit for decomposing of manure ingredients

7.2.2 Bioassay test to select effective acaricide

Toxicological effectiveness of an insecticide chlorpyrifos (Dursban 480E ®) and an acaricide, Avirmec 1.8E ® (abamectin) against *M. progresivus* motile stages was pre-tested and the latter found more efficacious (Fig.7.2). The bioassays were carried in concentrations of 0.187, 0.375, 0.75 and 1.5ml chemical active ingredient in water. Four

leaf replicates were collected from a cassava field plot infested with *M. progresivus* actives between 50-100 mites/ leaf and a similar control set. The control set was treated with distilled water bearing no chemical concentrate. The other two sets were treated with abamectin and chlorpyrifos in the described toxicological concentrates of similar distilled water. It was found out that the abamectin was more efficacious than chlorpyrifos as shown in Figure 5.1 after data cleaning adjustments following Abbott (1925) formula;

$$\%C = (N - T)100/Na$$

where, *%C* = corrected percentage (%), N = populations, T = treated, *Na* = number controlled after treatment.

It was for this reason the acaricide, abamectin was selected for use in the present studies in addition to its low toxicity to predacious mites (Zhang & Sanderson, 1993).
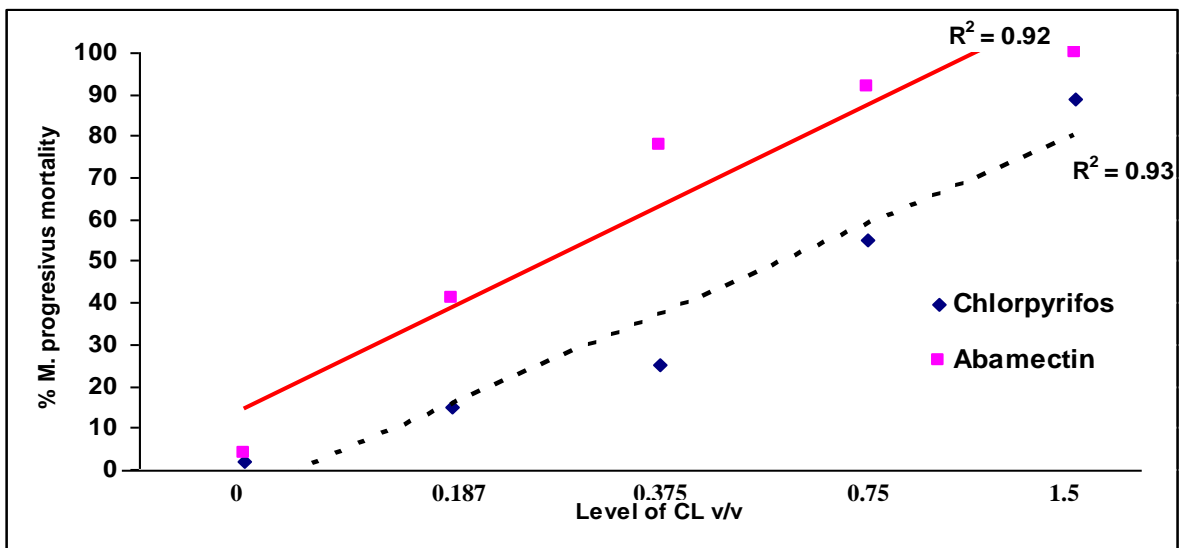
Figure 7.2: Mortality (%) of *Mononychellus progresivus* on abamectin and chlorpyrifos water concentrates.

7.2.3 *Typhlodromalus aripo* **mass production**

The predatory mite *T. aripo* was collected from a cassava field at Kenya Agricultural Research Institute- Kiboko Station (02º 12.872 S, 037º 42.960 E, 934m asl) of the eastern region. Fifty cassava apices infested with *T. aripo* were transferred in a cool box and delivered to the laboratory at KARI-Katumani for mass rearing in a week's time of multiplication. Four plastic containers of 23cm-height x 24cm-diameter were filled-half way with water. In the water some 8-10 apices of the x-Mariakani cultivar of 30-35cm stem lengths were stood in the containers with the apex part above the water. The cultivar x-Mariakani plant shoot is usually tri-branched resulting to three apices per shoot. Between 10 and 12 apices of cassava had about 100 *T. aripo* actives which were introduced on 10 plants per site of the predacious mite treatment.

7.2.4 **Experimental design and treatments**

A local cultivar, X-Mariakani was used at all the sites with spacing of 1m x1m between plants and four-meter paths between treatment blocks consisting of plots of same size and shape. Inorganic fertilizer formulated (at factory) in the element ratio of Nitrogen: Phosphorus: Potassium (NPK 17:17:17) was applied at 50kg ha^{-1} as a subplot treatment in the four main treatments. High nutrient compost treatment was applied at nutrient concentration level of 2:1:1 (NPK) at the rate of 2.8 t ha^{-1} for comparison. The Table 7.1 below show compost manure nutrient element and specific soil chemical compositions from the four sites. Micronutrient foliar spray (Copper, Zinc and manganese) was applied

(40L/Ha) at all the plots in the first four months to enrich soil trace element nutrient
(Sanginga & Woomer, 2009).

Table 7.1: Soil and manure chemical properties at Katumani (LM4), Mtwapa (CL3), Kiboko (LM5) and Embu (UM3) plots October 2010

Site plot	Macronutrients						Micronutrients				Soil Texture		
	(g/kg)						(mg/kg)				(%)		
	PH	N	P	K	Ca	Mg	Fe	Cu	Zn	Mn	Sand	Clay	Silt
Katumani	4.7	3.4	0.02	0.9	3.3	0.2	195.0	2.3	3.3	118.7	59	30	11
Mtwapa	4.9	1.9	0.01	0.1	1.7	0.1	57.8	0.1	3.3	80.7	69	30	1
Kiboko	7.4	1.6	0.04	0.2	1.5	0.3	0.2	0	0	-	61	18	11
Embu	6.7	3.1	0.01	0.5	2.0	0.4	0.2	0.0	0.0	1.64	42	56	2
*Manure	8.4	20.4	7.1	8.8	19.1	1.4	7.7	0	0	172.8	-	-	-

*Manure material composting ratio was 40:3:20 tons of goat, chicken droppings and cassava fodder ensiled in a pit for two months.

The mite management experimental design was four treatments of predacious mite *T. aripo*, abamectin, chlorpyrifos and control treatment (farmers' practice) as main plots of three replicates in a complete randomized block design (CRBD) (Fig. 7.3). The subplots were of compost manure, mineral fertilizer (NPK: 17:17:17) and control. The application rates of abamectin and chlorpyrifos were according to recommended rates (for vegetable crops) of 62.5 and 50 g active ingredient /ha (or 300 and 250ml/ha), respectively for spider mites control. The predacious mite *T. aripo* was obtained from KARI-Kiboko field and introduced later (100 individual on 10 plants) at all the sites on predacious mite treatments when the crop was four months after planting. Cassava root yield was assessed by taking weight (kg) of 10 plants per subplot from the middle five rows at the

eighth month of crop establishment. A spring weighing balance (Make: Spring-Dial Hoist scale) was used for taking weight.

