

**RELATIONSHIP BETWEEN MITES INFESTATION LEVELS IN KENYAN TEA TO  
OVERHEAD VOLATILE ORGANIC COMPOUNDS AND THEIR VARIATIONS WITH  
SEASONS, REGIONS AND SELECTED AGRONOMIC PRACTICES**

**BY**

**JENIPHER AKINYI ODAK**

**A THESIS SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE  
DEGREE OF DOCTOR OF PHILOSOPHY IN CHEMISTRY**

**SCHOOL OF PHYSICAL AND BIOLOGICAL SCIENCES**

**MASENO UNIVERSITY**

**© 2016**

**DECLARATIONS**

I declare that this thesis is my original work and that all sources that I have used or quoted have been acknowledged by means of references.

Jenipher Akinyi Odak .....Date.....  
(PG/PhD 041/2011)

This thesis has been submitted for examination with our approval as Supervisors.

Prof: Philip O. Owuor  
School of Physical and Biological Sciences  
Department of Chemistry  
Maseno University  
P.O. Box 333, Maseno, Kenya.

Sign.....Date.....

Prof: Francis N. Wachira  
Deputy Executive Director, Association for Strengthening Agricultural Research in Eastern and Central Africa  
P.O. Box 765, Entebbe, Uganda.

Sign.....Date.....

Prof: Lawrence O. Manguro  
School of Physical and Biological Sciences  
Department of Chemistry  
Maseno University  
P.O. Box 333, Maseno, Kenya.

Sign.....Date.....

## ACKNOWLEDGEMENT

I express my deepest and profound gratitude to my supervisors, Professors P. O. Owuor, L. A. O. Manguro and Professor Francis N. Wachira, for their keen interest, inspiration, sustained and perfect guidance during the study. My sincere gratitude goes to the Chairman, Department of Chemistry, Maseno University for his constant assistance and allowing me to use the departmental facilities; and to the technical staff of the Department for availing the equipment and materials in time. It is a great privilege to have been associated with International Centre for Insect Physiology and Ecology (ICIPE), Department of Chemistry which is well equipped with modern research facilities. I am thankful to Professor Baldwin I. Torto and Mr. X. Cheseto of ICIPE, (Department of Chemistry) for the training on overhead volatile organic compounds (OVOCs) collection and analyses and availing the facilities for trapping the OVOCs from the tea cultivars. Special thanks go to Tea Research Institute, Kenya Agricultural and Livestock Research Organization (Chemistry and Plant Protection Departments; especially Evelyne Cheramgoi) for providing facilities and conducive working environment during my laboratory work. Special unreserved gratitude goes to my mother Persila Odongo Odak for her sincere prayers and encouragements during the study period. My heartfelt appreciation is extended to my family members for their understanding, support, patience and perseverance. I am indebted to my colleagues for their constant assistance. Finally I sincerely thank God for his grace, which was sufficient all the time.

## **DEDICATION**

This thesis is dedicated to my beloved mother Persila Odongo Odak for her persistent prayers and guidance; and to my family who assisted me in every step of my success.

## ABSTRACT

Tea (*Camellia sinensis*) is a major cash crop in Kenya that suffers economic losses due to *Brevipalpus phoenicis* and *Oligonychus coffeae* infestations during droughts. Pesticides use on tea is prohibited and pests control is through cultural and agronomic practices. Resistance/susceptibility of imported and new cultivars to mites is unknown. Plants release overhead volatile organic compounds (OVOCs) that attract or repel insect pests. Some tea cultivars are preferred while others are resistant/tolerant to mites attack. Indeed even in the susceptible clones, infestations seem to be influenced by seasons, nitrogenous fertilizer rates and region of production. The purpose of the research was to investigate how OVOCs compositions/levels are influenced by seasons, region and the agronomic practices. The study was superimposed on ongoing clonal trials at Kangaita, Kipkebe and Timbilil and fertilizer trial on clone TRFK 6/8 at Timbilil A randomized complete block design was used. Mites were extracted using mite brushing machine and counted under dissecting microscope. OVOCs were trapped by adsorbents then analysed by GC and GC-MS. Mites responses to VOCs were tested on Y-tube olfactometer. There were significant ( $p \leq 0.05$ ) variations in clonal mite infestations. Sixteen clones were resistant/tolerant while five were susceptible to mites. Kangaita recorded higher ( $p \leq 0.05$ ) infestations than Kipkebe and Timbilil. Mites levels directly correlated to maximum temperature ( $r = 0.81$ ,  $P \leq 0.05$ ) and inversely correlated to relative humidity ( $r = -0.73$ ,  $P \leq 0.05$ ) and rainfall ( $r = -0.184$ ,  $P \leq 0.05$ ). The infestations were linearly correlated GLVs and inversely correlated with most aromatics and terpenoids. Susceptible and resistant clones released high amounts of GLVs and both aromatics and terpenoids respectively. More and high levels of OVOCs were released during dry season, declined in rainy and slightly increased during cold season. There were significant ( $p \leq 0.05$ ) variations in mite population due to N- rates. High infestations occurred on 0 and 300 while low levels on 150 - 225 kg N/ha/year. All GLVs increased while some aromatic and terpenoid compounds decreased with increase in N- rates. Mites were significantly ( $P \leq 0.05$ ) attracted by GLVs and repelled by aromatics and terpenes. These results indicate that high levels of GLVs (in dry season and at high fertilizer rates) predispose cultivars to mites infestations while aromatics and terpenes (in resistant cultivars and at recommended Kenyan fertilizer rate) induce resistance. Resistant cultivars are recommended for commercial exploitation in mite prone areas while breeding/selection programmes should incorporate OVOCs profiles to develop tea cultivars that resist mites attack.

## TABLE OF CONTENTS

TITLE PAGE.....	i
DECLARATION PAGE.....	ii
ACKNOWLEDGEMENT.....	iii
DEDICATION.....	iv
ABSTRACTS.....	v
TABLE OF CONTENTS.....	vi
LIST OF ABBREVIATIONS AND ACRONYMS.....	ix
DEFINITION OF TERMS .....	x
LIST OF TABLES.....	xiii
LIST OF FIGURES.....	xv
LIST OF APPENDICES.....	xvii
<b>CHAPTER ONE: INTRODUCTION.....</b>	<b>1</b>
1.1 Background to the study.....	1
1.2 Statement of the problem.....	9
1.3 Broad study objectives.....	10
1.4 Specific Objectives.....	10
1.5 Null hypotheses.....	11
1.6 Justification of the Study.....	11
<b>CHAPTER TWO: LITERATURE REVIEW.....</b>	<b>12</b>
2.1 Mites problems in tea farms.....	12
2.2 Tea clones.....	16
2.3 Pest management in tea farms.....	18
2.4 Plant volatiles.....	21
2.4.1 Green leaf volatiles (GLVs).....	22
2.4.2 Aromatics.....	24
2.4.3 Terpenes.....	27
2.4.3.1 Monoterpenes.....	29

2.4.3.2	Homoterpenes.....	31
2.4.3.3	Sesquiterpenes.....	32
2.4.4	Variability in the volatile organic compounds profiles in susceptible and resistant plant cultivars.....	35
2.4.5	The influence of seasons and region of production on the composition or levels of volatile organic compounds in relation to insect pests attack.....	38
2.4.6	Effect of nitrogenous fertilizer application rates on VOCs in relation to pests attack.....	41
2.4.7	Plant volatiles, insect pests attraction and/or repellency.....	42

**CHAPTER THREE: METHODOLOGY.....45**

3.1	Area of study.....	45
3.2	Meteorological data collection.....	45
3.3.1	Cultivar evaluation (Mites).....	46
3.3.2	Cultivar evaluation (OVOCs).....	47
3.4	Influence of rates of nitrogenous fertilizer application on OVOC levels or composition in relation to mites infestations.....	47
3.5	Volatile analysis and identification.....	48
3.6	Behavioral responses of mites to trapped volatile organic compounds.....	49
3.6.1	Collection of mites and rearing.....	49
3.6.2	Odour sources.....	50
3.6.3	Behavioral responses of mites in the Y-tube olfactometer.....	50
3.7	Statistical Analyses.....	51

**CHAPTER FOUR: RESULTS AND DISCUSSIONS.....52**

4.1	Determination of mites infestations levels in the imported and new clones in clonal adaptability trials and their characterization as resistant or susceptible to mites....	52
4.2	Determination of the contents and variations in the overhead volatile organic compounds (OVOCs) in selected susceptible and tolerant clones and their relationship with mites attack.....	65
4.2.1	Susceptibility/resistance of the selected tea cultivars to mites.....	65
4.2.2	Identification of volatile organic compounds emitted by the cultivars using GC-MS.....	69

4.2.3	Variations in volatile organic compounds emitted by the selected cultivars and their relationship with mites infestations.....	71
4.3	Influence of seasons and region of production of tea varieties on the composition or levels of the volatile organic compounds in relation to mites attack.....	83
4.4	Effect of nitrogenous fertilizer rates on the overhead volatile organic compounds composition or levels in relation to mites attack.....	124
4.5	Responses of red spider mites (RSM) and red crevice mites (RCM) to volatile organic compounds associated with <i>Camellia cinencis</i> .....	133
 <b>CHAPTER FIVE: SUMMARY, CONCLUSIONS, RECOMMENDATIONS AND SUGGESTIONS FOR FURTHER STUDIES.....</b>		<b>138</b>
5.1	Summary.....	138
5.2	Conclusions.....	139
5.3	Recommendations.....	140
5.4	Suggestions for further studies.....	141
<b>REFERENCES.....</b>		<b>143</b>
<b>APPENDIX.....</b>		<b>172</b>



## LIST OF ABBREVIATIONS AND ACRONYMS

Amsl	Above mean sea level
CDP-ME	4-Diphosphocytidyl-2-C-methyl -erythritol
CDP-ME <sub>2</sub> P	4-(Cytidine-5 -diphospho)-2-C-methyl-D-erythritol phosphate
DAHP	3-Deoxy-D-arabino-heptulosonate-7-phosphate
DMAPP	Dimethylallyl diphosphate
DMNT	Dimethyl-1, 3, 7-nonatriene
DXP	1-Deoxy-D-xylulose 5-phosphate
EU	European Union
eV	Electron volts
FAO	Food and Agriculture Organization
FPP	Farnesyl diphosphate
GC	Gas chromatography
GLVs	Green leaf volatiles
GPP	Geranyl diphosphate
HIPVs	Herbivore induced plant volatiles
HMBPP	<i>E</i> -4-Hydroxy-3-methylbut-2-enyl diphosphate
HMG-CoA	3-Hydroxy-3-methylglutaryl CoA
ICIPE	International Centre for Insect Physiology and Ecology
IPM	Integrated pest management
IPP	Isopentenyl diphosphate

IUPAC	International Union of Pure and Applied Chemistry
JMT	Jasmonic acid carboxyl methyl transferase
MEP	Methylerythritol pathway
MRL	Maximum residual level
MS	Mass spectrometry
MVA	Mevalonic acid
OVOCs	Overhead volatile organic compounds
PEP	Phosphoenolpyruvate
Phe	Phenylalanine
RCM	Red crevice mite
RSM	Red spider mite
TRFK	Tea Research Foundation of Kenya
TRIT	Tea Research Institute of Tanzania
UV	Ultraviolet light
VOCs	Volatile organic compounds

## **DEFINITIONS OF TERMS**

**Clones/Cultivars/ Genotype:** Tea plants/varieties that are derived from one mother bush by a method of vegetative propagation and maintained through cultivation. They have the same genetic constitution adapted to specific environmental and cultural conditions.

**A semiochemical or inforchemical:** is a generic term used for a chemical substance or mixture that carries a message for purpose of communication

## LIST OF TABLES

Table 1:	European Union MRLs on tea for some pesticides.....	19
Table 2:	Summary of the weather parameters and mites infestation levels in the three sites.....	54
Table 3:	Changes in the dynamics of mites with clones in the three sites.....	55
Table 4:	Changes in the dynamics of red crevice mites with clones and months in Kangaita.....	57
Table 5:	Changes in the dynamics of red spider mites with clones and months in Kipkebe.....	59
Table 6:	Changes in the dynamics of red spider mites with clones and months in Timbilil.....	61
Table 7:	Correlation coefficients (r) between weather parameters and mite population in the three sites.....	64
Table 8;	Changes in the dynamics of mites with selected clones and months in the three sites.....	67
Table 9:	Compounds identified, their retention time and IUPAC names.....	74
Table 10a:	Changes in mite infestations and overhead green leaf volatile compounds (GLVs) and their relationships in tea cultivars in Kangaita during dry season.....	78
Table 10b:	Clonal variations and the relationships between mites infestations and overhead volatile aromatic compounds in Kangaita during dry season.....	79
Table 10c:	Changes in and relationships between clonal overhead monoterpenes and mites levels in Kangaita during dry season.....	80
Table 10d:	The variations and relationship between sesquiterpenes released by 11 tea varieties and mite infestations levels in Kangaita during dry season.....	81
Table 11a:	Changes in mite infestations and overhead green leaf volatile compounds (GLVs) and their relationships in tea cultivars in Kangaita during rainy season.....	88

Table 11b:	Clonal variations and the relationships between mites infestations and overhead volatile aromatic compounds in Kangaita during rainy season.....	89
Table 11c:	Changes in and relationships between clonal overhead monoterpenes and mites levels in Kangaita during rainy season.....	90
Table 11d:	The variations and relationship between sesquiterpenes released by 11 tea varieties and mite infestations levels in Kangaita during rainy season.....	91
Table 12a:	Changes in mite infestations and overhead green leaf volatile compounds (GLVs) and their relationships in tea cultivars in Kangaita during cold season.....	92
Table 12b:	Clonal variations and the relationships between mites infestations and overhead volatile aromatic compounds in Kangaita during cold season.....	93
Table 12c:	Changes in and relationships between clonal overhead monoterpenes and mites levels in Kangaita during cold season.....	94
Table 12d:	The variations and relationship between sesquiterpenes released by 11 tea varieties and mite infestations levels in Kangaita during cold season.....	95
Table 13a:	Changes in mites infestations and overhead green leaf volatile compounds (GLVs) and their relationships in tea cultivars in Kipkebe during dry season.....	97
Table 13b:	Changes in and relationships between clonal overhead aromatics and mites levels in Kipkebe during dry season.....	98
Table 13c:	Changes in and relationships between clonal overhead monoterpenes and mites levels in Kipkebe during dry season.....	99
Table 13d:	The variations and relationship between sesquiterpenes released by 11 tea varieties and mite infestations levels in Kipkebe during dry season.....	100
Table 14a:	Changes in mite infestations and overhead green leaf volatile compounds (GLVs) and their relationships in tea cultivars in Kipkebe during rainy season.....	101
Table 14b:	Clonal variations and the relationships between mites infestations and overhead volatile aromatic compounds in Kipkebe during rainy season.....	102

Table 14c:	Changes in and relationships between clonal overhead monoterpenes and mites levels in Kipkebe during rainy season.....	103
Table 14d:	The variations and relationship between sesquiterpenes released by 11 tea varieties and mite infestations levels in Kipkebe during rainy season.....	104
Table15a:	Changes in mite infestations and overhead green leaf volatile compounds and their relationships in tea cultivars in Kipkebe during cold season.....	105
Table 15b:	Clonal variations and the relationships between mites infestations and overhead volatile aromatic compounds in Kipkebe during cold season.....	106
Table 15c:	Changes and relationships in clonal overhead monoterpenes and mites levels in Kipkebe during cold season .....	107
Table15d:	The variations and relationship between sesquiterpenes released by 11 tea varieties and mite infestations levels in Kipkebe during cold season.....	108
Table16a:	Changes in mite infestations and overhead green leaf volatile compounds (GLVs) and their relationships in tea cultivars in Timbilil during dry season.....	111
Table16b:	Clonal variations and the relationships between mites infestations and aromatic OVOCs in Timbilil during dry season.....	112
Table 16c:	Changes in and relationships between clonal overhead monoterpenes and mites levels in Timbilil during dry season.....	113
Table 16d:	The variations and relationship between sesquiterpenes released by 11 tea varieties and mite infestations levels in Timbilil during dry season.....	114
Table 17a:	Changes in mite infestations and overhead green leaf volatile compounds (GLVs) and their relationships in tea cultivars in Timbilil during rainy season.....	115
Table 17b:	Clonal variations and the relationships between mites infestations and overhead volatile aromatic compounds in Timbilil during rainy season.....	116
Table 17c:	Changes in and relationships between clonal overhead monoterpenes and mites levels in Timbilil during rainy season.....	117

Table 17d:	The variations and relationship between sesquiterpenes released by 11 tea varieties and mite infestations levels in Timbilil during rainy season.....	120
Table 18a:	Changes in mite infestations and overhead green leaf volatile compounds (GLVs) and their relationships in tea cultivars in Timbilil during cold season.....	121
Table 18b:	Clonal variations and the relationships between mites infestations and overhead volatile aromatic compounds in Timbilil during cold season.....	122
Table 18c:	Changes in and relationships between clonal overhead monoterpenes and mites levels in Timbilil during cold season.....	123
Table 18d:	The variations and relationship between sesquiterpenes released by 11 tea varieties and mite infestations levels in Timbilil during cold season.....	124
Table 19a:	Effect of nitrogenous fertilizer rates on the emissions of green leaf volatile organic compounds in relation to mites levels.....	128
Table 19b:	Influence of nitrogenous fertilizer rates on the emissions of aromatic compounds in relation to mites levels.....	129
Table 19c:	Effect of nitrogenous fertilizer rates on the emissions of monoterpenes in relation to mites levels.....	130
Table 19d:	Effect of nitrogenous fertilizer rates on the emissions of sesquiterpenes in relation to mites levels.....	132
Table 20:	Response of red spider mites and red crevice mites to various classes of VOCs.....	134

## LIST OF FIGURES

Figure1:	Red crevice mite.....	13
Figure 2:	Tea leaf attacked by red crevice mite.....	13
Figure3:	Red spider mite.....	15
Figure 4:	Tea leaves turn brown and defoliate due to red spider mites attack.....	15
Figure: 5	Biosynthesis of green leaf volatiles.....	23
Figure 6:	Biosynthesis of aromatics.....	25
Figure: 7	Biosynthesis of GPP from acetyl coenzyme A and FPP from pyruvate and glyceraldehyde-3-phosphate.....	27
Figure 8:	Biosynthesis of monoterpenes.....	30
Figure 9:	Biosynthesis of sesquiterpenes.....	33
Figure: 10	Biosynthesis of diglycoside, the form in which VOC is stored in tea leaves...35	
Figure 11:	Y-tube olfactometer set up.....	50
Figure 12:	Mass spectrum of cedrol in the headspace of <i>Camellia sinensis</i> .....	69
Figure 13:	Fragmentation pattern of cedrol.....	70



## LIST OF APPENDICES

<b>Appendix I:</b> Typical gas chromatogram for OVOCs from <i>Camellia sinensis</i> .....	174
<b>Appendix II:</b> Compounds identified, their retention time (RT) and IUPAC names.....	175

## CHAPTER ONE: INTRODUCTION

### 1.1 Background to the study

Tea *Camellia sinensis* (L) O. Kuntze, is one of the major agricultural commodities traded globally and the most popular beverage, being the second largest drink consumed in the world after water (Gardner, Ruxton & Leeds, 2007). In Kenya, tea is a major cash crop and the leading export crop, foreign exchange earner and source of livelihood (Anonymous, 2011). The crop is grown in east and west of Rift Valley in Kenya with distinct seasons which are classified as cold and wet (March to May); cold and dry (June to August) and warm and dry (December to February) (Ng'etich, Stephens & Othieno, 1995; Ngetich & Stephens, 2001). The growth of tea industry and subsequent increase in export of processed tea from the principal tea growing areas of the world such as India, China, Kenya, Sri Lanka, Taiwan, Nepal and Japan has been attributed to use of high yielding varieties and appropriate agronomic inputs. Despite the use of high yielding varieties and appropriate agronomic inputs, in some regions high yields are not attained due to insect pests infestations. Insects particularly mites are the most damaging pests, causing approximately US \$500 million to \$1 billion yield losses annually (Agnihotrudu, 1999) and in some cases, yield losses can be up to 100% (Muraleedharan & Chen, 1997).

Among the several mites species, red crevice (*Brevipalpus phoenicis* Geijskes) and red spider (*Oligonychus coffeae* Nietner) mites are serious pests of tea that cause economic yield losses during drought (Ahmed, Chowdhury, Haque & Mamum, 2012; Kumara, Babub, Rahmana & Roobakkumara, 2011). These mites have been recorded in all tea growing areas in Kenya in low to moderate infestations (Sudoj, 1991). Although tea in Kenya has been relatively pest free compared to, for example, tea in India or Sri Lanka, serious outbreaks have sporadically been reported. Sporadic yield losses caused by mites have been estimated to be about 50%, especially during prolonged drought in all growing areas in Kenya, the

situation being serious around the Mount Kenya region (Sudoj, Khaemba & Wanjala, 1994). The mites have the ability to multiply rapidly during hot and dry season with short life cycles, enabling a rapid build-up of their population (Roy, Muraleedharan & Mukhopadhyay, 2014; Sudoj, 1997). Climatic factors such as temperature, rainfall and relative humidity influenced mites infestation levels in Bangladesh (Ahmed et al., 2012). Distinct seasons occur in the tea growing regions in Kenya (Ng'etich et al., 1995; Ngetich et al., 2001) with distinct variations in temperatures, rainfall and relative humidity. It is not known if these climatic factors could lead to mites odour cues that could be responsible for the differences in mites attack to tea in the different parts of Kenya at different times of the year.

The tea improvement programme in Kenya is achieved through breeding and selection of cultivars for high yields, acceptable quality and tolerance to biotic and abiotic stresses including pests infestations (Kamunya et al., 2010c) . Significant genetic variability exists among tea cultivars (Banerjee, 1987; Ghosh, 2001) to which pests react differentially. For example, certain tea clones are susceptible to mites (STC 5/3, AHPCG28U864, TRFK 303/259, TRFK 55/56 and TRFK 54/40) while others exhibit resistance/tolerance (EPK C/12, TRFK 303/1199 and TRFK 6/8) (Sudoj et al., 1994; Sudoj, 1997; Sudoj et al., 2011a). The Kenya tea industry imported some tea varieties from Tanzania (Anonymous, 2008); in addition, the industry has developed clones with potential for commercial exploitation. These clones are being evaluated for suitability in different regions at Timbilil, Kipkebe and Kangaita (Kamunya, Chalo, Korir, Kiplang'at & Wachira, 2010a) before their release to farmers. These clones have not been evaluated for tolerance/resistance or susceptibility to mites and it is not known if such tolerance/resistance or susceptibility varies with location of production and time of the year.

Tea is grown in different ecological zones, with wide variations in biotic and abiotic stresses (Owuor, Obanda, Nyirenda, Mphangwe, Wright & Apostolides, 2006) including

pests infestations. As a result there have been measures to control pests (Borthakur et al., 2005; Elmoghazy et al., 2011; Mamun and Ahmed, 2011; Roy et al., 2014; Gurusubramanian, 2005). Synthetic pesticides are effective in controlling pests in tea farms especially during their outbreak (Roy et al., 2014). However, prolonged and extensive use of pesticides results in negative effects such as destruction of natural enemies of pests, development of resistance in insect pests and environmental pollution. Besides, undesirable pesticide residues in made tea may occur which may be above the maximum residual level (MRL) that make tea illegal to sell in the international markets (Elmoghazy, El-Saiedy & Romeih, 2011). Pesticide residues in tea are a serious concern globally, necessitating WHO and European Union to set maximum residue limits (MRLs) for pesticides and several countries are revising the MRLs downward (FAO, 2014). Tea being the most popular beverage and is the second largest drink consumed in the world next to water (Gardner et al., 2007) is monitored continuously of any pesticide residues. Various international agencies have fixed the MRL values for tea growing countries, a fact that has affected the trade of tea (FAO, 2014). Pruning is the main cultural practice used that affects the intensity of outbreaks, as it removes a large part of the foliage and stem harboring the mites (Gurusubramanian, 2005). The mites that remain on the few old leaves of the tea bushes multiply rapidly with the rise in temperature resulting in subsequent heavy infestations (Ahmed et al., 2012). Biopesticides such as pyrethrins are attractive for pest management because they are environmentally safe, selective, biodegradable, economical and renewable (Mamun & Ahmed, 2011) but they are slow acting and labile in sunlight a fact that has limited their use outdoors (Antonious, 2004). Use of natural enemies in biological control suppresses pest population and prevents the pest from attaining critical level (Borthakur, Rahman, Sarmah & Gurusubramanian, 2005). However, key pests are many and varied; this requires use of several natural enemies in combination, a strategy that is difficult and expensive to apply

(Akio & Hiroshi, 2001). Integrated pest management (IPM) method uses a combination of techniques including use of resistant varieties (Roy et al., 2014). For the Kenya tea industry, use of pesticides on tea is prohibited (Anonymous, 2002; Othieno, 1988) and control of pests is strictly through use of cultural practices including use of resistant/tolerant tea cultivars and agronomic inputs that deter pests attack (Sudo, Cheramgoi, Langat & Wachira, 2011; Sudo et al., 1994; Sudo, 1996). Resistant/tolerant clones have been selected over susceptible varieties as part of IPM in the management of mites in tea farms (Sudo et al., 2011; Sudo et al., 1994; Sudo, 1996). However, little is known on the biochemical substances released by tea varieties that make them resistant or susceptible to mites.

An alternative method to reduce pest pressure is to identify key plants that deter or repel the herbivores (Morley, Finch & Collier, 2005). Plants use indirect defences such as overhead volatile organic compounds (OVOCs) to repel herbivores or attract predators and parasitoids of the herbivores feeding on them (Penaflo, Erb, Miranda, Werneburg & Bent, 2011). VOCs are plant secondary metabolites that have no fundamental role in the maintenance of life processes in the plants, but are important for the plant to interact with its environment for adaptation and defense (Ramakrishna & Ravishankar, 2011). The VOCs are released from four major biosynthetic pathways, namely the shikimate, the mevalonic acid, the methylerythritol phosphate and lipooxygenase that release aromatic, mono-, homo- and sesquiterpenoids and green leaf volatile compounds, respectively. Phytophagous insects recognize their hosts by the specific VOC/mixture which is used to decide whether the plants are suitable for feeding (Najar-Rodriguez, Bellutti & Dorn, 2013). For example, *Bemisia tabaci* preferred cultivated tomato varieties over wild tomatoes, which was attributed to high levels of the monoterpenes being released by wild tomato plants (Simmons, Gurr & Trichomes, 2005). Caterpillars performed best on the highest-yielding variety that showed reduced induction of volatile sesquiterpenes compared to ancestral varieties suggesting that

breeding in cranberry compromised plant defences (Rodriguez-Saona et al., 2011). Field evaluation of gerbera cultivars showed that the cultivar with lower relative production of terpenes exhibited the highest mite intrinsic rate of population increase (Krips, Willems, Gols, Posthumus, Gort & Dicke, 2001) . Zhang *et al* (Zhang, Wang, Lin, Lu & Luo, 2015) observed that the chemical characteristics of the plant species that repelled *Chlorophorus caragana* and did not suffer from damage in the field mainly consisted of terpenes. Aromatic compounds have also been implicated in plant defence against insect pests (James & Price, 2004; Zhu & Park, 2005)). Flea beetles (*Epitrix hirtipennis*) were more abundant on green leaf volatile (GLV) -producing wild type plants compared to plants with reduced hydroperoxide lyase activity (Halitschke, Stenberg, Kessler, Kessler & Baldwin, 2008). Similarly, *Uschistus heros* preferred soybean pods that released high amounts of GLVs for feeding and oviposition over deficient cultivars (Silva, O-Panizzi, Blassioli-Moraes & Panizzi, 2013). Tea plant produces OVOCs belonging to monoterpenes, sesquiterpenes, homoterpenes, GLVs and aromatics (Han, Zhang & Byers, 2012) which have been implicated in plants defense mechanism against pests (Bruce, Midega, Birkett, Pickett & Khan, 2010; Hägg , Zagrobelny & Bak, 2013; Khan, Midega, Amudavi, Hassanali & Pickett, 2008; Scala, Allmann, Mirabella, Haring & Schuurink, 2013). It has not been established if variability in the OVOCs composition or levels exists among the different tea cultivars and if such differences in OVOCs contribute to susceptibility/resistance of tea cultivars to red spider and red crevice mites.

Variations in mites infestation levels with temperature, rainfall and relative humidity (Ahmed et al., 2012) as well as region of production (Sudoj et al., 2011) have been reported. These patterns of variations in attack could be related to herbivore-plant interactions that are influenced by the plant defence mechanisms. Plants produce and emit metabolites that influence predators attack or repulsion (Pare & Tumlinson, 1999). The changes in mite

infestations could in part be related to the variations in the levels of the OVOCs emitted by the tea cultivars. Both biotic and abiotic factors affect the emissions of these plant metabolites. Leaves normally release small quantities of volatile chemicals, but when a plant is damaged by herbivorous insects, more volatiles are released (Pare & Tumlinson, 1997). Plant emission of VOCs changes with weather conditions (Cheng, Cheng & Chang, 2010). Dry weather conditions favour the biogenesis of VOCs (Rawat & Gulati, 2008). For example, lipoxygenase activity responsible for production of green leaf volatile compounds (Pare et al., 1999; Robinson & Owuor, 1992), increases during water deficit periods (Sofo, Dichio, Xiloyannis & Massia, 2004). Lower soil moisture content experienced in dry season reduces photosynthesis and growth of plants (Coley, 1998). Irradiation effects of higher sunshine during the dry season and increased leaf temperatures (Lin, Chen & Harnly, 2008) increase the biosynthesis of VOCs (Hartikainen et al., 2012; Rawat et al., 2008). High humidity and warm weather conditions during rainy season favour faster growth (Coley, 1998; Kannaste, Pazouki, Suhhorutsenko, Copolovici & Niinemets, 2013) accompanied with lower dry matter accumulation per shoot volume (Ng'etich, Stephens & Othieno, 2001; Ngetich et al., 2001) with low enzyme activity (Muthumani, Verma, Venkatesan & Senthil Kuman, 2013) thus reducing the biosynthesis of secondary metabolites. Cold season is marked with scanty rainfall, low temperatures and moderate humidity. Low temperatures cause slow shoot growth rates and favours longer accumulation of secondary metabolites (Rawat et al., 2008). The variations and relationship between mites infestations and OVOCs with seasons have not been assessed.

Tea in Kenya is grown in altitudes ranging from 1300 m to 2700 m amsl (Owuor, 1999). Geographical conditions influence VOCs biogenesis in plants (Tura, Failla, Bassi, Attilio & Serraiocco, 2013), for example, average air temperature, rainfall (both distributions and amounts), sunshine hours and cloud cover affects tea growth (Kumara, Bisen, Choubey,

Singh & Bera, 2015; Squire, 1978; Squire, 1979) and vary with geographical locations. But in Kenya, apart from the variations in rainfall distribution in the east and west of the Rift Valley, other variations in climatic factors are minimal (Owuor et al., 2011). Teas grown at higher elevations produce larger amounts of VOCs (Mahanta, Baruah, Owuor, Murai, Horita & Tsushida, 1988; Owuor, Obaga & Othieno, 1990b). Similarly, same clone grown in different locations varies in chemical composition (Kwach, Kamau, Msomba, Muhoza & Owuor, 2014; Kwach, Kamau, Owuor, Wanyoko, Msomba & Muhoza, 2011; Kwach, Owuor, Kamau, Msomba & Uwimana, 2016; Kwach, Owuor, Kamau, Wanyoko & Kamunya, 2013; Owuor, Horita, Tsushida & Murai, 1987a; Owuor et al., 2011; Owuor, Tsushida, Horita & Murai, 1988). It is not documented if the differences in VOCs composition/levels are responsible for the differences in mites levels in east and west of Rift Valley (Sudoj et al., 2011; Sudoj et al., 1994).

Nitrogenous fertilizer application is the second most expensive agronomic input in tea production (Ruto, Wanyoko & Othieno, 1994) after harvesting. Application of nitrogenous fertilizer to tea is mandatory since it enhances plant vigour (Sudoj, 1991; Sudoj, 1993; Sudoj, Wanyoko, Owuor & Lang'at, 2001b) and increases yield by promoting vegetative growth and shoot density (Atijegbe, Nuga, Lale & Nwanna, 2013; Sarwar, Ahmad, Hamid, Khan & Khurshid, 2007) thus making tea production an economic venture through increased yields (Msomba, Kamau, Uwimana, Muhoza & Owuor, 2014; Owuor, Kamau, Kamunya, Msomba, Jondiko & Uwimana, 2013a). Indeed application of nitrogenous fertilizers (NPKS 25: 5: 5:5) to tea at rates between 150 and 200 Kg N/ha/year, increased plant vigour and induced tolerance to attack by the mites, but rates above 400 Kg N/ha/year encouraged buildup of mites (Sudoj, 1991; Sudoj, 1993). Similar observations were made by Atijegbe *et al* (Atijegbe et al., 2013) who reported that the higher the fertilizer application rates (as N.P.K 15.15.15) the greater the vegetative growth and the higher the incidences of insect pest species.



Nitrogen fertilization rate had a significant effect on the emissions of VOCs and the overall odour blend of the compounds in corn (Sandrine, Gouinguene & Turlings, 2002). Increasing nitrogen fertilizer application rates increased the concentrations of GLVs in *Brassica napus* (Veromann et al., 2013) and processed black tea (Owuor, Ng'etich & Obanda, 2000; Owuor, Othieno, Horita, Tsushida & Murai, 1987d; Owuor, Othieno, Odhiambo & Ng'etich, 1997) and their precursors, unsaturated fatty acid levels (Okal, Owuor, Kamau & Manguro, 2012b; Owuor, Munavu & Muritu, 1990a; Owuor, Okal, Kamau, Msomba, Uwimana & Kamunya, 2013c) but decreased the levels of aromatic and terpenoid compounds in black tea (Owuor et al., 2000). However, such studies were conducted on black tea that had undergone many biochemical transformations during processing. Levels of the OVOCs in tea have not been evaluated, although there appears to be a relationship between nitrogen fertilizer application rates, OVOCs emissions and insect pest incidences as observed in *Brassica napus* (Veromann et al., 2013).

While much is known about how volatile organic compounds influence quality of tea varieties (Obanda & Owuor, 1995; Owuor et al., 1987a; Owuor, Obanda, Othieno, Horita, Tsushida & Murai, 1987b; Owuor & Odhiambo, 1993; Owuor & Orchard, 1990c; Owuor, Othieno, Horita & Tsushida, 1987c; Robinson et al., 1992) due to location of production (Horita & Owuor, 1987; Owuor, Obanda, Nyirenda & Mandala, 2008a; Owuor et al., 1988), agronomic and processing (Owuor & Obanda, 1996a; Owuor, Orchard & McDowell, 1994; Takeo, 1984) practices, little is known on emissions of OVOCs from tea varieties and particularly on their specific variability in relation to mite infestations levels despite key infochemicals eliciting strong olfactory responses to mites (De Boer, Snoeren & Dicke, 2005; Dicke, 1986; Ishiwari, Suzuki & Maeda, 2007; Shamoda, 2010). There is no information on the responses of red spider and red crevice mites to the VOCs associated with tea plants,

whether VOCs are responsible for the susceptibility or resistance of tea clones to the two mites species.

## **1.2 Statement of the problem**

Tea infestations by mites especially red spider and red crevice mites reduce yield. Some tea cultivars are resistant/tolerant while others susceptible to the infestations. The newly developed and imported clones from Tanzania in clonal adaptation trials in Kangaita, Timbilil and Kipkebe have not been evaluated for susceptibility or resistance/tolerance to mites infestations. There is need to characterize the clones as resistant/susceptible to mites to enable release of the clones for commercial exploitation. Although clones vary in the levels of resistance/susceptibility, the causes of variations have not been identified. Yield reduction caused by mites infestations can be large. Control measures are therefore necessary during such heavy infestations. Use of organosynthetic pesticides to control mites may lead to undesirable pesticide residues in made tea which may be above the maximum residual level (MRL) leading to rejection in the international markets. With the present growing international markets' concern on the health hazards posed by toxic residues in tea, there is need for alternative methods of insect pests' control. Botanical, biological and cultural methods of pests control equally have drawbacks. There is need for selection criteria for resistant clones over susceptible ones. It has not been evaluated if OVOCs composition can be an alternative method of selecting tea for mites resistance/tolerance.