NPK	MN	Ỏ	NPK	MN	Ỏ	NPK	MN	Ỏ	NPK	MN	Ỏ
R1	R3	R3	R1	R3	R2	R1	R3	R2	R1	R3	R2
T. a	*T. a*	*T. a*	C	C	C	Aba	Aba	Aba	Chlor	Chlor	Chlor
NPK	MN	Ỏ	NPK	MN	Ỏ	NPK	MN	Ỏ	NPK	MN	Ỏ
R2	R2	R1	R2	R1	R3	R2	R1	R3	R2	R1	R3
T. a	*T. a*	*T. a*	C	C	C	Aba	Aba	Aba	Chlor	Chlor	Chlor
NPK	MN	Ỏ	NPK	MN	Ỏ	NPK	MN	Ỏ	NPK	MN	Ỏ
R3	R1	R2	R3	R3	R1	R3	R2	R1	R3	R2	R1
T. a	*T. a*	*T. a*	C	C	C	Aba	Aba	Aba	Chlor	Chlor	Chlor

Figure 7.3: Randomized complete block design (RCBD) of cassava crop under treatments of abamectin (Avirmec 1.8 EC), chlorpyrifos (Dursban 480E), predacious mite *Typhlodromalus aripo* and soil fertility factor at the sites of Kiboko (LM5), Katumani (LM4), Embu (UM3) and Mtwapa (CL3).

Main plot treatments; *T. a = Typhlodromalus aripo*, C = no insecticide treatment, Aba = Abamectin, Chlor = Chlorpyrifos. **Subplots:** NPK 17:17:17 mineral fertilizer, MN = manure fertilizer (npk=2:1:1), Ỏ = no fertilizer application.

7.2.5 Sampling mite densities and identification

Sampling of predacious mites per plant apex and number of CGM per leaf of the third mature leaf was recorded every month from each of the sites, Kiboko, Katumani, Embu and Mtwapa following procedure by Yaninek *et al* (1989). The purpose was to compare prey-predator densities during the production periods. Density of CGM was counted and recorded from the underside of the third mature leaf of cassava apex with the aid of head loop lenses of mag.x4. Any phytoseiid found on the sample leaf was picked with hair camel brush (size 000) and preserved in a vial of 70% alcohol for later identification. The plant apex was thoroughly observed for predacious mite *T. aripo* motiles which inhabit on cassava apices. Collected specimens were counted and preserved in labeled vials of 70% alcohol. The specimens were mounted on slides using Hoyers' mountant and identification carried out using El-Banhawy and Knapp (2011) and Chant and McMurtry (2005) keys for mites' identification.

7.2.6 Site climatic conditions

The separate agro-ecological zones of the four study sites were selected on the criteria of mean climatic conditions as defined by Jaetzold *et al.* (2007). Throughout the crop production, mean climatic factors defined each site overall conditions (Table 7.2). The site at Embu (UM3) was fairly cool and wet with mean monthly temperature of 17 °C and rainfall of 123mm. Katumani (LM5) was warm and dry with mean monthly temperature of 19.5 °C and rainfall of 69mm. Kiboko (LM5) was hot and dry with mean monthly temperature of 25 °C and rainfall of 53mm in addition to irrigation supplement of 160mm/ month. The site at Mtwapa (CL3) was warm, humid with mean monthly

temperature of 26 ℃ and rainfall of 106mm. The Embu site plot had heavy loam soil,

Katumani and Kiboko sandy loam while Mtwapa had sandy soil type.

Table 7.2: Mean (SE) monthly climatic variables at Kenya agro-ecological zones. Source: Kenya National Meteorological Department

Year	Site	Rainfall (mm)	Temp (°C)	RH (%)	Description
2011	Kiboko (LM5)	62.0 (14.4)*	24.8 (3.5)	82.9 (7.1)	Hot, dry
	Katumani (LM4)	68.4 (11.5)	19.5 (6.1)	64.1 (5.2)	Warm, dry
	Embu (UM3)	122.0 (9.3)	18.2 (5.2)	63.4 (11.2)	Cool, wet
	Mtwapa (CL3)	104.8 (13.3)	26.8 (4.6)	77.3 (2.1)	Warm, wet
2012	Kiboko (LM5)	36.6 (9.5)*	23.6 (3.2)	83.9 (8.6)	Hot, dry
	Katumani (LM4)	41.7 (7.4)	19.9 (2.6)	58.6 (5.4)	Warm, dry
	Embu (UM3)	102.6 (8,2)	17.7 (3.8)	63.8 (6.2)	Cool, wet
	Mtwapa (CL3)	96.7 (6.1)	26.6 (4.5)	76.0 (4.9)	Warm, wet
2013	Kiboko (LM5)	32.6 (6.4)*	25.2 (7.3)	74.9 (9.6)	Hot, dry
	Katumani (LM4)	61.2 (5.8)	22.5 (9.1)	58.6 (7.7)	Warm, dry
	Embu (UM3)	89.3 (5.9)	18.3 (5.4)	63.8 (5.3)	Cool, wet
	Mtwapa (CL3)	94.4(16.2)	26.3 (6.2)	76.0 (6.4)	Warm, wet

* Supplementary irrigation at Kiboko of 960mm during the production period.

Analysis of variance (ANOVA-GLM) of Fishers least Significant Difference (LSD) was

carried on log-transformed data (x+1). SAS Version 8 (1999-2001) was used to

determine whether the mean CGM densities and yield difference from the treatment plots

were significant at 5% level. Treatments efficacious control of CGM was determined by

separation of means of CGM numbers and yield significance by employment of SNK

Post Hoc Test. Actual mean data values were later presented on the tables. Microsoft

Excel (2003) was used to illustrate regression correlation (r) of pest mite and the

predacious mites' population trend during the study period for the various treatments of

predacious mite *T. aripo*, abamectin, chlorpyrifos and control treatments.

7.3 Results

7.3.1 Effect of compost manure and inorganic fertilizer

Both inorganic and manure had insignificant ($F_{2, 8} = 2.1$; $P > 0.05$) effect to CGM density at Kiboko (Table 7.3). Nevertheless highest pest densities were significantly ($F_{2, 11} = 189$, $P < 0.0001$ and $F_{3, 11} = 201$, $P < 0.0001$) scored on control treatments at Kiboko followed by Katumani site of >90<130 mites/leaf. Mtwapa had < 50 mites/leaf while Embu was < 10mites /leaf. *M. progresivus* density increased exponentially from April y to October at Kiboko and Katumani of $R^2 = 0.59$ and $R^2 = 0.81$ respectively (Fig.5.3). At the sites of Embu and Mtwapa, *M. progresivus* density correlations were low $R^2 = 0.08$ and 0.18, respectively.