Variations of VOCs with seasons, clones, and region of production in processed tea, and mites attack on tea have been reported. Higher mites infestation of tea occurs in the east than the west of Rift Valley in Kenya during warm and dry seasons. It is not documented if there is seasonal variations in overhead VOCs and if such possible variations change with clones, region of production and seasons. Also it is not known if the mites dynamics are

related to the composition of overhead VOCs. Application of nitrogenous fertilizers (200 Kg N/ha/year) to tea induce tolerance to mites but rates above 400 Kg N/ha/year encourage the buildup of mites on tea. Factors responsible for this have not been established. However, fertilizer varying rates influence volatile components of made tea. The effect of nitrogenous fertilizer rates on OVOCs composition of clonal tea in relation to mites' infestation has not been evaluated. Similarly, the response of red spider and red crevice mites to VOCS associated with *C.sinensis* are unknown.

### **1.3 Broad Study Objective**

The broad objective was to evaluate mites infestation levels in the imported and newly developed clones and investigate how overhead volatile organic compounds compositions or levels are influenced by tea agronomic practices and to relate the OVOCs levels and composition to mites infestations.

### **1.4 Specific Objectives**

1. To determine red spider and red crevice mites infestation levels in the forty-five (new, imported and commercial) clones in Kangaita, Kipkebe and Timbilil and characterize the clones as resistant or susceptible to mites.
2. To determine the levels and variations in the overhead volatile organic compounds (OVOCs) in infested and resistant clones.
3. Establish the influence of seasons and region of production of tea varieties on the composition or levels of the volatile organic compounds and relate seasonal OVOCs levels or composition to mites attack.
4. To determine if nitrogenous fertilizer rates affect the OVOCs composition or levels and relate the effect to red spider mites infestations in Timbilil (on clone TRFK 6/8).

5. Establish the response (repellency/attraction) of mites to trapped overhead volatile organic compounds from different tea varieties.

### **1.5 Null hypotheses (H<sub>0</sub>)**

1. There is no difference in levels of mites infested in the imported and new clones in clonal adaptability trials in Kangaita, Kipkebe and Timbilil hence cannot be characterized as resistant or susceptible to mites.
2. There are no variations in OVOCs levels in susceptible and resistant clones and no relationships exist between susceptibility/resistance of tea varieties to mites attack and overhead volatile organic compounds levels or composition.
3. Seasons and regions of production of tea varieties do not influence the composition or levels of OVOCs.
4. Nitrogenous fertilizer rates do not affect the OVOCs composition or levels.
5. Mites are neither attracted nor repelled by trapped overhead volatile organic compounds from different tea varieties.

If the null hypotheses are not realized, then the alternative shall be accepted.

### **1.6 Justification of the Study**

Identification of tea cultivars and agronomic inputs that mitigate mites attack can lead to elimination of the use of pesticides in tea hence reducing cost of production to farmers as well as enhancing acceptability of teas in the international markets and reducing environmental pollution. Knowledge of compounds responsible for susceptibility/resistance can help in the development of safe synthetic chemicals to mimic the natural plant biochemical for use in the management of mites. Identification of mites resistant cultivars for commercial exploitation in mites prone areas to mitigate yield loss due to mites attack

## CHAPTER TWO: LITERATURE REVIEW

Tea *Camellia sinensis* (L) O. Kuntze, belonging to the family *Theaceae*, is grown on over 2.71 million hectares in more than 34 countries across Asia, Africa, Latin America and Oceania (FAO, 2005). In Kenya, tea is grown on the foothills of Aberdare Ranges and Mount Kenya in the east and the Mau Ranges, Nandi, Kisii and Kakamega Hills in the west of the Great Rift Valley at altitudes ranging from 1300 m to 2700 m amsl (Anonymous, 2002; Owuor, 1999). The crop is an important player in the economies of the producing countries as it is a major economic activity creating employment to both skilled and unskilled labour especially in the rural areas where economic activities are scarce. For example, in Kenya, tea industry and its allied activities support directly over 500,000 families each on the average supporting 6 members (Ogola & Kibiku, 2004). Being a perennial monoculture crop *C. sinensis* provides stable microclimate and continuous supply of food for pests. Pest infestations are a major problem in most tea growing areas causing yield losses, with losses up to 100% having been reported (Muraleedharan et al., 1997). Globally, 1031 species of arthropods are associated with the intensively managed *C. sinensis* (Muraleedharan, 1992). The leaf and shoot feeders such as mites are the crucial pests in terms of yield reduction in tea (Zeiss & Braber, 2001) necessitating pest reduction strategies.

### 2.1 Mites problems in tea farms

Several species of mites attack tea, including red crevice or scarlet (*Brevipalpus phoenicis* Geijskes), red spider (*Oligonychus coffeae* Nietner), yellow tea (*Polyphagotarsonemus latus* Bank), purple (*Calacarus carinatus* Green) and bud (*Brevipalpus californicus* Bank) mites (Ahmed et al., 2012; Kumara et al., 2011). Among the several mites species, red crevice and red spider mites are of economic importance in tea causing crop losses in many countries (Ahmed et al., 2012; Sudoi et al., 2011). Although tea

in Kenya has been relatively pests free compared to for example tea in China, India or Sri Lanka, sporadic yield losses caused by mites infestations (especially red crevice and red spider mites) have been estimated to be up to about 50%, during prolonged drought (Sudoj et al., 2011; Sudoj et al., 1994; Sudoj, 1996)

The red crevice mite belongs to the family *Tenuipalpidae*. The mite is reddish brown and purple in colour. The body is elliptical, flat, and light to reddish orange (Figure 1). It passes through an egg (oval shaped, about 0.1 mm), a four- legged larval stage, a six-legged nymphal stage (Protonymph and deutonymph) and eight-legged adults (Zeiss et al., 2001). The number of setae on the venter is not constant but increases from four, five, seven and eight pairs on the larva, protonymph, deutonymph and the adult, respectively. Larval, protonymph and deutonymph stages have active periods during which they feed, grow and disperse (Zeiss et al., 2001). Normally mites attack the upper surface of mature leaves, feeding on chlorophyll of the maintenance foliage making the leaves to dry and curl (Figure 2). In severe cases, young leaves are also attacked leading to defoliation and occasional death of tea bushes (Ahmed et al., 2012; Kumara et al., 2011). The pest is widely distributed in India, Bangladesh, Sri Lanka, Taiwan, Burundi, Rwanda, Kenya, Malawi, Uganda, and Zimbabwe (Gotoh & Nagata, 2001). In Kenya it is predominant in the east of the Rift Valley where it causes yield losses during prolonged drought (Sudoj et al., 2011).



Fig 1: Red crevice mite



Fig 2: Tea leaf attacked by red crevice mite

The red spider mite, *Oligonychus coffeae* belongs to the family *Tetranychidae*. The mite has a simple, oval-shaped body (Figure 3) with front parts being red and hind part purple (Zeiss et al., 2001). It passes through an egg, a four-legged larval stage, a six-legged nymphal stage (Protonymph and deutonymph) before transforming into eight-legged adults. Parthenogenesis is known to occur; consequently, all mite stages can be found at a given time (Roy et al., 2014). The larvae, nymphs and adult mites cause damage. These have needle-like mouth parts and feed by piercing the leaves of the host plant and sucking out the fluid from the plant cells, causing the older leaves to fall off. Usually the red spider mites prefer old leaves but during heavy infestations and drought young leaves may be attacked (Sudo, 1997). During red spider mite infestation, yellowish spots appear along the mid-rib of tea leaves and occasionally on petioles. As feeding continues the spots turn brown and eventually the leaf becomes deeply bronzed/necrotic (Figure 4). Severe infestation of spider mites results in massive defoliation especially in hot and dry weather (Roy et al., 2014). Among the mites species, *O. coffeae* is considered to be the most serious pest of tea, causing considerable loss in tea production (Ahmed et al., 2012; Kumara et al., 2011; Roy et al., 2014). It has been recorded in all tea growing areas in Kenya in low to moderate infestations but mainly in the west of Rift Valley (Sudo, 1997; Sudo et al., 2011).

The weather factors such as temperature, relative humidity, rainfall, sunshine hours etc. are very important for the productivity of tea as well as outbreak of pests in tea (Ahmed et al., 2012). The population of red spider and crevice mites are seasonally variable and dependent on the prevailing agro-climatic conditions (viz. temperature, rainfall and relative humidity) (Ahmed et al., 2012; Sudo et al., 2011). These weather factors exercise a dominating effect on the fecundity, incubation period, reproductive capacity, longevity and development of mite pests (Ahmed et al., 2012; Haque, Begum, Naher & Wahab, 2007; Sudo, 1997). High temperature, low relative humidity, high sunshine hours have been

positively correlated with the infestation of red spider mite and red crevice mite in tea (Ahmed et al., 2012; Haque et al., 2007; Sudoi, 1997).



Fig 3: Red spider mite



Fig 4: Tea leaves turn brown and defoliate due to red spider mites attack

Due to higher temperature the development of *O. coffeae* occurred rapidly and the duration of different developmental stages was shortened except the deutonymphal period (Haque et al., 2007). Prolonged dry weather which is characterized by high temperature, low humidity and low rainfall, normally increases the mites incidences (Haque et al., 2007). Like temperature, relative humidity was positively correlated with the infestation of red spider mites in tea (Ahmed et al., 2012). There was a negative effect of rainfall on infestations of the two species of mites and this was attributed to heavy rainfall which might have washed out the mite population from the leaves (Ahmed et al., 2012; Sudoi et al., 2011). During the cold weather the duration of all the developmental stages of *O. coffeae* were longer (Haque et al., 2007) and the mites were present in very small numbers on few old leaves of the tea bushes (Ahmed, 2012; Ahmed et al., 2012). The seasons in the tea growing regions in Kenya can be classified as cold and wet (March to May); cold and dry (June to August) and warm and dry (December to February) (Ng'etich et al., 1995; Ngetich et al., 2001). The tea areas in the east of the Rift Valley have a weekly bimodal rainfall distribution in April/May and October/November, while areas in the west of Rift Valley have rains most of the year, except



in the months of January and February when there is drought. Climatic factors such as temperature, rainfall and relative humidity influenced mites infestation levels in Bangladesh (Ahmed et al., 2012). Distinct seasons occur in the tea growing regions in Kenya (Ng'etich et al., 1995) with distinct variations in temperatures, rainfall and relative humidity. It is not documented if these climatic factors may influence mites levels and if such influence vary in the different parts of Kenya and at different times of the year.

## **2.2 Tea clones**

Tea was first introduced in Kenya around 1904 in form of seed (Owuor, 1999). The early introductions were highly variable forming the initial populations of mixed genotypes. Uniformity and stability in yield and quality of the mixed genotypes could not be maintained; hence this necessitated the search for more uniform high yielding tea cultivars. The first set of clones was released in 1964 (Mamati, Wachira & Njuguna, 2001). The production of tea clones at Tea Research Foundation of Kenya (TRFK) has been done in three phases. The first phase of the tea improvement was done by mass selection among introduced seedling jats based on morphological characteristics (Owuor, 1999). The initial selections were based on similarity to the Assam varieties, vigour, density of plucking points and shoot size. The clones selected for high yields, were compared mainly to seedling tea, and later clone TRFK 6/8 for quality. Initially a clone was released when it had yields greater than TRFK 6/8 or with quality lower than TRFK 6/8 but better than seedling tea or a clone with better quality than TRFK 6/8 and yields greater by 175% the yield of seedling tea (Mamati et al., 2001). Using these criteria, clones TRFK 6/8, TRFK 7/3, TRFK 7/9, TRFK 7/14, TRFK 11/4, TRFK 12/12, TRFK 12/19, TRFK 31/8, TRFK 31/11, TRFK 31/27, TRFK 31/28, TRFK 31/29, TRFK 54/40, TRFK 55/55, TRFK 55/56, TRFK 56/89, TRFK 100/5 and TRFK 108/82 were released.

The second phase involved breeding through hybridization of selected parental stocks. The early stages of this phase involved evaluation of maternal half-sib progenies (i.e. only maternal parent was known) of selected parents with the second phase involving the evaluation of full-sib crosses (both maternal and paternal parents known) of selected maternal parents. The stocks were initially selected for yield and quality compared to the seedling stocks and clone TRFK 6/8 but has since been diversified to include other desirable attributes with respect to the growth and management of tea (Kamunya et al., 2010b). The half-sib selected clones with the progenitor maternal parent TRFK 6/8 were TRFK 303/35, TRFK 303/152, TRFK 303/156, TRFK 303/179, TRFK 303/186, TRFK 303/199, TRFK 303/216, TRFK 303/231, TRFK 303/248, TRFK 303/259, TRFK 303/352, TRFK 303/366, TRFK 303/388, TRFK 303/577, TRFK 303/745, TRFK 303/791, TRFK 303/978, TRFK 303/999, TRFK 303/1199, TRFK 347/314, TRFK 347/326, TRFK 347/336 and TRFK 347/573.

The later phase involved selections from bi-colonial full-sib progeny which resulted in the release of clones TRFK 337/3, TRFK 337/138 and TRFK 338/13. Tea improvement efforts however, have shifted emphasis towards developing clones with all the important attributes and where possible develop varieties with a combination of majority of the desired traits in a single clone (Kamunya et al., 2010a). This is driven by lack of sufficient suitable land for tea production due to competition from human settlement and other competing enterprises. The progenies are assessed for the traits contributing to yield and black or green tea quality, and with tolerance attributes to various biotic and abiotic stress factors such as drought, pests, diseases, high soil pH, cold and frost as well as other properties such as good rooting ability, fast oxidation (fermentation) and vigour (Kamunya et al., 2010b). Additionally the breeding programme incorporates medicinal properties of tea as its pharmacological potential is widely acknowledged. For example, certain tea catechins have been associated with boosting the body's immunity and to exhibit anti-cancer properties while

polyphenols (flavanols and anthocyanins) have antioxidant properties and so help in combating chronic diseases like cancer as well as cardiovascular diseases (Kamunya et al., 2010c). A number of the important attributes in tea have been considered in developing new clones of tea grown at the test sites in Timbilil, Kipkebe and Kangaita (Kamunya et al., 2010b). The susceptibility or resistance to mites of these new clones as well as the imported tea varieties from Tanzania (Anonymous, 2008) is unknown. The search for clones with desired traits continues.

### **2.3 Pest management in tea farms**

Under the different ecological zones, tea is subjected to different biotic and abiotic environmental stresses. The tea breeding/selection research programme release tea varieties to tea industries, some of which are resistant/tolerant while others are susceptible to pests including mites. Consequently, there have been measures to control pests. Cultural practices including keeping pest prone sections free from weeds and alternate host plants, stoppage of cattle trespass inside the tea sections to prevent migration of mites from infested areas to uninfested areas and pruning have been used to control the build-up of mites population (Gurusubramanian, 2005). Pruning is the main cultural practice used; it affects the distribution of mites and the intensity of outbreaks, as it removes a large part of the foliage and stem harboring the mites (Gurusubramanian, 2005). Although pruning removes all stages of mites, a few remain on the few old leaves and the small leaves at the base of the tea bushes that multiply rapidly with the rise in temperature resulting in subsequent heavy infestations (Ahmed et al., 2012).

Mites control in tea is mainly achieved by the use of synthetic pesticides especially during their outbreak. The prolonged and extensive use of pesticides result in several negative effects such as destruction of beneficial organisms including natural predators, parasitoids

and pollinators, development of resistance in insect pests, phytotoxicity, environmental pollution, increase in production costs and undesirable pest residues in made tea which may be above the maximum residual level (MRL) (Elmoghazy et al., 2011). The EU MRL levels of some commonly used pesticides are presented in Table 1.

Table 1: European Union MRLs on tea for some pesticides

Pesticide	MRL	Pesticide	MRL	Pesticide	MRL
Residue	(mg/kg)	Residue	(mg/kg)	Residue	(mg/kg)
Methidathion	0.1*	Thiamethoxam	20	L-cyhalothrin	1
Clothianidin	0.7	Cypermethrin	0.5	Fenazaquin	10
Fenpropathrin	2	Bifenthrin	5	Thiacloprid	10
Chlorpyrifos	0.1*	Flubendiamide	0.02*	Acetamiprid	0.1
Deltamethrin	5	Dicofol	20	Chlorfenapyr	50
Propargite	5	Hexythiazox	4	Glyphosate	2.0
Endosulfan	30	Ethion	3	Oxyfluorfen	0.05*
Etoxazole	15	Spiromesifen	50	Fenpyroximate	0.1*
Permethrin	0.1*	Flufenoxuron	15		

\*Level at or about the limit of determination (LOD)

**Source:** (FAO, 2014)

Tea is one of the major agricultural commodities traded globally. The beverage being the most popular drink and the second largest drink consumed fluid in the world next to water (Gardner et al., 2007) is monitored regularly for detection of any pesticide residues. Various

international agencies like Environmental Protection Agency (EPA), Food and Agricultural Organization (FAO), World Health Organization (WHO), European Union (EU) etc. have fixed the MRL values for tea growing countries (FAO, 2014). The EU has increased the number of pesticides regulated for tea over the years, and the number currently stands at 454 pesticides (FAO, 2014).

The continued use of pesticides has affected the trade of tea. For example, China's exports to major European partners decreased significantly over the last two decades, especially after 2000. This was attributed to the increasing number of pesticides used by tea producers detected in China tea for export to the EU (FAO, 2014). For Kenyan tea, control of tea pests is by use of cultural practices. However, if infestation level is high and application of pesticides is mandatory, the pesticides must be applied under close supervision of the Tea Research Foundation of Kenya as outlined in the Tea Growers Handbooks (Anonymous, 2002).

Plant derivatives (biopesticides) such as neem products are attractive alternatives to synthetic chemical insecticides for pest management because they are environmentally safe, selective, biodegradable, economical and renewable (Mamun et al., 2011). However, they have drawbacks as slow acting and being labile in the presence of the UV component of sunlight and this has greatly limited their use outdoors (Antonious, 2004). For example the half-lives of botanical, pyrethrins on field-grown tomato and bell pepper fruits were only 2 hours or less (Antonious, 2004).