Table 7.3: Mean (±SD) cassava green mite population densities observed under different treatments in different agro-ecological zones

Site	AEZ	Fertilizer	*T. aripo* No. CGM	Abamectin No. CGM	Chlorpyrifos No. CGM	Control No. CGM	*F*	*P*
			Cassava green mite management treatment		
Kiboko	LM5	NPK	125.2 ± 12.6a^A	3.2 ± 1.2a^B	3.4 ± 0.5a^B	128.1 ± 23.0a^A	211.2	< 0.0001
		Manure	123.6 ± 9.4a^A	2.1 ± 0.8b^B	4.2 ± 1.8b^B	119.4 ± 9.9a^A	189.3	< 0.0001
		Control	124.3 ± 11.2a^A	1.8 ± 0.4b^B	1.9 ± 0.8ab^B	109.1 ± 12.4a^A	79.1	0. 0006
		F	2.1	18.9	136.7	2.8		
		P	0.2518	0.0074	< 0.0001	0.1694		
Katumani	LM4	NPK	83.6 ± 23.2bA	1.8 ± 0.6a^B	0.9 ± 0.7c^B	85.2 ± 11.9c^A	201.8	< 0.0001
		Manure	96.4 ± 8.7a^A	2.3 ± 1.0a^B	1.4 ± 0.6b^B	97.3 ± 15.7a^A	79.5	< 0.0001
		Control	97.7 ± 4.7a^A	1.5 ± 0.8a^B	1.6 ± 0.5a^B	87.2 ± 8.2b^A	66.1	< 0.0001
		F	24.0	12.8	83.0	213.8		
		P	0.0047	0.0149	0.0004	0.0001		
Embu	UM3	NPK	6.4 ± 3.4a^B	0.6 ± 0.2b^C	0.4 ± 0.1a^C	9.1 ± 5.2a^A	18.3	0.0012
		Manure	5.9 ± 2.7b^A	0.8 ± 0.1b^B	0.2 ± 0.0a^B	5.2 ± 4.3b^A	22.9	0.0009
		Control	6.2 ± 2.5a^A	1.3 ± 0.7a^B	0.3 ± 0.1a^B	5.3 ± 3.4b^A	44.0	0.0004
		F	23.4	4.5	0.7	8.3		
		P	0.0049	0.0860	0.6238	0.0324		
Mtwapa	CL3	NPK	11.8 ± 9.3a^B	1.9 ± 0.4b^C	1.4 ± 0.6b^C	44.4 ± 24.2ab^A	118.2	< 0.0001
		Manure	13.5 ± 7.4a^B	2.2 ± 0.6a^C	1.8 ± 0.2a^C	41.0 ± 16.2b^A	39.7	< 0.0001
		Control	12.9 ± 2.9a^B	1.7 ± 0.5c^C	1.6 ± 0.8ab^C	47.0 ± 12.5a^A	49.4	< 0.0001
		F	1.3	53.0	12.5	6.5		
		P	0.4019	0.0010	0.0156	0.0483		

Mean values with different letters in the same columns denote significant difference ($P < 0.05$, df = 2, 8; LSD-GLM) both within fertilizer columns and among mite management treatments. Different upper letters denote significant ($p < 0.05$) across treatments at 5% level (df = 3, 11).

7.3.2 Effect of *Typhlodromalus aripo* to pest and yield

Phytoseiid *T. aripo* had most significant ($F_{3, 11}$ = 118.2; P < 0.0001) suppression of CGM densities at Mtwapa (Table 7.3). Similarly other sites had lesser significant Kiboko ($F_{3, 11}$ =79.1; P < 0.05) and Katumani ($F_{3, 11}$ = 79.5; P < 0.0001). In the *T. aripo* treatment, the highest predator densities were observed at the warm humid site of Mtwapa at a peak number of 7.5 *T. aripo* actives /plant apex in the month of October (Figure 7.3). The irrigated plot at Kiboko followed with a peak of 6.2 *T. aripo* /apex in the month of August. *T. aripo* growth in the field plots increased with increase of months of production from April at Kiboko, Embu and Mtwapa. The highest *M. progresivus* density reduction was observed at Mtwapa of 27% on the *T. aripo*-NPK subplot treatment. A 32% *M. progresivus* density reduction was observed on the *T. aripo*-manure subplot at the same coastal plot. Agro-ecological zone factors influenced CGM and T. aripo densities at the sites (Table 5.4). The predacious mite species at Mtwapa and Kiboko was 90% *T. aripo* while at Katumani and Embu the common phytoseiid species recovered from cassava was *E. fustis*. The *T. aripo* treated plot at Kiboko led with highest significant (yield at 34.2 t ha^{-1}, followed by Mtwapa at 31.5 t ha^{-1}. The least yield of < 20 t ha^{-1} was realized at Katumani where *T. aripo* could not establish in the field.

7.3.3 Effect of abamectin and chlorpyrifos to pest and yield

Over 90% CGM density was reduced significantly (P<0.001) in abamectin and chlorpyrifos treated plots at all the sites (Fig 5.3 and Table 5.3). Both abamectin and chlorpyrifos treatment indicated low *T. aripo* re-emergence at the sites within the two months interval of spray (Fig.5.3). Root yield at the four sites significantly (P<0.05)

varied according to treatments in the separate agro-ecological zones (Table 5.3). While *T. aripo* mite treatment led with highest yield at 34.2 tons per hectare, abamectin and mineral fertilizer (NPK) followed second at 33.0 t ha^{-1} at Kiboko. At Embu, root yield was not significantly (>0.05) different among the treatments, where mean tonnage was 24.6-29.2 t ha^{-1}. The site at Katumani showed significant (P<0.05) yield increase in manure and abamectin integrations at 18.2 t ha^{-1} while control was at 10.1 t ha^{-1}. The treatment of chlorpyrifos had close and similar results as abamectin on pest control at the four sites. Each agro-ecological zone influenced CGM density as indicated in Table 5.4.
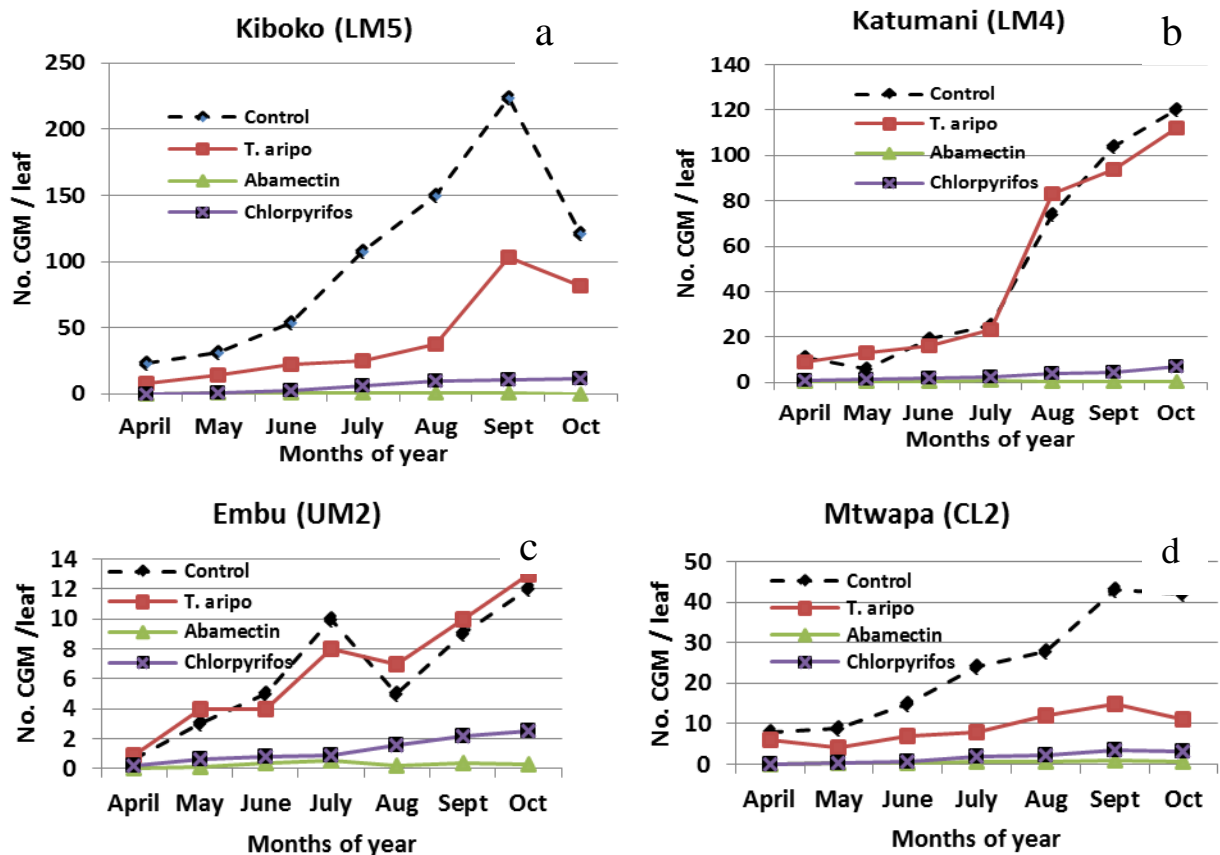
Figure 7.3: Effects of different treatments on cassava green mite (CGM) density in four agro-ecological zones of Kenya (2011-2013)**.**