Biological control involving natural enemies plays an important role in the tea pest population suppression and prevents the pest from attaining critical level. Larvae and adults of *Sthethorus gilvifrons*, *Verania vincta*, *Jauravia quadrinotata* and *Scymnus sp*, staphylinid beetle, *C. carnea*, and predatory mites *Agistemus hystrix*, *Exothorhis caudate*, *Cunaxa sp* are important natural enemies of tea mites (Borthakur et al., 2005)). Key pests are many and

varied thus in order to cope with the multiple pest problems, growers must use several natural enemies in combination, a strategy that is difficult and expensive for application (Akio et al., 2001).

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, habitat manipulation, modification of cultural practices, and use of resistant varieties. Integrated management has been adopted to control these mite pests, involving cultural, mechanical, physical, biological and chemical methods (Roy et al., 2014). The occurrences of mites reduce with rise in nitrogen fertiliser rates (Sudo, 1993). There has been clone bias in the mites infestations, clones TRFK 54/40, TRFK 303/259, TRFK 55/56, STC 5/3 are susceptible to *B. phoenicis* while TRFK 6/8 and TRFK 303/1199 are resistant (Sudo, 1997). Resistant/tolerant clones have been selected over susceptible varieties as part of IPM in the management of mites in tea farms. Thus growers are advised to use the plant resistant/tolerant clones and apply adequate amounts of nitrogenous fertilizer as preventive methods of controlling the mite (Sudo, 1997). Screening of the new and imported clones may provide selection criteria for incorporating these clones in the IPM programme in the management of mites. However, little is known on the biochemical substances released by tea varieties that make them resistant or susceptible to mites.

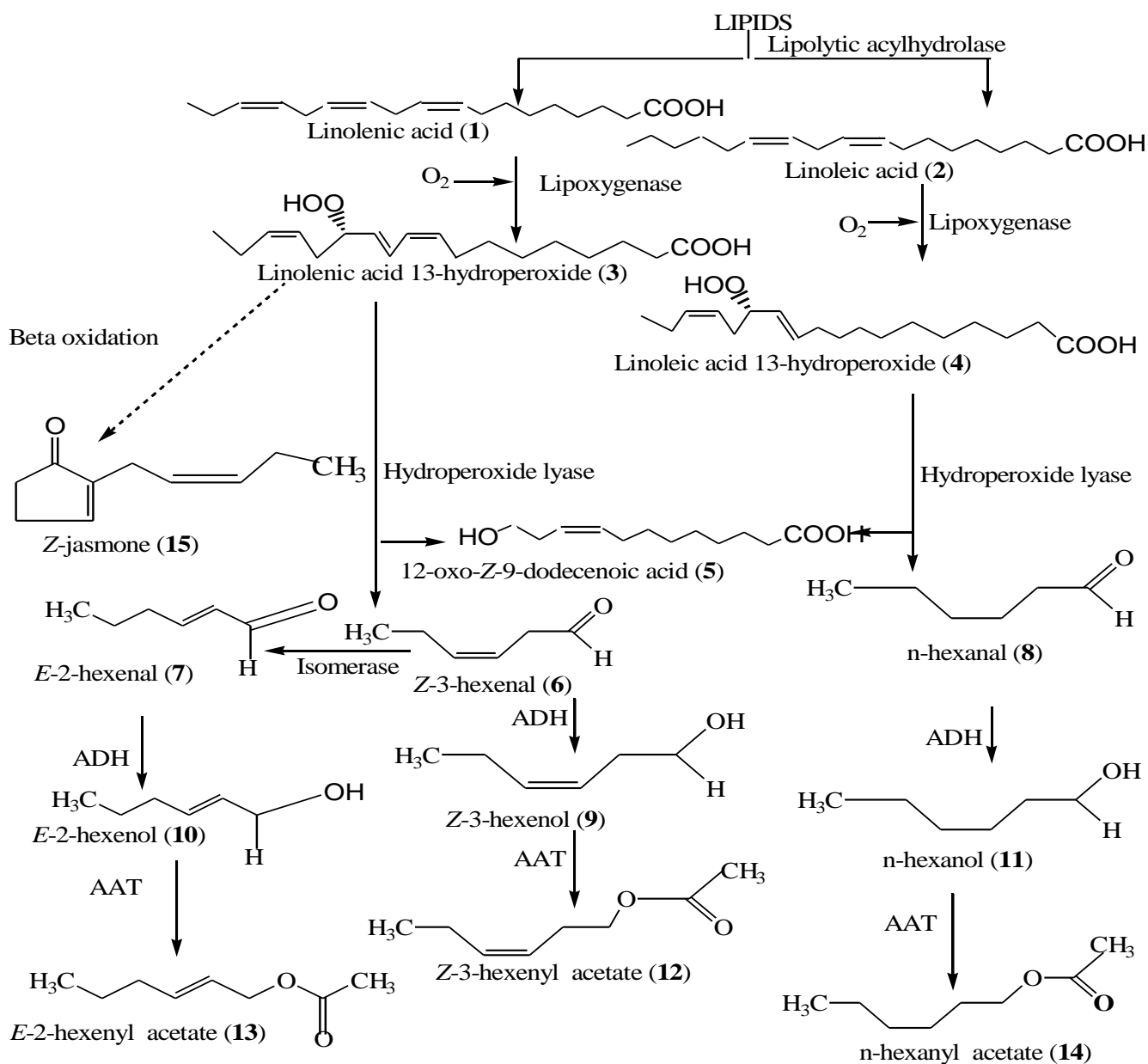
## **2.4 Plant volatiles**

Plant volatile organic compounds (VOCs) are defined as any organic compound with vapor pressure high enough under normal conditions to be vaporized from plant into the atmosphere (Yuan, Himanen, Holopainen, Chen & Stewart, 2009). Plants are capable of biosynthesizing and releasing an array of volatile organic compounds derived from a diverse set of primary metabolites that include amino acids and fatty acids (Lee, 2010). The volatile

compounds are biosynthesized mainly via four biochemical pathways: the lipoxygenase pathway for green leaf volatiles (GLVs) (Figure 5), shikimic acid pathway for aromatic volatiles (Figure 6), methylerythritol pathway (MEP) for monoterpenoids (Figure 8) and mevalonic acid (MVA) pathways for sesquiterpenoids (Figure 9) (Niinemets, Kannaste & Copolovici, 2013).

#### **2.4.1 Green leaf volatiles (GLVs)**

Green leaf volatiles are short-chain acyclic aldehydes, alcohols and esters that form as a result of the catalysis by hydroperoxide lyases and alcohol dehydrogenases (ADH) (Shen et al., 2014). The first step of this metabolic route is catalyzed by lipases that deacylate galactolipids to release free  $\alpha$ -linolenic (**1**) or linoleic (**2**) acids. In the second enzymatic step, C<sub>13</sub>-lipoxygenases catalyze the addition of oxygen to  $\alpha$ -linolenic (**1**) and linoleic (**2**) acids to form, linolenic acid 13-hydroperoxide (**3**) and linoleic acid 13-hydroperoxide (**4**) respectively. These hydroperoxides instead of losing water are cleaved to form two fragments of twelve (oxoacids), 12-oxo-*Z*-9-dodecenoic acid (**5**) and six carbon (C<sub>6</sub>-aldehydes) units, *Z*-3-hexenal (**6**), respectively (Fig 5) (Hatanaka, Kajiwa & Sekiya, 1987; Hatanaka, Kajiwara, Matsui & Toyota, 1992; Hatanaka, 1995). These aldehydes can be converted into the corresponding alcohols and esters by alcohol dehydrogenases and alcohol acyltransferase, respectively. The hexenyl family compounds including *E*-2-hexenal (**7**), *n*-hexanal (**8**), *Z*-3-hexenol (**9**), *E*-2-hexenol (**10**), *n*-hexanol (**11**), *Z*-3-hexenyl acetate (**12**), *E*-2-hexenyl acetate (**13**) and *n*-hexanyl acetate (**14**) are formed as outlined in Figure 5. 9*S*- or 13*S*-hydroperoxides can undergo  $\beta$ -oxidation to form products such as *Z*-jasmonone (**15**) (Niinemets et al., 2013).



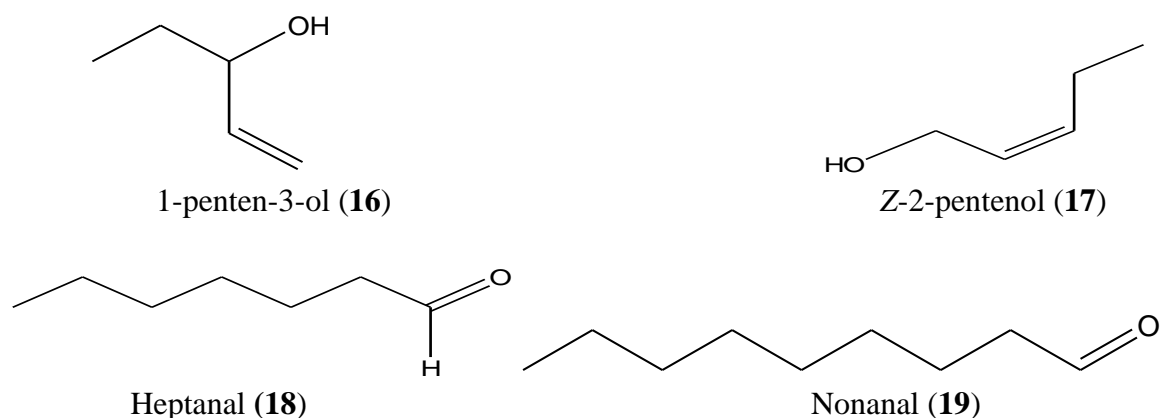
*ADH, alcohol dehydrogenase; AAT, alcohol acyltransferase.*

Figure: 5 Biosynthesis of green leaf volatiles (Shen et al., 2014; Singh et al., 2015).

C<sub>5</sub> compounds such as 1-penten-3-ol (16) and Z-2-pentenol (17) are formed through a pathway involving two separate lipoxygenases reactions (Shen et al., 2014; Singh & Sharma, 2015). Other GLVs such as heptanal (18) and nonanal (19) are also present in tea (Robinson et al., 1992). Prolonged drought stress combined with high leaf temperatures can induce lipid peroxidation (Jardine et al., 2015). Terpenoid emissions may initially be stimulated but decline following photosynthetic membrane peroxidation and the loss of net carbon assimilation. Thus, if volatile terpenoids act as effective antioxidants to reduce photosynthetic



membrane peroxidation during stress, this antioxidant system can be overwhelmed and/or is no longer active during excessive reactive oxygen species accumulation associated with lipid peroxidation (Jardine et al., 2015). This implies that the combined effect of high leaf temperatures and water deficits can result in a strong decrease in photosynthesis and the emissions of volatile terpenoids. The collapse of photosynthesis and the volatile terpenoids antioxidant system under high temperature and desiccation stress particularly during drought is associated with membrane peroxidation and the emissions of GLVs (Jardine et al., 2015). It is not documented if the attack of tea in Kenya by red spider and red crevice mites during prolonged drought is related to the GLVs emissions by the tea cultivars.



#### 2.4.2 Aromatics

Coupling of phosphoenolpyruvate (PEP) (20) and D-erythrose 4-phosphate (21) gives the seven-carbon 3-deoxy D-arabino-heptulosonic acid 7-phosphate (DAHP) (22) (Figure 6). Elimination of phosphoric acid from DAHP (22) followed by an intramolecular aldol reaction generates 3-dehydroquinic acid (23). 3-Dehydroshikimic acid (24) undergoes dehydration and reduction steps to form shikimic acid (25). After a series of reactions, chorismate (26) is formed which is later converted to phenylalanine (Phe) (27). In the first step of phenylpropanoid biosynthesis, phenylalanine (Phe) (27) is converted to *trans*-cinnamic acid (28) in a reaction catalyzed by phenylalanine ammonia-lyase. In the next step a variety of hydroxycinnamic acids, aldehydes and alcohols are formed from *trans*-cinnamic acid (28) via

a series of hydroxylation and methylation reactions, which occur at the level of hydroxycinnamic acid esters and their corresponding aldehydes and alcohols. Benzenoid compounds also originate from *trans*-cinnamic acid (**28**). Their synthesis requires the shortening of *trans*-cinnamic acid (**28**) side chain by a C<sub>2</sub> unit.

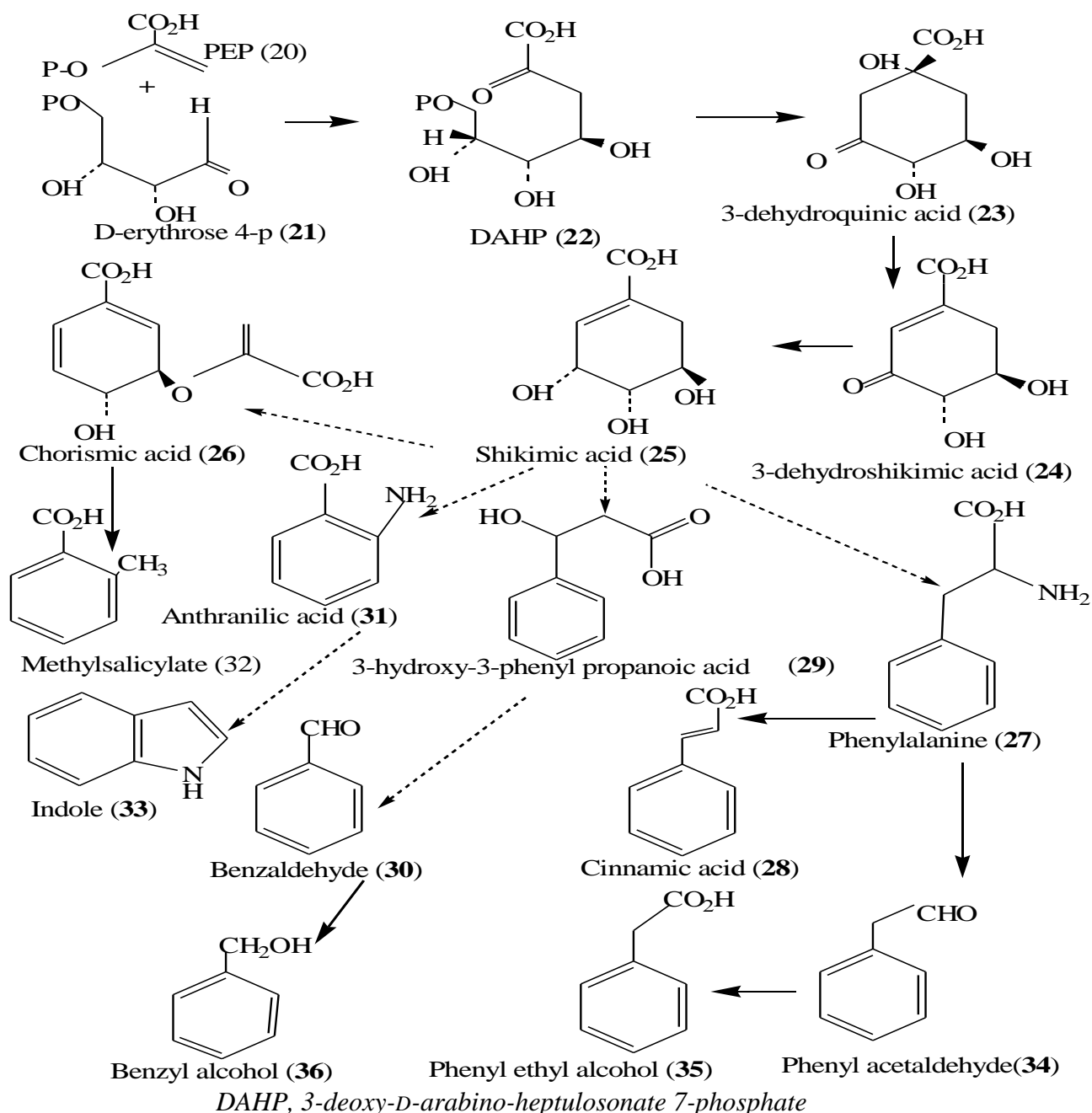


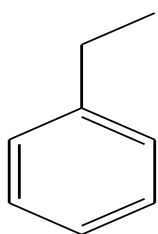
Figure 6: Biosynthesis of aromatics (Dudareva et al., 2006; Maeda et al., 2010).

The side chain shortening could occur via a CoA-dependent- $\beta$ -oxidative, CoA-independent-non- $\beta$ -oxidative pathways, or via the combination of the two mechanisms. For example, the

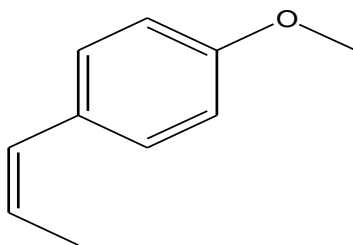
CoA-independent–non- $\beta$ -oxidative pathway involves hydration of the free *trans*-cinnamic acid (**28**) to 3-hydroxy-3-phenyl propanoic acid (**29**) and side chain degradation via a reverse aldol reaction with formation of benzaldehyde (**30**). Chorismic acid (**26**) and anthranilic acid (**31**) are intermediates in the biosynthesis of methyl salicylate (**32**) and indole (**33**), respectively. *Trans*-cinnamic acid (**28**) is the precursor to several benzenoid and phenyl propanoid volatile compounds including phenyl acetaldehyde (**34**), phenyl ethyl alcohol (**35**) and benzyl alcohol (**36**) as outlined in Figure 6 (Boatright et al., 2004; Dudareva, Negre, Nagegowda & Orlova, 2006; Maeda et al., 2010).

Other aromatic compounds produced by the shikimate pathway include ethyl benzene (**37**), *Z*-anethole (**38**), benzothiazole (**39**), benzophenone (**40**) *p*- xylene (**41**), *o*- xylene (**42**), indene (**43**), naphthalene, (**44**) azulene (**45**), acetophenone (**46**),  $\alpha$ - methyl styrene (**47**), diphenyl ether (**48**) and phenol (**49**) (Maeda et al., 2010).

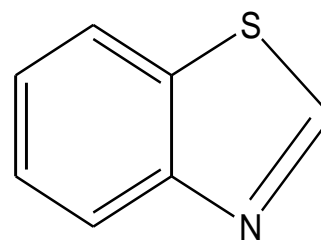
Aromatic compounds protect plants against pests (Borg-Karlson et al., 2003; Fujita, Fujita & Kubo, 2007; Han & Chen, 2002). Several aromatic compounds have been associated with tea plants (Chen, Olson & Ruberson, 2010; Owuor et al., 1987a; Owuor et al., 1987d; Owuor et al., 1997). It is necessary to determine if the cultivars emit aromatic compounds and relate them to mites attack



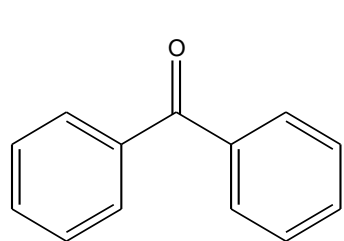
Ethylbenzene (**37**)



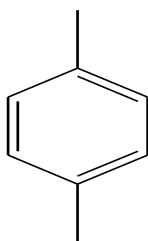
*Z*-anethole (**38**)



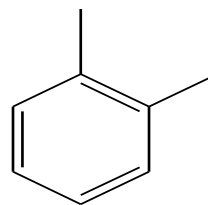
Benzothiazole (**39**)



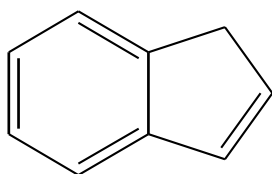
Benzophenone (40)



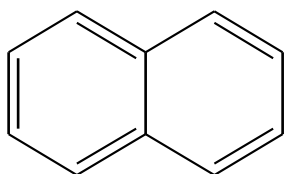
p- xylene (41)



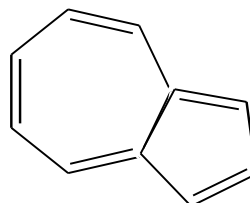
o- xylene (42)



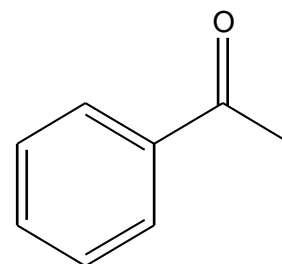
Indene (43)



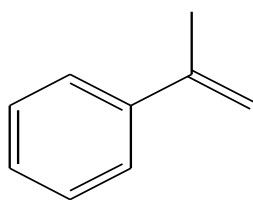
Naphthalene (44)



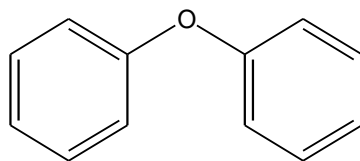
Azulene (45)



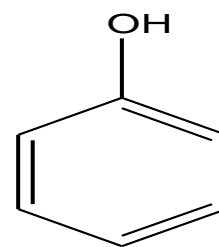
Acetophenone (46)



$\alpha$ - methyl styrene (47)



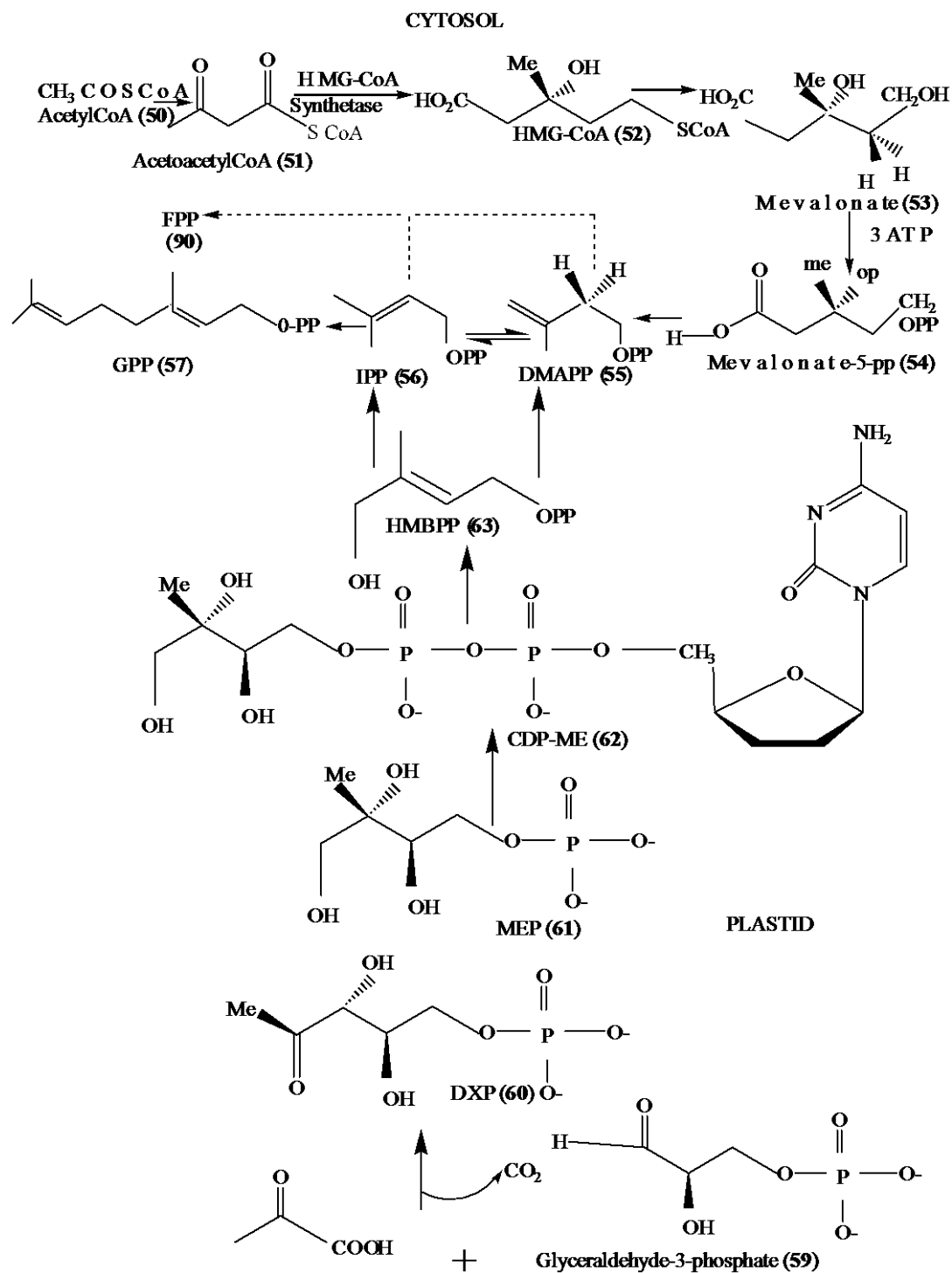
Diphenyl ether (48)



Phenol (49)

### 2.4.3 Terpenes

Terpenoid compounds originate from dimethylallyl diphosphate (DMAPP) (55) and isopentenyl diphosphate (IPP) (56) which are synthesized via the cytosolic MVA and the plastidial MEP pathways (Fig 7). The MVA pathway starts with the condensation of three units of acetyl CoA (50) which leads to the synthesis of 3-hydroxy 3-methylglutaryl CoA (HMGCoA) (52), which later on produces mevalonic acid (53). The mevalonic acid (53) is converted to isopentenyl diphosphate (IPP) (56) through phosphorylation and decarboxylation. Coupling of IPP (56) with DMAPP (55) gives rise to geranyl diphosphate (GPP) (57) the precursor of monoterpenes (Figure 7).



*DXP*, deoxyxylulose-5-phosphate; *MEP*, methylerythritol-4-phosphate; *DMAPP*, dimethylallyl diphosphate; *FPP*, farnesyl diphosphate; *HMG-CoA*, 3-hydroxy-3-methylglutaryl CoA; *IPP*, isopentenyl diphosphate; *CDP-ME*, 4-diphosphocytidyl-2-C-methyl-erythritol; *HMBPP*, (*E*)-4-hydroxy-3-methylbut-2-enyl diphosphate.

Figure: 7 Biosynthesis of GPP from acetyl coenzyme A and FPP from pyruvate and glyceraldehyde-3-phosphate (Lange et al., 2013).

In contrast, the plastidial MEP pathway starts in plastid by the condensation of pyruvate (**58**) and glyceraldehyde-3-phosphate (**59**), which leads to the synthesis of 1-deoxy D-xylulose 5-phosphate (DXP) (**60**). The DXP (**60**) is reduced to methyl erythritol 4-phosphate (MEP) (**61**) (Lange & Ahkami, 2013; Singh et al., 2015). After MEP (**61**) is formed, the pathway proceeds *via* 4-diphosphocytidyl-2-C-methyl-erythritol (CDP-ME) (**62**) which undergoes a series of reaction to form hydroxymethylbutenyl 4-diphosphate (HMBPP) (**63**). In the cytosol, IPP (**56**) is converted into DMAPP (**55**) by the action of IPP isomerase, but in the plastid, isomerism does not occur, IPP (**56**) and DMAPP (**55**) are formed independently from a common intermediate. The hydroxymethylbutenyl 4-diphosphate (HMBPP) (**63**) is directly converted into the IPP (**56**) and DMAPP (**55**) mixture by the enzyme isopentenyl diphosphate and dimethylallyl diphosphate synthases. A condensation of one molecule of DMAPP (**55**) with two molecules of IPP (**56**) generates farnesyl diphosphate (FPP) (**90**), the direct precursor of most sesquiterpenes (Singh et al., 2015)

#### 2.4.3.1 Monoterpenes

Monoterpenes may be envisaged as consisting of two isoprene (C<sub>5</sub> carbon) units, formed by head-to-tail condensation to produce a C<sub>10</sub> branched chain or ring. The mechanism of the reactions proceeds by ionization of the geranyl diphosphate (**57**) to form geranyl cation (**64**) (Figure 8). Addition of water to the cation forms geraniol (**65**) and linalool (**66**). Microorganisms, especially fungi, are very versatile biocatalysts for the production of a wide range of flavour and fragrance compounds from cheap natural precursors such as terpenoids. For example, furanoid *trans*-(2*R*, 5*S*) linalool oxide (**67**) and furanoid *cis*-(2*S*, 5*R*) linalool oxide (**68**) are formed by epoxidation of (±) linalool (**66**) by *Corynespora cassicola* (Bormann, Maria, Etschmann & Schrader, 2012). Linalyl cation (**71**) forms when the phosphate group is eliminated from linalyl diphosphate (cisoid) (**70**).

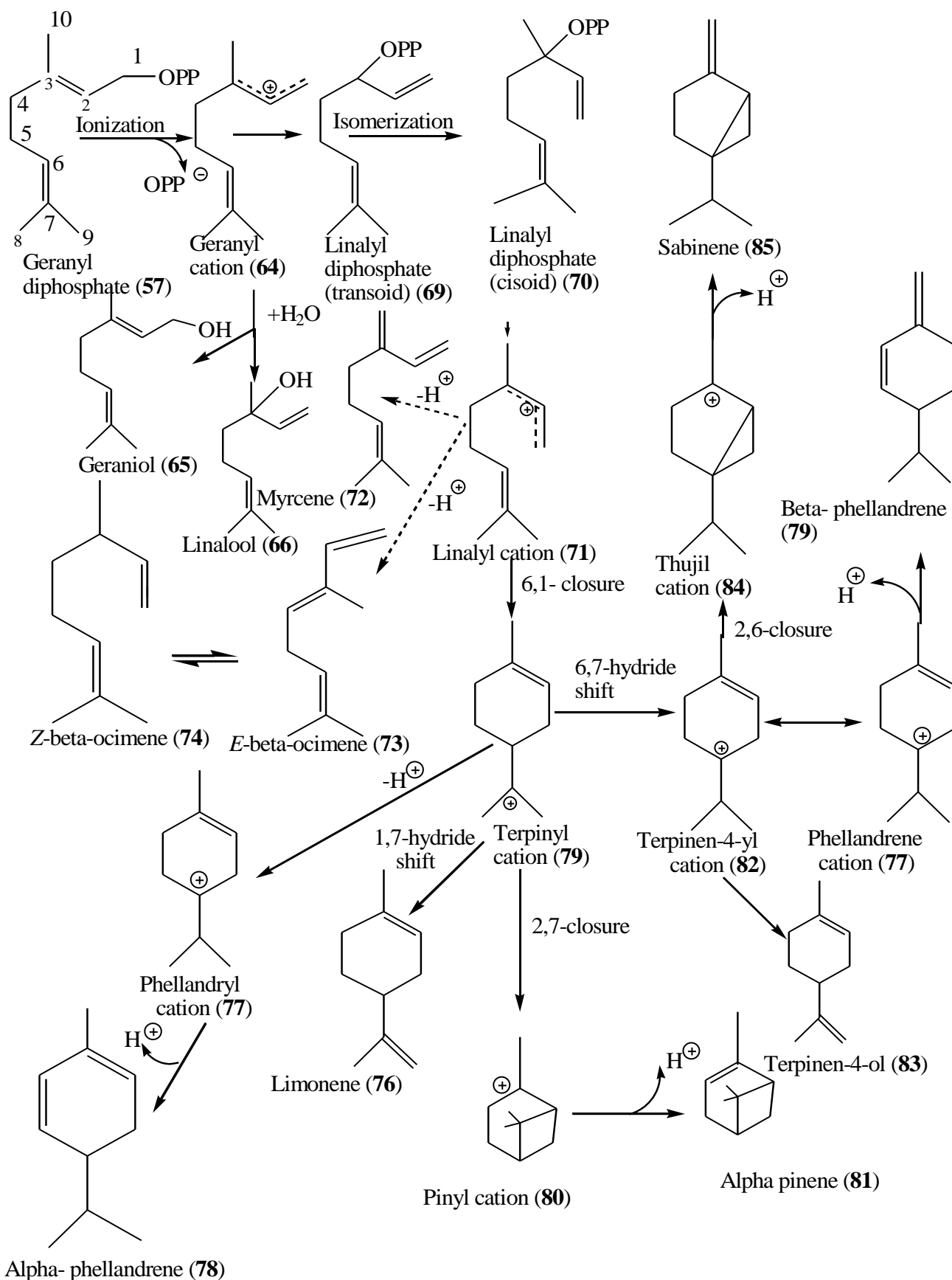
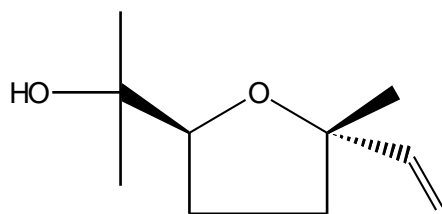
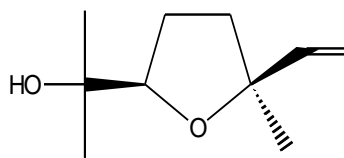


Figure 8: Biosynthesis of monoterpenes (Degenhardt, Tobias & Köllne, 2009; Lange et al., 2013).

Loss of a proton from linalyl cation (**71**) leads to the formation of myrcene (**72**), *E*- $\beta$ -ocimene (**73**) and its isomer *Z*- $\beta$ -ocimene (**74**).



*E*-Linalool oxide furanoid (**67**)



*Z*-Linalool oxide furanoid (**68**)

Similarly, linalyl cation (**71**) undergoes 1, 6- closure to form terpinen-4-yl cation (**75**) from which limonene (**76**) is formed as a result of 1,7 hydride shift. Loss of a proton from terpinyl cation (**75**) forms phellandryl cation (**77**) that further loses a proton to form  $\alpha$ -phellandrene (**78**) and  $\beta$ -phellandrene (**79**). A 2,7- closure of terpinyl cation (**75**) forms pinyl cation (**80**) that loses a proton to form  $\alpha$ -pinene (**81**). Terpinen-4-ol (**83**) is derived from terpinen-4-yl cation (**82**) which is formed when terpinyl cation (**75**) undergoes a 6,7 hydride shift. Terpinen-4-yl cation (**82**) undergoes a 2,6- closure to form a bicyclic thurjil cation (**84**) which forms sabinene (**85**).

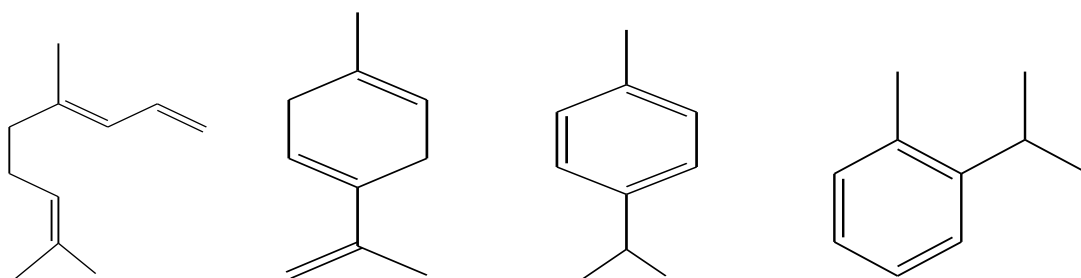
Limonene (**76**) can be converted to *p*-mentha-1,3,8-triene (**86**) in the presence of P450 which forms *p*-cymene (**87**) and the isomer *o*-cymene (**88**) in the presence of both water and light (Lucker et al., 2004).

#### 2.4.3.2 Homoterpenes

The homoterpenes are also formed from IPP intermediate. For example the irregular acyclic ( $C_{11}$ ), *E*-4,8-dimethyl-1,3,7-nonatriene (DMNT) (**89**) is derived from its 15 carbon precursor, farnesyl pyrophosphate (FPP) (**90**) by a series of enzymatic steps with the overall loss of four carbon units. The biosynthesis proceeds via two enzymatic steps; the formation



of tertiary C<sub>15</sub>- alcohol precursor, *E*-nerolidol (**99**) followed by an oxidative degradation catalyzed by a cytochrome P450 monooxygenase (Tholl, Sohrabi, Huh & Lee, 2011).