Table 7.4: Cassava green mite density response to different treatments at different sites

Site		April	May	Jun	Jul	Aug	Sept	Oct
Kiboko	*DF*	3,11	3,11	3,11	3,11	3,11	3,11	3,11
	F	84.7	70.0	77.5	188.1	137.5	146.5	22.4
	P	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0012
Katumani	*DF*	3,11	3,11	3,11	3,11	3,11	3,11	3,11
	F	57.2	25.6	60.7	18.4	45.4	51.9	37.1
	P	< 0.0001	0.0006	< 0.0001	0.0020	0.0004	0.0003	0.0008
Embu	*DF*	3,11	3,11	3,11	3,11	3,11	3,11	3,11
	F	21.8	5.9	1.6	2.7	1.7	41.2	35.3
	P	0.0012	0.0257	0.2867	0.1271	0.2648	0.0006	0.0009
Mtwapa	*DF*	3,11	3,11	3,11	3,11	3,11	3,11	3,11
	F	46.4	6.1	5.3	85.5	24.9	108.7	110.4
	P	0.0002	0.0236	0.0336	< 0.0001	0.0022	< 0.0001	< 0.0001

Overall environmental effect significance ($p < 0.05$)(LSD, GLM) to varied treatments for control of cassava green mite at 5% level at specific sites during the production months of April-October.

7.3.4 Effect of integration of pest management components to yield

Integration of abamectin and fertilizers (manure and inorganic) led to significant yield increase at Kiboko ($F_{3, 11} = 68.4$, $P <0.05$), Katumani ($F_{3, 11} = 53.1$; $P < 0.05$) and Mtwapa ($F_{3, 11} = 9.4$; $P < 0.05$) (Table 7.4). Similarly, *T. aripo* presence in the field significantly ($F_{3, 11} = 9.4$; $P < 0.05$) increased root yield in the NPK treatment at Mwapa. Further, the phytoseiid significantly ($F_{3, 11} = 8.6$; $P < 0.05$) led to increase of root yield when integrated with manure fertilizer. In contrast, at Embu there was no significant ($F_{3, 11} =2.7$; $P >0.05$) with inorganic or manure ($F_{3, 11} = 0.8$; $P > 0.05$) integration with abamectin.

In the midlands zones (LM5), combination of *T. aripo* and manure in the presence of supplemental irrigation increased the root yield from 28.2 to 34.2 tons / ha — an increase of 6 tons/ha or 21%. In the coastal lowlands zone (CL3) at Mtwapa in the same *T. aripo* and manure combination treatment, yield increased from 27.8 to 31.5tons /ha, an increase of 4.7 tons /ha or 13% more root yield. In the upper midlands at Embu (UM3), *E. fustis* and mineral fertilizer treatment had yield increase from 25.4 to 29.2 tons /ha, an increase of 15%. At Katumani (LM4), abamectin combined with manure led to yield of 18.2 tons/ha in comparison to 15.5 non-predator treated plot, an increase of 17%. Overall, environmental factors influenced cassava root yield more than the integration components applied at each site (Fig.7.4).

Table 7.4: Mean (±SE) root yield (tons/ha) under different cassava green mite *Mononychellus progresivus* pest management and fertility treatments at different sites

Site	Fertilizer	*T. aripo* Yield (t-ha⁻¹)	*Chlorpyrifos* Yield (t-ha⁻¹)	*Abamectin* Yield (t-ha⁻¹)	*Control* Yield (t-ha⁻¹)	*Mean* Yield (t-ha⁻¹)	*F*	*P*
Kiboko-LM5	NPK	30.0 ± 4.7aB	29.7 ± 3.7aB	33.0 ± 1.6aA	29.9 ± 2.5aA	30.7 ± 1.6aB	*9.2*	*0.0341*
	Manure	34.2 ± 3.2aA	32.2 ± 2.2aB	30.7 ± 4.1aC	28.2 ± 2.9aD	31.3 ± 2.5aC	*68.4*	*<0.0001*
	Control	29.2 ± 2.6aA	25.4 ± 3.6baC	27.3 ± 4.3bB	22.4 ± 7.3bC	26.1 ± 2.9aB	*102.3*	*<0.0001*
	F	1.4	1.8	6.4	22.2	2.4		
	P	0.3895	0.2957	0.0494	0.0054	0.1618		
Katumani-LM4	NPK	14.5 ± 3.3aA	13.4 ± 2.4aA	14.5 ± 3.6bA	13.4 ± 6.2bA	13.9 ± 0.6abA	*3.9*	*0.1331*
	Manure	12.1 ± 2.8bC	13.5 ± 2.9aBC	18.2 ± 1.2aA	15.5 ± 1.6aB	14.8 ± 2.6aB	*53.1*	*0.0007*
	Control	10.3 ± 2.3cB	13.6 ± 5.1aB	17.5 ± 2.7aA	10.1 ± 2.4cB	12.9 ± 3.4bB	*26.8*	*0.0003*
	F	27.9	8.1	11.5	41.3	12.1		
	P	0.0035	0.0340	0.0181	0.0016	0.0167		
Embu-UM3	NPK	29.2 ± 5.6aA	28.6 ± 1.9aA	28.5 ± 3.9aA	24.6 ± 1.4aA	27.7 ± 2.1aA	*2.7*	*0.4116*
	Manure	26.2 ± 3.2aA	28.7 ± 3.2aA	27.2 ± 1.7aA	25.7 ± 1.8aA	26.9 ± 1.3aA	*0.8*	*0.8612*
	Control	24.8 ± 2.7aB	26.6 ± 1.6bA	25.4 ± 2.3aAB	26.6 ± 1.3aA	25.9 ± 0.9aA	*18.2*	*0.0005*
	F	0.7	20.9	1.7	1.3	0.8		
	P	0.6226	0.0060	0.3077	0.4081	0.5872		
Mtwapa-CL3	NPK	28.4 ± 0.9abA	24.6 ± 2.9bB	28.2 ± 1.5aA	25.4 ± 2.6bB	26.7 ± 1.9bA	*9.4*	*0.0287*
	Manure	31.5 ± 5.2aA	30.2 ± 3.1aB	31.2 ± 1.9aA	27.8 ± 1.8aB	30.2 ± 1.7aA	*8.6*	*0.0462*
	Control	24.8 ± 4.2bB	27.4 ± 4.5abA	24.6 ± 1.4bB	27.7 ± 2.6abA	26.1 ± 1.7bB	*11.3*	*0.0221*
	F	5.6	4.4	17.3	1.9	3.0		
	P	0.0615	0.0896	0.0088	0.2657	0.1597		

Mean values with different lower case letters in the same columns denote significant difference ($P <0.001$, LSD (GLM) at 5% level, df = 2, 8).
Likewise different upper case letters within rows indicate significance difference ($P < 0.05$, df = 3, 11).