DMNT (**89**)    1,3,8-p-menthatriene (**86**)    p-cymene (**87**)    o-cymene (**88**)

### 2.4.3.3 Sesquiterpenes

Ionization of FPP (**90**) to the farnesyl cation (**91**) is the first step in the biosynthesis of a large number of sesquiterpenes (Figure 9). Farnesyl cation (**91**) undergoes series of reactions to form *E*- $\beta$ -caryophyllene (**94**), germacrene D (**95**) and *E*- $\beta$ -farnesene (**96**). Isomerization of farnesyl cation (**91**) generates the nerolidyl cation (**97**) which forms nerolidol (**99**), humulene (**100**) and longifolene (**101**) after series of reactions which include cyclizations and hydride shifts.

Terpene skeletal diversity arises not only from the number of terpene synthases, but also from the ability of these catalysts to form multiple products from a single substrate. In addition to their main product, nearly half of all characterized terpenes synthases also form significant amounts of additional products (Degenhardt et al., 2009). The tremendous diversity of volatile terpenoids in plants is generated through the action of terpene synthases (TPSs) many of which have the distinctive ability to synthesize multiple products from a single substrate. In addition, many TPSs accept more than one substrate (Bleeker et al., 2011), which expands the diversity of produced terpenoids by directing bifunctional enzymes to different compartments with a sesquiterpene varying range of available substrates (Gutensohn, Orlova, Nguyen, Davidovich-Rikanati, Ferruzzi & Sitrit, 2013). In addition to a wide range of volatile terpenoids formed directly by TPSs, terpenoid diversity is further

increased by other enzymes that are capable of modifying the TPS products via hydroxylation, dehydrogenation, acylation, or other reactions (Bleeker et al., 2011)

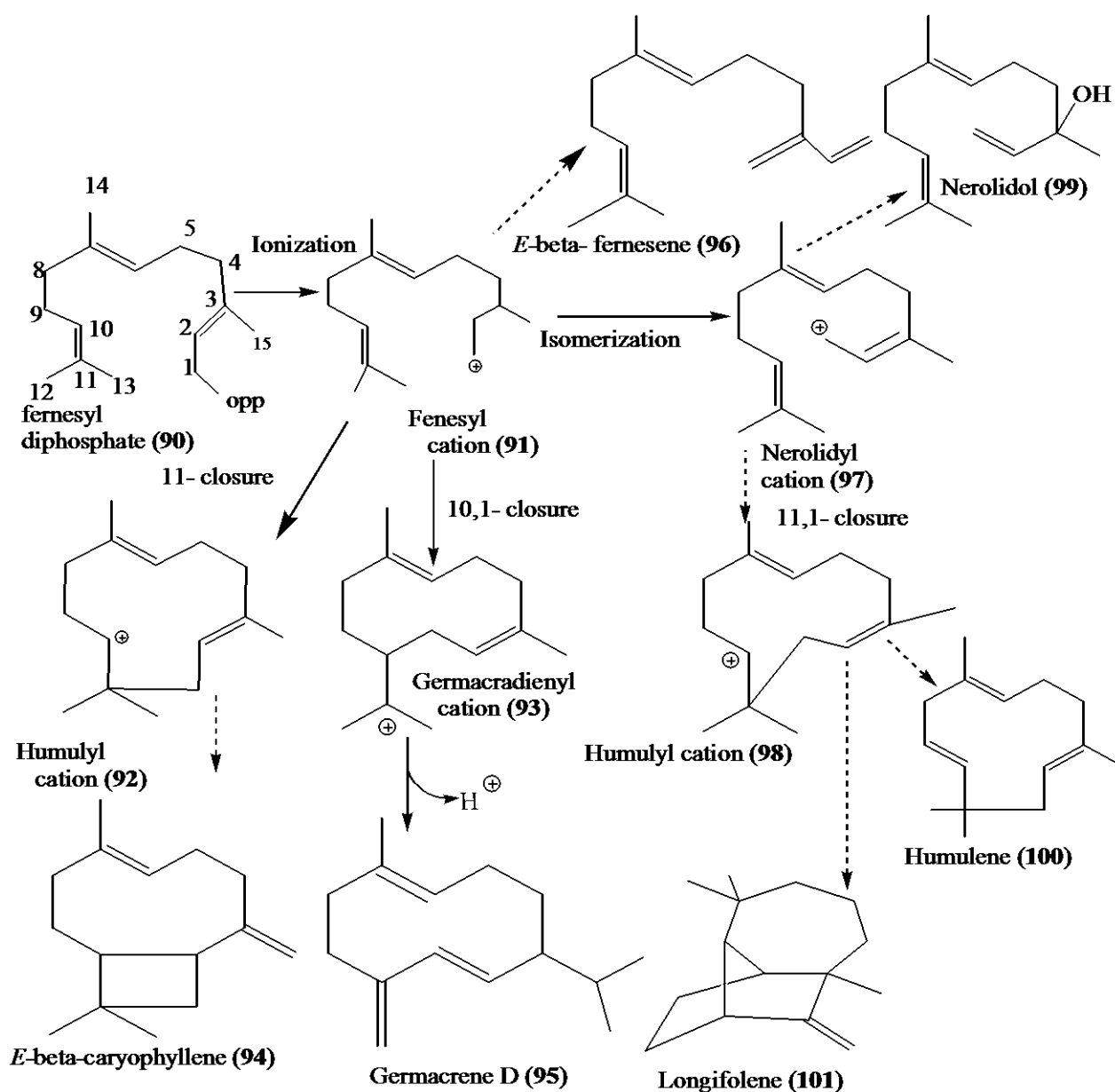
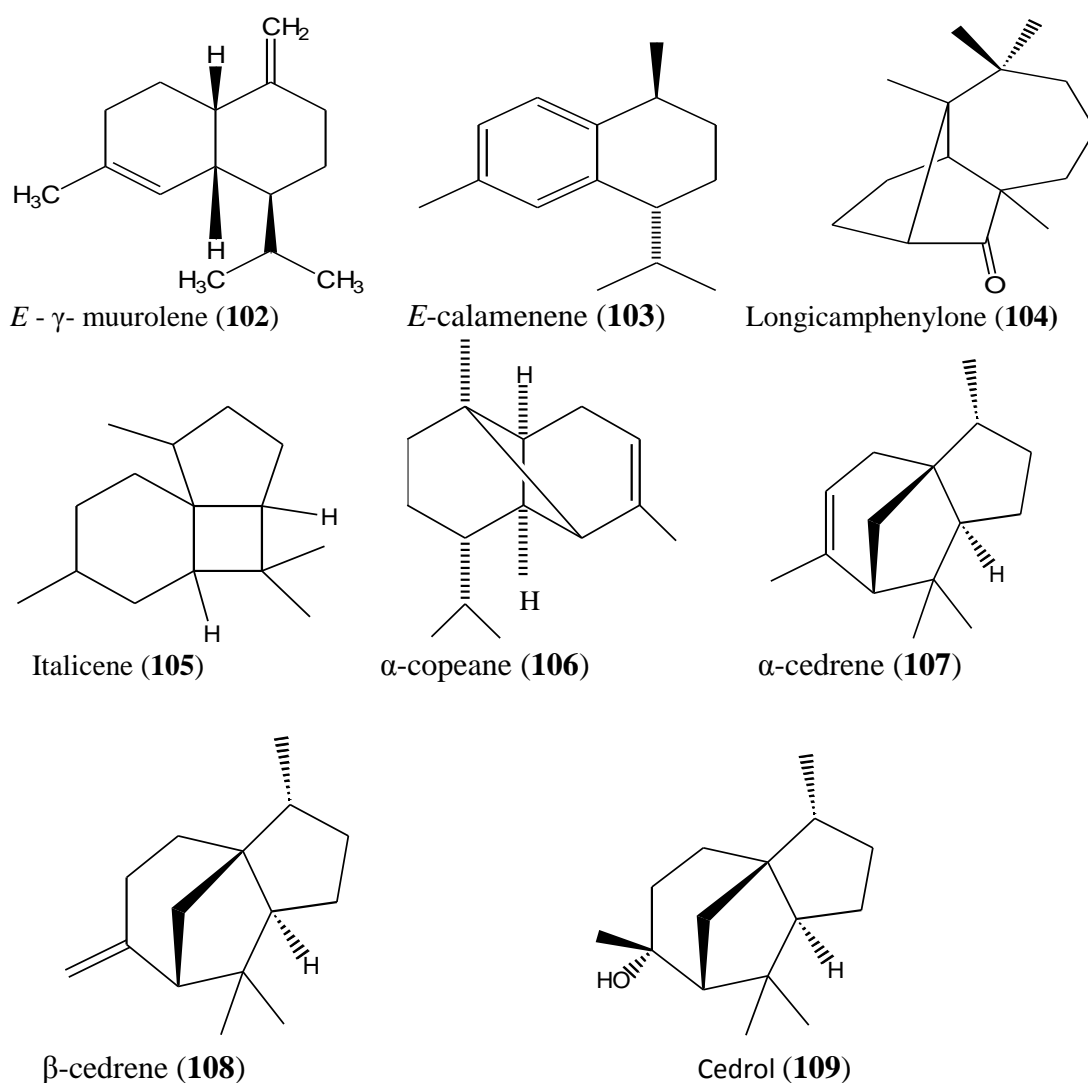


Figure 9: Biosynthesis of sesquiterpenes (Bleeker et al., 2009; Singh et al., 2015).

. Other sesquiterpenes formed in the biosynthetic pathway include *E*-γ-muurolene, (102) *E*-calamenene (103), longicamphenylene (104), italicene (105) α-copeane (106), α-cedrene (107), β-cedrene (108) and cedrol (109). A large proportion of VOCs are terpenes, either monoterpenes or sesquiterpenes and these compounds are highly diverse (Degenhardt et al., 2009). Possible value of complex terpenes mixtures is that a diverse combination of

terpenes may help provide protection against a diversity of herbivores, parasites and competitors and reduce the potential number of herbivore species. Secondly, having a diverse mixture allows each plant to play a slightly different defense strategy than its conspecific neighbors, reducing the likelihood for an enemy to evolve resistance. Additionally, compounds may act synergistically to provide greater toxicity or deterrence than the equivalent amount of a single substance (Gutensohn et al., 2013).



Terpenes are toxic, feeding deterrents and repellents to plant pests (Mazid, Khan & Mohammad, 2011; Oluwafemi, Dewhurst, Veyrat, Powers & Bruce, 2013). The relationship

between the terpenes released by tea plants and red spider and red crevice mites has not been established.

Tea plants store volatile organic compounds in the leaves in the form of water-soluble diglycosides, primarily as  $\beta$ -primeverosides (6-*O*- $\beta$ -D-xylopyranosyl- $\beta$ -D-glucopyranosides). VOCs are converted by UDP-glycosyltransferases into  $\beta$ -primeverosides by sequential glucosylation and xylosylation, respectively as illustrated in Figure 10 (Ohgami et al., 2015). Plants release OVOCs in response to biotic and abiotic stresses (Scala et al., 2013). The relationship between OVOCs and resistance/tolerance or susceptibility of tea cultivars to red spider and red crevice mites is unknown.

#### **2.4.4 Variability in the volatile organic compounds profiles in susceptible and resistant plant cultivars**

Volatile organic compounds are released by plants and can consist of hundreds of compounds, such as terpenoids, green leaf volatiles and benzenoids which have been shown to act as repellents and/or attractants for herbivores and their natural enemies (Dicke & Baldwin, 2010; Fatouros, Lucas-Barbosa, Weldegergis, Pashalidou, van Loon & Dicke, 2012; Unsicker, Kunert & Gershenzon, 2009). The chemical composition and proportion of such VOCs not only depend on the biotic and abiotic stress factors, but also on plant genetic background (Anjum, Xie, Wang, Saleem, Man & Lei, 2011). Phytophagous insects recognize their hosts by the specific VOC mixture which is used to decide whether the plants are suitable for feeding (Najar-Rodriguez et al., 2013). Many plants have natural defenses that repel pests, for example, a number of terpenes show high repellent activity against insect pests. These include  $\alpha$ -pinene (**81**), limonene (**76**) (Nerio, Olivero-Verbel & Stashenko, 2010), nerolidol (**99**) and cedrol (**109**) (Yatagai, Makihara & Oba, 2002). The sweet potato whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae) was repelled by plants that had significant

increases in the production of monoterpene, cymene (**87**); sesquiterpenes,  $\alpha$ -copaene (**106**) and aromatic compound, methyl salicylate (**32**) (Saad, 2015).

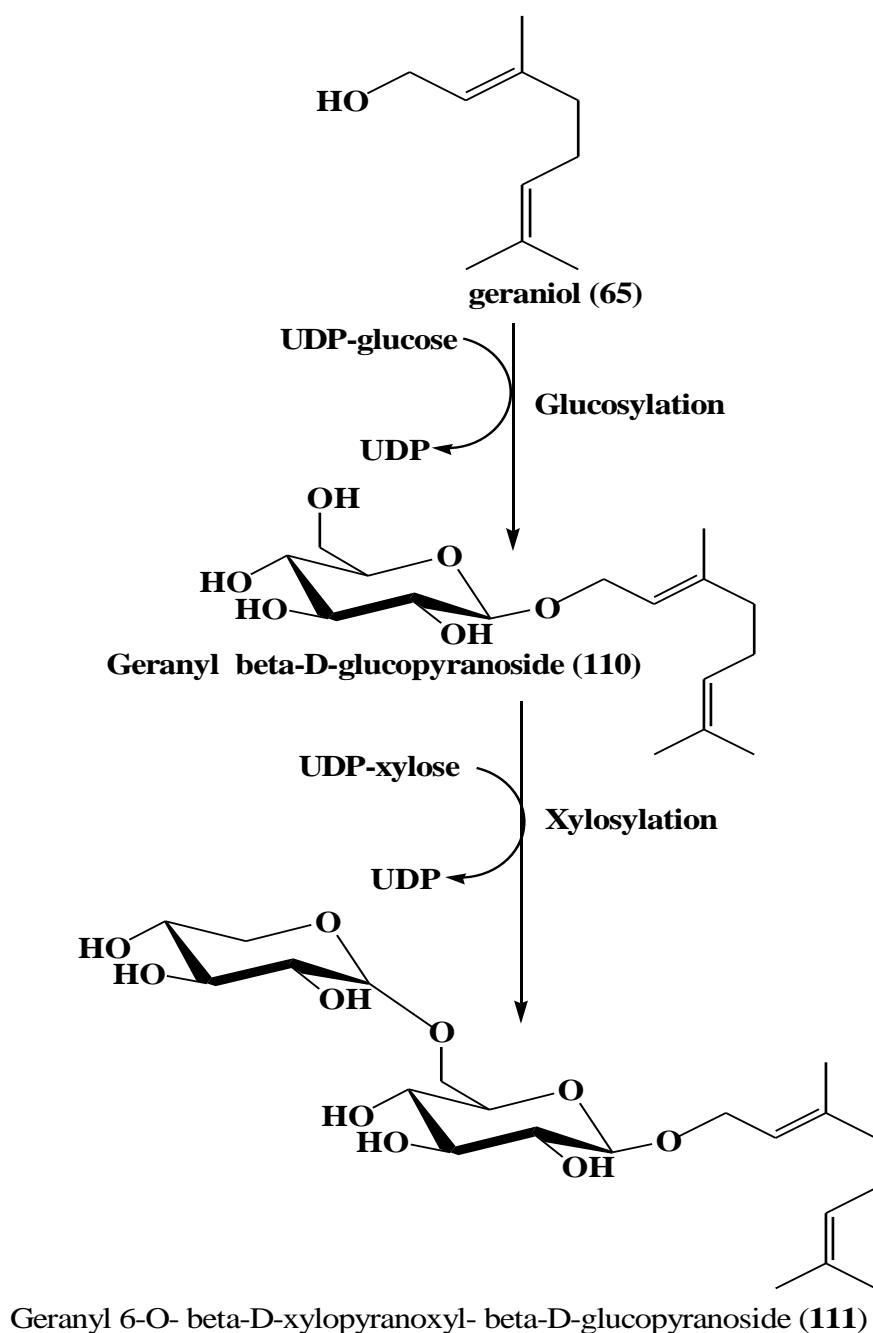


Figure: 10 Biosynthesis of diglycoside, the form in which VOC is stored in tea leaves (Ohgami et al., 2015).

Similarly, *B. tabaci* preferred cultivated tomato varieties over wild tomatoes, which was attributed to high levels of the monoterpenes *p*-cymene (**87**) and myrcene (**72**) being

released by wild tomato plants (Simmons et al., 2005). In another study using a Y-tube olfactometer bioassay the leafhopper, *Cicadulina storey* preferred maize seedlings without the Z-jasmone (**15**) pre-treatment. The pre-treatment led to a significant increase in emission of terpenes *E*- $\beta$ -caryophyllene (**94**), *E*- $\beta$ -farnesene (**76**) and *E*-4,8-dimethyl-1,3,7-nonatriene (**89**), known to act as herbivore repellents (Oluwafemi et al., 2013). Evaluation of four cultivars that differed in resistance, as expressed in terms of spider mite intrinsic rate of population increase showed that the cultivar that had a lower relative production of terpenes exhibited the highest mite intrinsic rate of population increase (Krips et al., 2001). In another study caterpillars performed best on the highest-yielding variety that showed reduced induction of volatile sesquiterpenes compared to ancestral varieties suggesting that breeding in cranberry compromised plant defences (Rodriguez-Saona et al., 2011). Zhang *et al* (Zhang et al., 2015) observed that the chemical characteristics of the plant species that repelled *Chlorophorus caragana* and did not suffer from damage in the field mainly consisted of terpenes and a small amount of GLVs. Aromatic compounds have been implicated in plant defence (Honda, Omura & Hayashi, 1998; James et al., 2004; Reddy & Guenero, 2004; Zhu et al., 2005). Zhang *et al* (Zhang et al., 2015) observed that Caragana plant species that released acetophenone (**46**) in its headspace did not suffer damage from *Chlorophorus caragana* in the field and methyl salicylate (**32**) showed antifeedant activity against pine weevils (Borg-Karlson, Nordlander, Mudalige, Nordenhem & Unelius, 2006). Flea beetles (*Epitrix hirtipennis*) were more abundant on GLV-producing wild type plants compared to plants with reduced hydroperoxide lyase activity (Halitschke et al., 2008). Similarly, *Uschistus heros* preferred soybean pods that released high amounts of GLVs for feeding and oviposition over deficient cultivars (Silva et al., 2013). The identification of plants that provide semiochemicals beneficial to crops, such as repellents for the insect pests and/or attractants to parasitoids and other natural enemies, is important for pest management in the

field. Repellents emitted by plants in the ecosystem can be utilized to reduce insect pest populations. For example, stem borer control, involved release of attractant VOCs; hexanal (**8**), *E*-2-hexenal (**7**), *Z*-3-hexen-1-ol (**9**), *Z*-3-hexen-yl acetate (**12**) from the trap plants and repellent VOCs *E*- $\beta$ -ocimene (**73**), *E*- $\beta$ -caryophyllene (**94**), humulene (**100**), *E*-4,8-dimethyl-1,3,7-nonatriene (**89**),  $\beta$ -cedrene (**108**) from the intercrops (Khan et al., 2008). Cai *et al* (Cai, Dong, Wang & Chen, 2012) demonstrated that tea plants released mixture of OVOCs consisting of terpenoids, green leaf volatiles and aromatic compounds. Variability in the OVOCs composition or levels exist among tea cultivars (Cai et al., 2012) however, it is not known if such differences in OVOCs contribute to susceptibility/resistance of tea cultivars to red spider and red crevice mites.

#### **2.4.5 The influence of seasons and region of production on the composition or levels of volatile organic compounds in relation to insect pests attack.**

The emission of VOCs is highly variable under different biotic and abiotic stresses (Copolovici, Kännaste, Pazouki & Niinemets, 2012; Holopainen & Gershenzon, 2010) and strength of the emission signal can be quantitatively related to the severity of both abiotic and biotic stresses (Niinemets et al., 2013). The levels of VOCs in plants are related to growth rate or yield, in tea, yields are usually low during the dry or very cold seasons and high during rainy seasons (Ng'etich et al., 2001; Rawat et al., 2008). Water availability affects the content and emission of secondary metabolites in plants and different plant species respond to water deficit differently (Kannaste et al., 2013). Dry and cool seasons cause slower shoot growth rate leading to high levels of VOCs while, wet season especially rainy period leads to fast tea growth which reduces VOCs. Growth under dry weather with cooler nights and desiccating winds favour the biogenesis of VOCs. Indeed, black tea volatile flavor compounds vary with time of the year (Owuor, 1992a; Owuor, 1992c; Owuor, 1999; Owuor,

Othieno, Robinson & Baker, 1991). Higher terpenoid levels especially geraniol (**65**) and linalool (**66**) and aromatics such as phenyl ethanol (**35**) and methyl salicylate (**32**) have been reported in the dry than rainy seasons (Gulati & Ravindranath, 1996). Even within the same plant species, the VOCs vary with the cultivars (Owuor, 1992b; Owuor et al., 1988). Lower soil moisture content experienced in dry season reduces photosynthesis and growth of plants (Coley, 1998) but favours accumulation of secondary metabolites (Rawat et al., 2008) such as VOCs. Lipoxygenase activity responsible for production of green leaf volatiles increases during water deficit periods (Sofa et al., 2004). Irradiation effects of higher sunshine during the dry season as well as increased leaf temperatures during drought could be another reason for the higher accumulation of particular phytochemicals in tea shoots (Jeyaramraja, Pius, Raj Kumar & Jayakumar, 2003; Lin et al., 2008). During prolonged drought, high leaf temperatures and water deficits result in strong decrease in photosynthesis and the emissions of volatile terpenoids (Jardine et al., 2015). The collapse of photosynthesis and the volatile terpenoids antioxidant system (that remove reactive oxygen species) results in membrane peroxidation and consequently the emissions of GLVs (Jardine et al., 2015). GLVs emissions are not only induced by high temperatures but also by fluctuations of light and temperature regularly observed in nature (Loreto, Barta, Brillì & Nogues, 2006).

Rainy season is marked with high humidity and warm weather conditions that favour faster growth accompanied with lower dry matter accumulation per shoot volume. Previous research identified that enzyme reactions controlling the dynamic metabolic systems in tea leaves are also responsible for changes in tea quality parameters during different seasons, with low enzyme activity in the rainy season (Muthumani et al., 2013) thus reducing the biosynthesis of secondary metabolites. Cold season is characterised with scanty rainfall, low temperatures and moderate humidity. Low temperatures cause slow shoot growth rates and favours longer accumulation of secondary metabolites (Rawat et al., 2008). These seasonal



changes affect plants further away from the equator much more than those close to the equator where seasonal variations are minimal (Owuor et al., 2011; Owuor et al., 2008a).

Geographical conditions influence VOCs biogenesis in plants, similarly, regions of cultivation and cultivars significantly affected the VOC composition (Owuor et al., 2008a; Tura et al., 2013). Teas grown at higher elevations possess superior aroma (Mahanta et al., 1988; Owuor et al., 1990b) but lower yields (Ng'etich et al., 2001; Othieno, Stephens & Carr, 1992). This is attributed to the slow rate of shoot growth due to cool temperatures at high altitudes. Indeed black teas from higher altitudes contained higher amounts of aromatic compounds and terpenes, which contribute positively to the aroma of black tea (Owuor et al., 1990b). The average air temperature, rainfall (both distributions and amounts), sunshine hours and cloud cover affects tea growth (Kumara et al., 2015; Squire, 1978; Squire, 1979) and hence VOCs. Tea in Kenya is grown in altitudes ranging from 1300 m to 2700 m amsl (Owuor, 1999). The chemical and quality variations occur due to the variation in the genetic make-up of the plants, even when they are grown under similar conditions in one environment (Cherotich, Kamunya, Alakonya, Msomba, Uwimana & Owuor, 2013a; Cherotich, Kamunya, Alakonya, Msomba, Uwimana & Owuor, 2014; Cherotich et al., 2013c; Kwach et al., 2014; Owuor, 2014; Owuor et al., 2000). Variations in environment and growing conditions cause variations in VOCs in plants. There is a decrease in growth rate of same cultivars of tea with rise in altitude, even within a radius of only 10 km where the variations in environmental conditions were considered minimal (Obaga, Squire & Langat, 1988; Squire, Obaga & Othieno, 1993). Indeed overall tea yields decrease with increase in altitude (Cherotich et al., 2013a; Cherotich et al., 2014; Cherotich et al., 2013c; Kwach et al., 2014; Kwach et al., 2016). In Kenya, where there is only a marginal difference in rainfall distribution, east and west of the Rift Valley, the same clone grown in different locations varies in chemical composition (Owuor et al., 1987a; Owuor et al., 1988). The effect of

seasons and region of production of tea cultivars on OVOCs has not been determined. In previous studies, mites infestations were higher in east than west of Rift Valley (Sudo, 1997; Sudo et al., 2011; Sudo et al., 1994). There is need to establish the effect of seasons and region of tea production on OVOCs emissions and relate to mites attack.

#### **2.4.6 Effect of nitrogenous fertilizer application rates on VOCs in relation to pests attack.**

Fertilizer is one of the major agro-inputs contributing to the cost of production and productivity in tea plantation. Nitrogen, potassium and phosphorous are three major nutrients required for the cultivation. Because tea is a leaf crop, nitrogen is the key element that promotes vegetative growth, improves shoot succulence, shoot size and leaf size (Sarwar et al., 2007). For most tea cultivars in Kenya, 100 to 150 kg N/ha/year range is the most economical (Owuor & Othieno, 1996b; Ruto et al., 1994). The recommended rate of fertilizer application in Kenya is 100 to 250 kg of nitrogen per hectare per year as NPKS 25:5:5:5 or NPK 20:10:10 (Anonymous, 2002; Othieno, 1988). In the Eastern Highlands of Kenya nitrogen application increased plant vigour and induced tolerance to attack by the red crevice mite (Sudo et al., 2001b). Application of nitrogenous fertilizers (NPKS 25: 5: 5:5) to tea that induced tolerance to the red crevice mite (*Brevipalpus phoenicis*, Geijskes) was found to be rates between 150 and 200 Kg N/ha/year. Rates above 400 Kg N/ha/year encouraged the buildup of mites on tea (Sudo et al., 2001b). In another study (Sudo, Khaemba & Wanjala, 1996), the levels of nitrogen in the leaf tissues increased with soil applied nitrogen and were related to the mite numbers on the leaves. Increasing N application increased the concentrations of GLVs such as 1-penten-3-ol (**16**), hexanal (**8**), Z-3-hexenal (**6**) and E-2-hexenal (**7**) (Wang, Huang, Liu & Jin, 2007). By contrast, increasing N supply decreased the concentration of an aromatic compound phenylacetaldehyde (**34**). Equally, there was an

increase in emission of several lipoxygenase pathway products from N-treated plants (Veromann et al., 2013). Fertilization rate had a significant effect on the emissions of VOCs (Sandrine et al., 2002). Plants that received little fertilization released significantly lower amounts of volatiles. The fertilization rate also had an effect on the overall odor blend of the compounds. Nitrogen fertilization significantly affected the composition and levels of plant VOCs and their attractiveness to pests (Cheng et al., 2010). Significant negative correlations between the abundance of *M. aeneus* larvae and  $\alpha$ -pinene (**81**), linalool (**66**), *E*- $\beta$ -farnesene (**96**), benzaldehyde (**30**) and positively correlated larval abundance with *Z*-3-hexenyl acetate (**12**), limonene (**76**) and indole (**33**) emission rates have been observed (Veromann et al., 2013). From a physiological point of view, increased growth rates due to fertilization can trade-off with carbon allocation to secondary metabolites, leading to reduced concentration of chemical defences in tissues of fast growing genotypes or plant species reduced resistance against herbivores in fertilized and fast-growing plants as observed in *P. pinaster* and *P. radiata* seedlings at field (Moreira, Sampedro, Zas & A., 2008). Nitrogen availability affects both the direct and indirect defence system of plants (Chen et al., 2010). Although a lot of studies have been carried out on clones and the two species of mites in Kenya as well as the effect of nitrogen fertilizer rates on the mites, no attempt has been made to determine whether the overhead VOCs influence clones resistance/tolerance or susceptibility. Similarly, no study has established the effect of nitrogen fertilizer rates on OVOCs in relation to mites infestations.

#### **2.4.7 Plant volatiles, insect pests attraction and/or repellency**

Repellency and/or attraction of insects to VOCs are investigated using behavioral assays such as Y-tube olfactometers. Colorado potato beetles, *Leptinotarsa decemlineata* (Say) was attracted to a blend of volatiles consisting of *Z*-3-hexenyl acetate

(12) and linalool (66) (Dickens, 2006). GLVs including hexanal (8), *E*-2-hexenal (7), *Z*-3-hexen-1-ol (9) and *Z*-3-hexenyl acetate (12) were reported to attract stem borer (Khan et al., 2008). *Z*- $\beta$ -ocimene (74), *E*- $\beta$ -ocimene (73) and *Z*-3-hexenyl acetate (12) attracted *Myllocerinus aurolineatus* (Coleoptera: Curculionidae) in Y-tube olfactometer (Sun, Wang, Cai, Jin, Gao & Chen, 2010). *Z*- $\beta$ -ocimene (73) was one of the most attractive volatiles to *Chlorophorus caragana* (Coleoptera: Cerambycidae) a trunk borer that feeds on *Caragana* shrubs (Zhang et al., 2015). *Z*-3-hexenyl acetate (12) attracted *Pantomorus cervinus* (Coleoptera: Curculionidae; (Wee, El-Sayed, Gibb, Mitchell & Suckling, 2008) while *E*- $\beta$ -ocimene (73) was attractant to insects in wheat (Buttery, Xu & Ling, 1985) and oats (Buttery, Ling & Wellso, 1982). The monoterpenes  $\alpha$ -pinene (81),  $\beta$ -phellandrene (79), linalool (66) and p-cymene (87) repelled *Nephotettix virescens* in a Y-tube olfactometer (Calumpang, Burgonio, Navasero & Navasero, 2013). *Fraxinus pennsylvanica* volatiles proved significantly repellent to gypsy moth larvae due to the combined repellencies of linalool (66), methyl salicylate (32), and *E*- $\beta$ -farnesene (96) (Marckovic, Norris, Phillips & Webster, 1996). Ethyl acetate extracts significantly reduced probing activity of *Aedes aegypti* (L.) that contained *E*- $\beta$ -caryophyllene (96), limonene (76),  $\alpha$ -pinene (81),  $\alpha$ -phellandrene (78) and myrcene (72) (Jaenson, Palsson & Borg-Karlson, 2006). Nerolidol (99) and cedrol (109) showed high repellent activity against *Cryptomeria* bark borer (Yatagai et al., 2002). Acetophenone (46) had a strong repellent activity on western pine beetles and the terpenes, *E*- $\beta$ -ocimene (73), *E*- $\beta$ -caryophyllene (94), humulene (100), (*E*)-4,8-dimethyl-1,3,7-nonatriene (89) (Erbilgin, Krokene, Kvamme & Christiansen, 2007) and  $\beta$ -cedrene (108) repelled stem borer (Khan et al., 2008). The monoterpenes p-cymene (87) and  $\alpha$ -phellandrene (78) repelled *Bemisia tabaci* when these compounds were applied on tomato (Bleeker et al., 2009) while linalool (66) repelled the aphid *Myzus persicae* in dual-choice assays (Aharoni et al., 2003) and other insects (Ayvaz, Sagdic, Karaborklu & Ozturk, 2010; Bowers, Ortega,

You & Evans, 1993). However, linalool (**66**) has also been reported as a male pheromone attractant to bee *Colletes cunicularius* (Borg-Karlson et al., 2003) and aphid (Pare et al., 1999). Ginger oil extract that repelled adult sweet potato whitefly, *Bemisia tabaci* in a vertical olfactometer experiment contained monoterpenes  $\alpha$ -phellandrene (**78**),  $\alpha$ -pinene (**81**) and myrcene (**72**) (Yang, Li, Wan, Liu & Johnson, 2010). Tea plants release over 600 VOCs that belong to GLVs, aromatic and terpenoid compounds amongst others (Robinson et al., 1992). Several of these compounds are released overhead tea plants (Cai et al., 2012) which could be affecting the response of mites attack to tea. Some cultivars are tolerant/resistant while others susceptible (Sudo, 1997; Sudo et al., 2011; Sudo et al., 1994). It is not documented if OVOCs released by tea cultivars attract and/or repel red spider and crevice mites to influence the response observed.

## CHAPTER THREE: METHODOLOGY

### 3.1 Area of study

The research was carried out in Kangaita Sub-station Tea Farm at Kerugoya, Kipkebe Tea Estate, Sotik and Timbilil Tea Estate, Kericho. Kangaita Tea Farm (Latitude 0°30'S, Longitude 37° 16'E, Altitude 2100m above mean sea level (amsl) is located on the slopes of Mt Kenya in the east of Great Rift Valley. It has a double maxima rainfall of 1500 mm – 1800 mm annually, which averagely occur between March – April and October – December. The region has relatively cool tropical annual temperature of 12°C – 19°C. The coldest months are July – August with mean temperature of 12°C – 19°C and the hottest being September and February with mean temp of 22°C – 24°C? Timbilil Estate (Tea Research Institute (formerly Tea Research Foundation of Kenya (TRFK) is in upper Kericho, (Latitude 0°22'S, Longitude 35° 21'E, Altitude 2180m amsl), in the west of the Great Rift Valley. It receives rains most of the year (an average of 1791mm annually), except in the months of January - February when there is drought. Kipkebe Tea Estate is located in Sotik (Latitude 0° 17'S, Longitude 35° 3'E, Altitude 1740m amsl) and receives annual rainfall ranging between 1300-1950mm while temperature ranges 16.6 - 20.4°C. The long rain starts in March -April while short rain starts in September -December.

### 3.2 Meteorological data collection

Monthly meteorological data of maximum and minimum temperatures, relative humidity and rainfall of the experimental areas (Kangaita, Timbilil and Kipkebe) were collected for eight months covering the three seasons (dry season, January- March; rainy season, April-.June and cold season, July-August) (Ng'etich et al., 1995). The environmental conditions during the long rains (April-.June) and short rains (September – November) are similar.

### 3.3.1 Cultivar evaluation (Mites)

The mites sampling was superimposed on on-going Clonal Field Trials at Kangaita, Timbilil and Kipkebe established in the year 2005 to evaluate the agronomic performance of the newly developed Kenyan (TRFK) clones, imported Tanzania (TRIT) clones relative to some commercial clones in production in Kenya (Anonymous, 2008). At each site, a randomized complete block design trial with three replicates, each plot consisting of 20 plants, planted at 1.22m by 0.75m planting spacing was laid out. The clones used were:- Tanzania clones: TRIT 201/16, TRIT 201/43, TRIT 201/44, TRIT 201/47, TRIT 201/50, TRIT 201/55, TRIT 201/70, TRIT 201/73, TRIT 201/75 and TRIT 201/82. New Kenyan clones: TRFK 18/19, TRFK 18/22, TRFK 18/3, TRFK 301/4, TRFK 301/5, TRFK 301/6, TRFK 303/1199, TRFK 303/178, TRFK 303/216, TRFK 303/259, TRFK 303/577, TRFK 371/3, TRFK 371/6, TRFK 371/8, TRFK 381/5, TRFK 400/4, TRFK 400/10, TRFK 430/5, TRFK 430/7, TRFK 430/61, TRFK 430/63, TRFK 430/90, TRFK 480/378, TRFK 481/200 and TRFK 481/272. Commercial clones: AHP SC12/28, AHP S15/10, AHP SC31/37, EPK C12, EPK TN15-23, TRFCA SFS150, TRFK 11/4, TRFK 12/19, TRFK 31/8 and TRFK 6/8. Susceptible clones TRFK 54/40 and STC 5/3 in Kangaita, Timbilil and Kipkebe from different plots were used as positive control. After bringing into bearing, the clones received recommended agronomic and cultural management inputs (Anonymous, 2002).

Determination of natural mites infestations levels on these clones was carried out according to (Sudoj et al., 2011). The numbers of mites on clones were assessed by sampling 10 maintenance leaves that were randomly plucked from 5 bushes per plot. The mites on the leaves were counted directly under a dissecting microscope after extracting them from the leaves using mite brushing machine (Model–Leedom Engineering, USA). The procedure was carried out in triplicate.