A
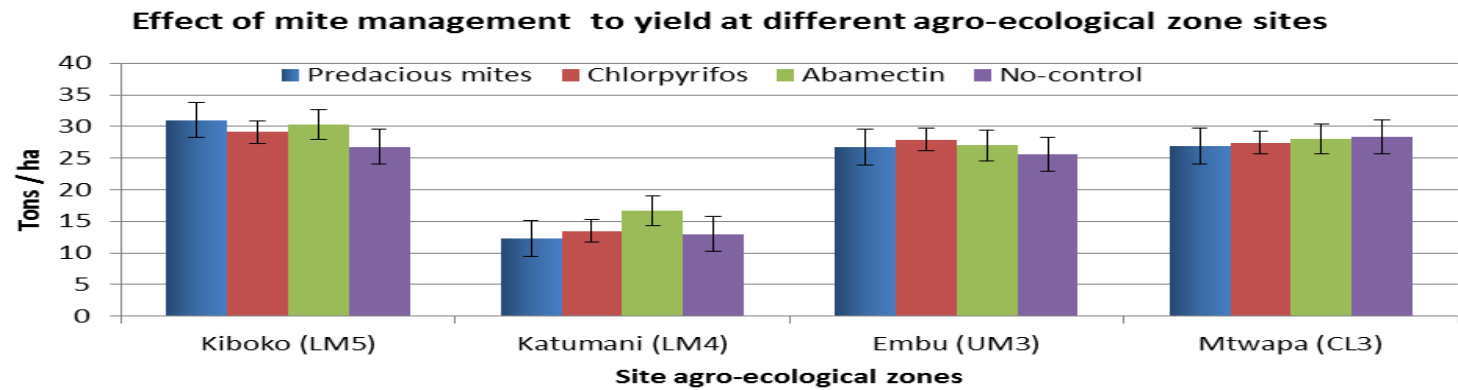
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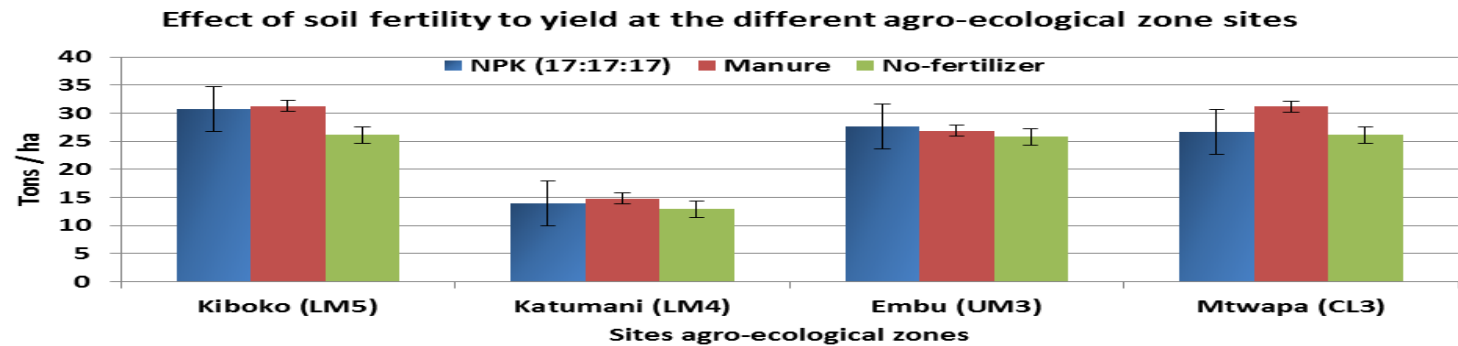
Figure 7.4: Comparable effects of (A) mite management and (B) soil fertility to yield (t ha^{-1}) at the different agro-ecological zone sites.

7.4 Discussion

The present study evaluated various components for integrated CGM management in different agro-ecological zones, where performance scored differed according to prevailing climatic conditions. Bonato *et al* (1994) reported high CGM mortalities from rain drop effect as one of the natural population control of the mite pest during the plant development period (Boudreaux, 1957). The exponential density growth of CGM found confirms these findings. This could be because *T. aripo* increased with increased moisture in the fields as reported by other ecological workers on phytoseiids (Bakker e*t al.,* 1993; Waltzer *et al.,* 2007). The high density of *T. aripo* a few months after introduction at the site in the coastal and eastern low midlands confirms these findings. The integrated pest management strategy was found successful in specific agro-ecological zones at Kiboko, Katumani and Mtwapa where abamectin and fertilizers increased yield with suppressed CGM densities. In the midlands zones (LM5), combination of *T. aripo* and manure in the presence of supplemental irrigation increased the root yield from 28.2 to 34.2 tons / ha — an increase of 6 tons/ha or 21%. Likewise in the coastal lowlands zone (CL3) at Mtwapa in the same *T. aripo* and manure combinations treatment, yield increased from 27.8 to 31.5tons /ha, 4.7 tons /ha or 13% more root yield. Similarly, in the upper midlands at Embu (UM3), *E. fustis* and mineral treatment had yield increase from 25.4 to 29.2 tons /ha, an increase of 15%. In the hilly midland zone at Katumani (LM4), abamectin combined with manure led to yield of 18.2 tons/ha in comparison to 15.5 non-predator treated plot, an increase of 17% in the same prevailing environmental conditions. In the cool upper midlands zone (Embu-UM3) the strategy would be to conserve the presence of *E. fustis* to suppress CGM density. Besides

the type of mite management treatments, environmental factors like rainfall and annual temperatures influenced final yield at the sites, with distinct comparable variables at Kiboko (LM5) and Embu (UM3).

On the other hand, environmental considerations is important on the part of farmers not to pollute the fields with chemical sprays which could lead to upsurge of more pest densities and higher leaf damage (Memarizadeh *et al.,* 2013). Otherwise studies on environmental effect of abamectin to mammal and fish has been justifiably indicated as low toxicity of residue level of less than 0.025ppm, on sprayed crops as the pesticide is photodegradable (Lasota & Dybas, 1990; Zhang & Sanderson, 1990)

From the present study manure and mineral nutrients have been found to increase cassava yield against high CGM densities at the dry lowlands climate. What would guide the farmer or pest manager would be observation and monitoring of both the beneficial phytoseiids presence and pest mite population density status in the prevailing conditions to make a decision on the cause of action; whether to implement a curative approach of chemical (acaricide) control to prevent crop damage or to leave in place the established production system of integrated mite management with increased soil fertility input in the presence of beneficial predacious mites.

CHAPTER 8

8.0 GENERAL DISCUSSION, CONCLUSSIONS AND RECOMMENDATIONS

8.1 General discussion

In most cases the status of methodology for pest assessment and estimation of crop losses is usually both incomplete and generally inadequate for support of an effective integrated pest management programme (Lima, 1998). There is need for uniform and reliable detection programme to assist in the effort. In the case of cassava, several factors determine crop yield; rainfall amount and reliability, pest complex and status as well as soil fertility and cultural practices by the farmer (Alves, 2002; Smaling *et al.*, 2002). Likewise, there are number of interactions which exemplify the need for an interdisciplinary approach to integrated pest management (IPM) as soil fertility and biotic factors (Komkoiewtz *et al.*, 1993; Fermont *et al.*, 2009). For example, in modern agriculture, cultural practices for pest management are used less extensively than their potential warrants. It is quite useful through interdisciplinary combination of cultural practices that these aspects are identified, modified and improved to achieve effective management of a particular pest. A successful development and demonstration would make cultural practices more attractive for adoption in field situations (Jeppson *et al*., 1975; Conn, 1981). Another important component of IPM is biological control programme which is often dependence upon the ability to introduce and manipulate natural enemies in a new environment (Waage, 2001). Such interdisciplinary approach involving sound biological aspects as in the present case of soil fertility, *T. aripo* and acaricide would deliver enhanced yield increase.

Since the introduction of CGM in East Africa in early 1970s, the assumption has been that the pest mite identity is *M. tanajoa* (Yaninek & Herren, 1988; Onzo *et al.*, 2012). The mite was reported to have been accidentally introduced on some cassava cuttings from Brazil, South America (Megevand *et al.,* 1987, Yaninek & Herren 1988). Murega (1989) demonstrated how the phytophagous mite populations from Uganda and Kenya were compatible after crossing the different population individuals getting successive progeny, thus making the conclusion that it was indeed one similar species. Navajas *et al.* (1994) demonstrated in a molecular study that indeed the cassava species in Benin and Congo was 100% similar to the Columbian *M. progresivus* species. While some workers might recommend a repeat study in most areas of the African Continent, the present study has analyzed the species both morphologically and molecular level and concluded that the CGM species in Kenya is indeed *M. progresivus*. Consideration of the morphological studies by Gutierrez (1987) and the molecular work by Navajas *et al.* (1994) the confusion on species identity is alleviated and concluded that *M. progresivus* is the cassava green mite species in Kenya, and probably the whole East Africa region as well as other countries of Africa.

The survey results on predacious mites diversity on cassava in Kenya has shown that two phytoseiid predators stand out as the most abundant in different regions of the country. The phytoseiid *T. aripo* was released in Kenya in 1995/96 and has been persistent in warn humid regions of the country (Kariuki *et al*., 2002). The phytoseiid has not been found in the high altitude cool areas of eastern and central. One phytoseiid *E. fustis* was found common on most cassava plants in coastal, eastern, central and western regions of the

country as the mite occurrence results have indicated. While some 30 species were identified from the specimens collected, only *T. aripo* and *E. fustis* were found most frequent on cassava plants and were probably the species suppressing *M. progresivus* densities on cassava at specific regions of the country (Kariuki *et al.,* 2000, 2002). Various studies have comparably demonstrated that *T. aripo* was more effective on CGM control than most indigenous species in Africa (Onzo *et al.,* 2005; Kariuki *et al.,* 2005; Zannou *et al.*, 2007). The cool regions of western and Embu were the regions where *T. aripo* was not found, where incidentally CGM was found in low densities (<10 mites/leaf) in such zones (Kariuki *et al.*, 2000). The justifiable concern for CGM management has been mainly in the marginal low and midland zones where interest for cassava cultivation is reportedly growing as increased climate change lead to conversion of earlier arable lands to more marginal lands (Maina *et al.*, 2012).

Kenya food security today is increasingly precarious as more marginal lands convert to semi-desert zones increasing low incomes for specific communities (FAO, 2010; Maina *et al*., 2012). A crop like cassava could be the only option for food security. The fact lies on reports that the crop's ability to tuberize with the least amount of rainfall (Malindagabo & Birandano, 1984; Gomez, 1991; Ferris *et al.*, 1997; Alves, 2002; Hillocks, 2002). In areas of eastern and north-eastern lowlands animal enterprises continue to be decreasing as rainfall amounts fall below 200mm per year. Figure 8.1 below shows shaded eastern and North-eastern region of the country where irregular torrents of rainfall have been reportedly occurring each year leading to less agriculture related activities of legumes and cereals (Maina *et al.*, 2012). While most legumes and

cereal abort without any yield, the solution could be found in cassava production; the plant roots continue expanding high quality carbohydrates for animal and human feed (Balagopalan, 2002; Shama & Suppan, 2011). However, the danger lies on high infestation by CGM during the dry spell of production period. This defines the importance of the present study where the option of planting cassava with manure compost would help trap soil water moisture for long in the ground resulting to higher root yield (Fermont *et al.*, 2009; Sanging & Woomer, 2009). The option of spray with abamectin and manure application would save the crop from increased drought effect when more tissue water is lost by CGM attack in the absence of drought sensitive phytoseiid predators (Lasota & Dybas, 1990; Youdeowei, 2002; Ayoola, 2006).

The study on CGM population growth on different cassava varieties has demonstrated that the spider mite prefers high leaf cyanogens glycoside content to low one. The finding is important as it translates to the fact that low cyanide cassava varieties were tolerant to CGM damage and hence safe for human consumption (Mutisya *et al.*, 2013). This is important information for breeders when breeding against CGM which is reported to cause 30 to 80% root yield (Yaninek *et al*., 1989). On the performance of *T. aripo* it was found that the phytoseiid has predator-prey ratio of 28 motiles or 62 egg cohorts per day consumption. Earlier on Gnanvossou *et al.* (2005) demonstrated *T. aripo* cohorts grew on castor oil pollen as plant alternative diet showing that the phytoseiids has both plant and herbivore diet range. Further, the information indicated that *T. aripo* would not starve in absence of its preferred prey *M. progresivus.* As with cassava, many of our crop protection problems require a multi-disciplinary approach to manage the specific pest

below injury levels (Lima, 1998). This would mean combining old and new techniques for an integrated basis with due consideration for the nature of principles associated with each of the pest species involved. The goal of the programme should be to integrate the biological and technical control measures to take maximum advantage of host plant tolerance /resistance, naturally-occurring biological and other environmental factors to prevent the pest organism from reaching injury levels. In the specific case of cassava, the major elements in an ecosystem which must be considered are the plant (cassava), the pest (CGM), the natural enemies (like *T. aripo*), the climate, the soil and the available pesticides. Even in its simplest form, as shown by such studies, there are many direct and indirect interrelationships that must be considered in the specific management programme (Komkoiewtz *et al.*, 1993; Ship *et al.*, 1997). In the past there has been a tendency to depend upon a single method, pesticides for pest control. The results have been disastrous with pest upsurge after development of pesticide resistance (Yaninek & Schulthess, 1993).

Fermont *et al*. (2009) showed that soil fertility was an important input for root yield increase on cassava production in East Africa. Amanullah *et al.* (2007) and Ande *et al.* (2008) found strong evidence of need to incorporate soil fertility in cassava production. This included even use of organic manure increasing yield quality of tubers (Amanullah et al., 2008). Continuous production systems were found to negatively to yield if no soil fertilizers were used (Ande *et al.* 2008). In the East Africa region little work on cassava agronomy has been done, specifically where agro-ecological zones are evaluated for a model applicable to each farming system (Fermont *et al.*, 2008).
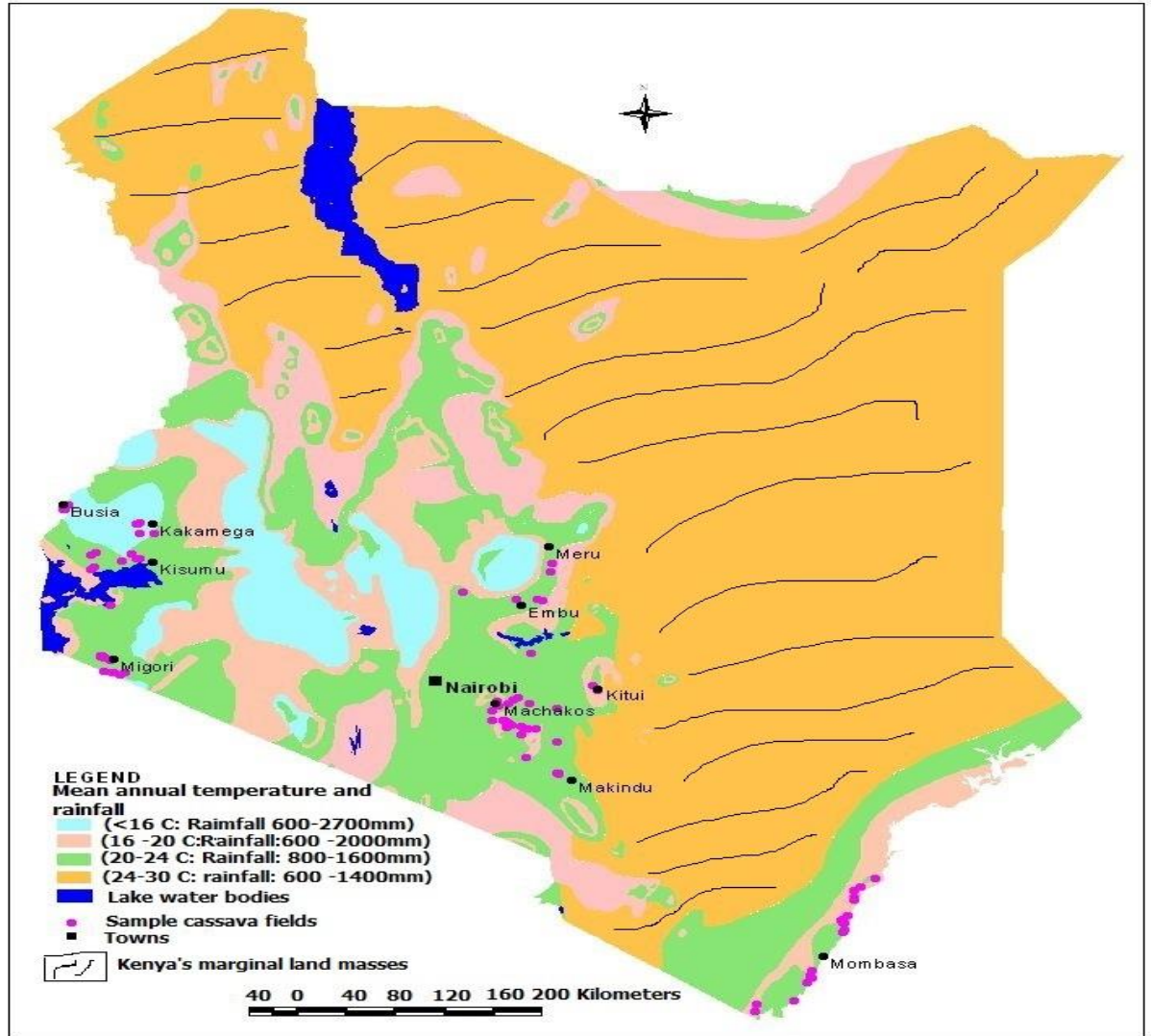
Figure 8.1: Map of Kenya landmasses unsuitable (shaded) for crop production.

The evaluation study on the different options of *M. progresivus* management on cassava

in different agro-ecological zones in Kenya led to insight information for effective

control of CGM. The low midlands (Kiboko and Katumani), the coastal lowlands (Mtwapa) and the upper midlands (Embu) strongly showed higher field persistence of predacious mites *T. aripo* and *E. fustis* in the warm humid regions of the country while in the low drier midlands acaricide spray was justified for control of the pest mite. The judicial use of acaricide would only be sustainable for a period since the spider mite group of pests has been reported to develop abamectin resistant in the Asian countries as in the case of *T. urticae* on tomatoes (Memarizadeh *et al.*, 2013).

Finally, the bio-meteorological accounting system is essential to maintain an organized input of data into plant growth model synchronized into the pest growth model for pro-active implementation of management strategy in specific agro-ecological zones. Plant growth and pest population dynamics are directly related to bio-meteorological inputs and, therefore meteorological data are the driving parameters of these models. Hence, such models would greatly aid in CGM management in specific agro-ecological zones of Kenya.

8.2 Conclusions

The general objective of the present study was to determine an integrated management strategy of the mite cassava green mite on the understanding that cassava is grown by resource poor farmers, hence integrated mite management use of fertilizer input would increase plant health and hence higher root yield. It was determined that *T. aripo* contributes to suppress *M. progresivus* densities below injury levels on the leaf photosynthetic area. Where the environment is too dry or too cold to enable survival of the predacious mites, judicial inclusion of an acaricide like abamectin was justifiable to

save plant leaf drop and apex die-back during the dry spell and the subsequent yield loss. Thus, the integration order would be:

(1) Soil fertility input at planting probably by use of manure or mineral fertilizer;

(2) Field predacious mites' conservation where the beneficial biological agents naturally occur, or augmentation of such phytoseiids from nearby fields to suppress *M. progresivus* densities early enough before injury level threshold status are attained, and finally as last option;

(3) Application of acaricide spray like abamectin which has low mammal and phytoseiid toxicity where prey densities are at threshold levels ($\geq$27 mites/ leaf) of injury to cassava variety under cultivation. This approach suits the low dry midlands of eastern Kenya while the cool areas need the fertility input. Probably, development of high yielding cultivars, low in cyanogens potential (<10mg/kg) will give cassava genotypes tolerance attribute to *M. progresivus* and solve yield loss in most farms in Kenya especially in the low humid regions of Kenya.

8.3 Recommendations

The recommendations issuing from the present study results are that farmers have various options for implementation of integrated *M. progresivus* management options.

The first choice is implementation of compatible management components like fertilizer / manure input in the presence of predacious mites, *E. fustis* and *T. aripo* in the cool and warm zones (UM2-4, LM1-2and CL2-3), respectively.

The option for the low dry midlands (LM4-5) is fertilizer /manure input and acaricide spray with the latter carried out during the dry months spell, before next rain season. Breeding programs would benefit farmers if they develop low cyanogens varieties of least attraction of *M. progresivus*. Environmental and social safe guard protocols for chemical use by farmers would need to be disseminated through extension of the Ministry of Agriculture.

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 APPENDICES

APPENDIX I

SURVEY FOR CASSAVA PHYTOPHAGOUS AND PREDACIOUS MITES (QUESTIONNAIRE)

1.0 BACKROUND

Sample site No………..Farmer's Name……………. Region (Province)………………

District………………..Division………………….. Location…………………………..

Sublocation……………..Village……………Agro-ecozone…………………………..

Altitude………………..GPS………………… Farm size……………………………

Cassava variety…………….. Time crop planted………………… Recorder…………..

Date…………………………..

2.0 LAND USE:

2.1 How much is your farm under cassava cultivation

Local variety………………….acres

Improved variety……………..acres

2.2 Which of the livestock enterprise do you have in your farm;

- Poultry…………………..
- Dairy/Beef cattle..............
- Goats……………………
- Sheep……………………
- Pigs…………………………
- Donkey…………………..
- Others (Specify)…………

3.0 CASSAVA CROPPING TYPE

3.1 Do you grow cassava crop pure or intercropped?

- Pure………………….

- Maize intercrop…….

- Cowpea intercrop…..

- Sorghum intercrop…..

- Other (Specify)………

4.0 FERTILIZER / MANURE USE

4.1 What type of fertilizer do you apply on cassava crop?

Type of fertilizer / manure / pulp / foliar	Month	Rates
A)		
B)		
C)		
D)		
E)		
F)		

5.0 DISEASES AND PESTS CONTROL ON CASSAVA

5.1 Diseases Control

5.1.1 Do you experience any disease problems?

(   ) YES

(   ) NO

If yes, which ones?

(a)……………..

(b)…………..

(C)………….

5.1.2 List down any pesticide (fungicide) you use to control any of the diseases

Pesticide (fungicide) Type Applied	Months	Rates
1)		
2)		
3)		
4)		
5)		

5.2 PEST CONTROL

5.2.1 Do you experience any pest problem on cassava crop?

( ) YES

( ) NO

If yes, which ones;

(a)……………….

(b)…………………

(c)………………..

(d)………………..

5.2.2 Do you apply insecticides (any pesticides) to control any of the pests?

( ) YES

( ) NO

If yes, which insects do you apply?

Insecticide	Month	Rates
1)		
2)		
3)		
4)		
5)		

5.2.3 Apart from insecticide (pesticide) which control method do you apply?

Method	Insect pest controlled	Comment on results
1)		
2)		
3)		
4)		

6. 0 WEED CONTROL

6.1 Do you experience any weed problem in your field?

( ) YES

( ) NO

If yes, which ones;

1)……………………………..
2)………………………………
3)……………………………..
4)………………………………

6.2 Do you apply herbicides to control weeds in the field?

(   ) YES

(   ) NO

If yes, which herbicides do you apply?

Herbicide	Month(s)	Rate
1)		
2)		
3)		
4)		

APPENDIX II

Cassava pests score-sheet per leaf and number of predatory mites on cassava apex

Site:………………………………… Date:………………………..

Plant No.	No. spider mite CGM / leaf	Damage score (1,2,3,4,5)	Other Pests / diseases (Specify)	Status: (%) field incidence	Phytoseiids / sample or / plant apex
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					
16					
17					
18					
19					
20					
21					
22					
23					
24					
25					
26					
27					
28					
29					
30					
CV%					
ŷ± sd					
Lsd (0.05)					

APPENDIX III

Publications

Present work Journal Articles

1. D. L. Mutisya, E. M. El Banhawy, C. W. Kariuki C. P. M. Khamala K.K.M. Fiaboe, Kungu, M.M. (2012). Effect of cassava green mite, *Mononychellus progresivus* on the development and reproduction of the introduced predatory mite, *Phytoseiulus longipes* (Acari: Phytoseiidae) at different temperatures. *Systematic and Applied Acarology*, *17(4): 378-383.*

2. D. L. Mutisya, C. P. M. Khamala, E.M. Banhawy, C. W. Kariuki. (2013). Cassava variety tolerance to spider mite attack in relation to leaf cyanide level. *Journal of Biology and Healthcare, 3 (5): 45-51*

3. D. L. Mutisya, E.M. Banhawy C. P. M. Khamala, C. W. Kariuki., D.W. Miano (2014). Determination of damage thresholds of cassava green mite (Acari: Tetranychidae) on different cassava varieties. *Journal of Plant and Pest Science, 1 (2): 79-86.*

4. D. L. Mutisya, E.M. Banhawy, C. P. M. Khamala, C. W. Kariuki, D. Odongo, A. Owiti.   (2014). Cassava green mite phylogenetic diversity from three geographical sites in Kenya. *Journal of Biodiversity & Environmental Sciences (JBES), 5(4): 504-510.*

5. D. L. Mutisya , El-Banhawy, E. M; Kariuki, C. W, Khamala C.P.M. 2014 *Typhlodromalus aripo* De Leon (Acari: Phytoseiidae) development and reproduction on major cassava pests at different temperatures and humidities: an indication of mite resilience. Acarologia

6. D. L. Mutisya, E. M. El-Banhawy, C. P. M. Khamala, C. W. Kariuki. 2015 Management of cassava green mite *Mononychellus progresivus* (Acari: Tetranychidae) in different agro-ecological zones of Kenya. Systematic & Applied Acarology

Under review

D. L. Mutisya, E. M. El-Banhawy, C,W. Kariuki, C. P.M. Khamala, A. Owiti. Mite species diversity in Kenya: phylogenetic divergence comparison of two phytoseiids inhabiting cassava infer diet and ecological preferences. Elsevier (Biological Control)

Published conference proceedings of the present work

1. **Mutisya,** D. L, E. M. Banhawy, C. P. M. Khamala, C.W Kariuki (2012). Casssava production in different agro-ecological zones in Kenya: The potential for higher yield on manure and inorganic fertilizer input. 13th KARI-Biennial proceedings, 22-26th Oct. 2012, Nairobi, Kenya.

2. **Mutisya,** D. L., C. P. M. Khamala, C. W. Kariuki and E. M. El Banhawy (2013). Influence of soil fertility and physico-chemical factors on pests and diseases on cassava in different agro-ecological zones. Proceedings of '*Linking Environmental Research to Kenya's Development Agenda 2030'.* 1st Technical University of Kenya Conference. 9-12th April, 2013.

3. **Mutisya,** D. L. Khamala, C. P. M., Kariuki, C. W., El Banhawy, E. M. (2013). Influence of agro-climatic conditions and fertilizer use on different pest mite management options in cassava production. In: Transforming rural livelihoods in Africa: How can land and water management contribute to enhanced food security and address climate adaptation and mitigation". The 27th Soil Science Society of East Africa and 6th Africa Soil Science Society Conference, Nakuru, Kenya 21st- 25th October 2013. 22pp.

4. **Mutisya, D.** L., El- Banhawy, E.M, Khamala, C.P.M, Kariuki, C.W., Molo, R. 2014**.** Determination of damage threshold of *Mononychellus progresivus* (Acari: Tetranychidae on different cassava varieties for improved pest management. Proceedings of Third Cassava Regional Center of Excellence Review and Scientific Conference. On "*Enhancing the competitiveness of the cassava sub-sector through regional collective action in research for development"*. 28th-30th July 2014, Kampala, Uganda, pp86-88.