### **3.3.2 Cultivar evaluation (OVOCs)**

Resistant/tolerant and susceptible clones screened in Section 3.3.1 together with susceptible clones STC 5/3 and TRFK 54/40 (Sudoj, 1997) which were used as positive control were evaluated for OVOCs. Volatile collection in the field was carried out according to the method of Chen *et al* (Chen, Whitehill, Bonello & Poland, 2011). Shoots of the tea varieties were individually enclosed in large oven bags (355 mm x 508 mm) (that had been conditioned in an oven at 170°C) together with two Teflon pipes and tied with elastic bands. Activated charcoal-purified air generated by a pump entered the bag through one pipe flowing at 100 ml min<sup>-1</sup>. The air exiting the bag passed through an adsorbent trap consisting of a 200 × 7 mm glass tube containing 30 mg Alltech Super-Q, 80/100 mesh (Alltech Associates Inc., Deerfeld, IL) adsorbent material. Collected volatiles were eluted from the Super-Q (N,N-Didecyl-N,N-dimethylammonium chloride) adsorbent with 1ml dichloromethane (HPLC grade; purity: 99%) and 80 ng of cumene internal standard added and then concentrated to 20 µl with a stream of nitrogen gas while cooling under ice. Additionally, blanks (odours collected from empty bags only) were collected to verify a lack of background presence of reported VOCs. Two replicates were made on different days but at the same time of day i.e. from 0700 to 1100 h. OVOCs collections were done at Kangaita, Timbilil and Kipkebe during dry, rainy and cold seasons.

### **3.4 Influence of rates of nitrogenous fertilizer application on OVOC levels or composition in relation to mites infestations**

The experiment was conducted in Timbilil to find out the optimum levels of NPKS fertilizer applied to clonal teas that will discourage mites' infestation and to relate the optimum fertilizer to VOC composition/levels. The experiment was superimposed on the ongoing nitrogenous fertilizer rates trial on clone TRFK 6/8 (Kwach *et al.*, 2014; Kwach *et*



al., 2016; Msomba et al., 2014). The trial is set in a randomized complete block design replicated three times. It is set in a factorial design with fertilizer N: P: K: S (25:5:5:5) rates 0, 75, 150, 225 and 300 Kg N/ha/year as a factor. Prior to setting up the experiments, the field had received 150 Kg N/ha/year while the control had not had any fertilizer application. Fertilizer application was done in the month of June and both mites screening and VOCs trapping were carried out in February in the year 2012 and 2013 respectively.

### **3.5 Volatile analysis and identification**

Both GC and GC-MS were used for analysis. The GC analysis was carried on a Shimadzu model GC-2010 equipped with flame ionization detector. A 50 m silica gel capillary column (film thickness 0.20  $\mu\text{m}$ , 0.25 mm inner diameter) was used. Oven temperature was programmed from 35 to 230°C with the initial temperature maintained for 5 min then 5 °C/min to 190 °C, then at 50°C/min to 230 °C, and followed by 5 min hold at 230 °C. The flow rate for the carrier gas ( $\text{N}_2$ ) was 3.0 ml/min and for detector gases 40.0 ml/min (hydrogen) and 400.0 ml/min (air), respectively. The detector temperature was set at 230.0°C. A sample volume of 1 $\mu\text{l}$  was injected in splitless mode. Analysis on GC-MS was conducted using an Agilent 7890A Series Gas Chromatograph coupled to a Mass Spectrometer (Agilent 5973 quadrupole detector). The gas chromatographic conditions were as follow: helium was used as the carrier gas at a flow rate of 1.25 ml/min; Inlet temperature 270°C, transfer line temperature of 280°C, and column (HP-5MS 30 m $\times$ 0.25 mm $\times$ 0.25  $\mu\text{m}$  film) oven temperature programmed from 35 to 280°C with the initial temperature maintained for 5 min then 10 °C/min to 280 °C for 10.5 min and the final temperature for 29.9 min 50 °C/min to 285 °C. Parameters for electron impact sample ionization were as follow: mass selective detector maintained at an ion source temperature of 250°C and quadrupole temperature of 180°C, electron energy, 70 eV; source temperature, 250°C. Fragment ions were analyzed

over 40-550 m/z mass range in the full scan. The compounds were quantified as ratio of the peak area of each compound to that of cumene (internal standard). Identification of compounds was by comparing the fragmentation pattern with mass spectral data in mass spectral library (NIST/EPA/NIH., 2008) and literature. Confirmation of compounds was done by injecting samples with VOCs authentic standards which led to the enhancement of the peak being analysed at a particular time. The authentic standard was then analysed alone. Formation of a peak with the same retention time and fragmentation pattern confirmed the identity of the compound being analysed.

### **3.6 Behavioral responses of mites to trapped volatile organic compounds.**

#### **3.6.1 Collection and rearing of mites**

Red crevice (*Brevipalpus phoenicis* Geijskes) and red spider (*Oligonychus coffeae* Nietner) mites were collected from tea plantations of TRFK, Kangaita (Latitude 0°30'S, Longitude 37° 16'E, Altitude 2100 m amsl) and Timbilil (Latitude 0°22'S, Longitude 35° 21'E, Altitude 2180 m amsl) respectively. Rearing of the mites was done in the greenhouse according to Sanjaya *et al* (Sanjaya, Virginia, Ocampo, Barbara & Caoili, 2013) with minor modifications. After field collection, the mites were immediately transferred onto 1-year-old potted susceptible tea plants (Sudoj, 1997) (TRFK 54/40 and STC 5/3 for red crevice and red spider mites respectively) grown under greenhouse conditions and were used as stock culture. From the stock, the adult mites were transferred onto cuttings of the same clones planted in 100 ml plastic beakers. Plastic beakers were kept under controlled conditions of  $25 \pm 1^{\circ}\text{C}$ ,  $75 \pm 5\%$  RH and 16L: 8D photoperiod. Withered and drying cuttings were regularly replaced.

### 3.6.2 Odour sources

Odour sources were of analytical grades purchased from Sigma-Aldrich (Purity: 99%). A number of VOCs chosen were released by the cultivars in substantial amounts implying that they may influence the susceptibility/resistance of cultivars to red crevice and red spider mites.

### 3.6.3 Behavioral responses of mites in the Y-tube olfactometer

The set up was carried out according to Calumpang *et al* (Calumpang et al., 2013) with some modifications. The base and arms of the olfactometer was each 10 cm long and the angle between the two arms 120°. The internal diameter was 1 cm and each arm was connected to either a glass flask containing an odour source or control flask.

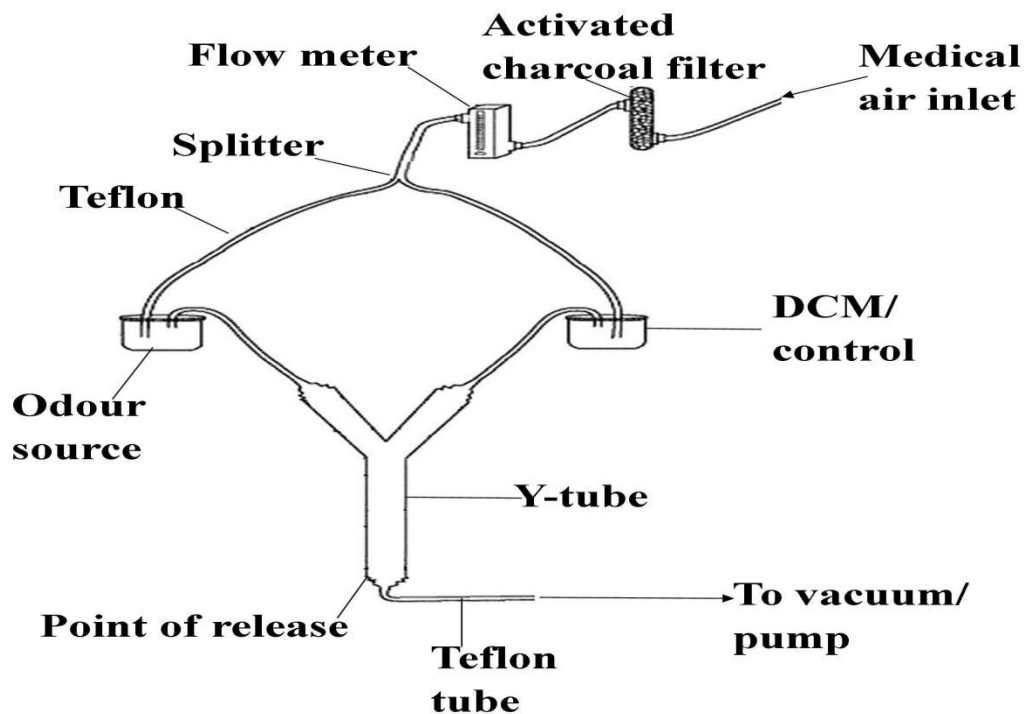


Figure 11: Y-tube olfactometer set up

A pump was connected to the base of the Y-tube to draw the air through an activated charcoal filter before entering the Y-tube so that it was purified of any contaminating odours as illustrated in Figure 11. This was conducted at room temperature ( $24 \pm 1^\circ\text{C}$ ) and 50-60 RH. The airflow through each of the arms was maintained at 100 ml/min. Twenty individuals of each mite species were tested against each odour source replicated three times. They were presented with a choice between the test odour and a control. The olfactometer arm chosen by the insects after a 5-min period were recorded. The olfactometer set-up was rinsed with soap water, and ethanol, and then air-dried and the treatment arms reversed after each replication to eliminate directional bias. Each VOC was tested at a concentration of 100  $\mu\text{g}$ . Each compound was delivered as 10  $\mu\text{l}$  sample placed on filter paper strips (7 x 38 mm Whatman no. 1).

### **3.7 Statistical analysis of results**

The obtained mites data were transformed,  $\log_e(x+1)$  to meet assumptions of normality and homogeneity of variances and then subjected to analysis of variance (ANOVA) using MSTAT-C statistical package. For mites infestations levels in clones, factorial three design was used with site as first factor, genotype as second factor and months as third factor. Analysis of mites levels in each site was done by factorial two design with genotype as the first factor and months as the second factor. For clone TRFK 6/8 trial, randomised complete block design was used in the analysis with fertilizer rates as a factor. Extent of variation in OVOCs was calculated as standard deviation/means x100. Free-choice bioassay data were also subjected to analysis of variance (ANOVA). The means were separated using least significant difference (LSD) at 5% level of probability and Excel Microsoft Office was used to perform regression analyses.

## CHAPTER FOUR: RESULTS AND DISCUSSIONS

### 4.1 Determination of mites infestations levels in the imported and new clones in clonal adaptability trials and their characterization as resistant or susceptible to mites

The three locations where the trials were conducted were characterized by differences in temperatures, rainfall, relative humidity and clonal tea mites infestations levels which also varied with time of the year (Table 2). The mites were highest in March in all sites which was the driest month characterised by high maximum temperature, low relative humidity and low rainfall (Table 2). In previous studies, variations in temperatures (Ahmed, 2012; Ahmed et al., 2012), rainfall (Ahmed, 2012; Ahmed et al., 2012; Sudoi et al., 1994) and relative humidity (Ahmed, 2012; Ahmed et al., 2012) were associated with changes in mites populations in tea farms. The variations in the weather parameters in this study led to changes in mites populations which varied with sites (Table 3). Kangaita, near Mount Kenya in the east of Rift Valley recorded higher ( $p \leq 0.05$ ) mites infestations than Kipkebe and Timbilil, in the west of Rift Valley. Despite the differences in clones used in this study, the trend was similar to previous observations where mite infestations were higher in the east than west of the Rift Valley in Kenya (Sudoj et al., 1994). The differences were therefore due to the environmental factors, not the cultivars. Thus, geographical region of tea production is a critical factor in mite infestations on Kenyan tea. Tea producers/plantations in the east of the Rift Valley therefore require cultivars resistant/tolerant to mites attack for realization of high yields.

In the west of the Rift Valley, the mite infestation levels in Kipkebe were higher ( $p \leq 0.05$ ) than those in Timbilil. Similar clonal variations in mites infestations had been observed in previous studies (Ahmed, 2012; Sudoi et al., 2011; Sudoi et al., 1994; Sudoi, Khaemba & Wanjala, 2001a; Sudoi, 1996). High temperatures and low rainfall have been associated with high mite infestations in tea in Bangladesh (Ahmed, 2012). The difference in

mite infestations in these two sites was attributed to differences in rainfall and temperatures (Table 2). Generally, Kipkebe site recorded higher monthly maximum temperatures and lower rainfall than Timbilil site. These results demonstrated that even in areas of low mite infestations, dry weather conditions and low rainfall could lead to high levels of the pests. The results demonstrate a possible future challenge for the tea industry in Kenya. Due to climate change, the tea growing areas in Kenya have been observing increased temperatures (Adhikari, Nejadhashemi & Woznicki, 2015; Bilham, 2011) and prolonged droughts (Stephens, Othieno & Carr, 1992). This implies that in future, mites infestations may increase. Areas already recording high mites population incidences may record much higher numbers, while areas currently recording low mite infestations incidences may witness increased incidences and level of the infestations (Table 3).

The clonal mites demonstrated that the cultivars under test were different in their tolerance/resistance or susceptibility to mites attack. Cultivar selection is therefore a viable option in the management of mites infestations in tea production. Clones AHP S 15/10, EPK TN 15-23, TRFK 18/3 and TRIT 201/16 ranked highest in terms of their susceptibility to mites while TRIT 201/50, TRFK 6/8, and TRFK 303/1199 were the most tolerant/resistant to mites (Table 3). Despite the lower ( $p \leq 0.05$ ) mite infestations levels in Kipkebe and Timbilil than in Kangaita, clones that had high or low levels of infestation were similar in all locations. However, the extent of the variations in infestations levels in different clones at different locations varied culminating in significant ( $p \leq 0.05$ ) interactions effects between the population of mites on clones and locations (Table 3). Thus, the change in magnitude of mite infestations in one cultivar cannot be used to predict the possible changes in different cultivar.

Table 2: Summary of the weather parameters and mites infestation levels in the three sites

Month	Site																	
	Kangaïta/Mount Kenya						Kipkebe/Sotik						Timbilil/Kericho					
	Max. temp (°C)	Min. temp (°C)	Mean temp (°C)	Mean rainfall (mm)	Mean RH (%)	Mean mite	Max. temp (°C)	Min. temp (°C)	Mean temp (°C)	Mean rainfall (mm)	Mean RH (%)	Mean mite	Max. temp (°C)	Min. temp (°C)	Mean temp (°C)	Mean rainfall (mm)	Mean RH (%)	Mean mite
Jan	23.5	9.0	16.3	17.2	38.0	18	28.7	11.0	19.9	0.0	56.0	6	25.7	7.7	16.7	0.5	46.0	3
Feb	21.4	9.1	15.3	19.7	35.0	21	28.4	11.7	20.1	26.8	70.4	9	26.3	9.1	17.7	82.9	46.0	5
Mar	23.9	10.3	17.1	40.3	38.0	23	28.7	10.1	19.4	27.7	70.4	12	27.5	8.5	18.0	50.1	44.0	8
Apr	18.8	13.5	16.2	449.6	55.0	18	26.7	12.3	19.5	398.4	82.2	7	27.5	8.5	18.0	514.4	44.0	3
May	19.3	13.5	16.4	692.0	46.0	10	27.0	12.2	19.6	146.1	80.6	3	23.1	9.0	16.1	249.4	70.0	3
June	16.9	12.7	14.8	88.4	61.0	2	26.0	11.2	18.6	226.9	84.8	2	22.2	9.7	16.0	178.3	75.0	1
July	14.6	11.9	13.3	49.1	72.0	2	26.6	11.6	19.1	160.9	83.1	2	21.8	10.4	16.1	122.6	79.0	1
Aug	16.4	10.7	13.6	190.5	46.0	2	26.6	11.1	18.9	4.8	80.9	1	22.8	9.4	16.1	97.7	70.0	1
Mean	19.5	11.3	15.4	193.4	48.9	12	27.3	11.4	19.4	124.0	76.1	5	24.6	9.0	16.8	162	59.3	3

RH = Relative humidity

Table 3: Changes in the dynamics of red crevice and red spider mites with clones in the three sites

Clone	Kangaita	Rank	Kipkebe	Rank	Timbilil	Rank	Mean clone	Stdev	Variations (%)
AHP SC 12/28	23 (2.76)	5	9 (2.01)	4	4 (1.51)	6	12 (2.09)	10	83
AHP S 15/10	27 (2.97)	3	11 (2.14)	1	5 (1.61)	2	14 (2.24)	11	79
AHP SC 31/37	12(2.19)	19	4 (1.56)	26	3 (1.38)	19	6 (1.71)	5	83
EPK C12	7(1.74)	33	7 (1.44)	11	3 (1.19)	34	6 (1.46)	2	33
EPK TN 15-23	29 (2.84)	2	7 (1.75)	9	3 (1.18)	35	13 (1.92)	11	85
TRFCA SFS 150	17 (2.50)	8	8 (1.86)	5	4 (1.40)	10	10 (1.92)	5	50
TRFK 11/4	14 (2.51)	12	6 (1.71)	15	4 (1.58)	5	8 (1.93)	7	88
TRFK 12/19	15 (2.37)	10	4 (1.40)	34	4 (1.35)	12	8 (1.70)	6	75
TRFK 18/19	15 (2.24)	11	4 (1.41)	33	4 (1.50)	7	8 (1.72)	6	75
TRFK 18/22	12 (2.31)	17	6 (1.76)	14	3 (1.33)	21	7 (1.80)	5	71
TRFK 18/3	26 (2.82)	4	11 (2.07)	2	5 (1.41)	4	14 (2.10)	11	79
TRFK 301/4	5 (1.66)	41	3 (1.28)	40	3 (1.15)	38	4 (1.36)	1	25
TRFK 301/5	9 (2.15)	27	6 (1.81)	13	4 (1.42)	9	6 (1.79)	3	50
TRFK 301/6	6 (1.62)	38	4 (1.29)	36	3 (1.10)	41	4 (1.34)	2	50
TRFK 303/1199	6 (1.61)	39	3 (1.32)	39	2 (1.11)	43	4 (1.35)	2	50
TRFK 303/178	7 (1.70)	34	3 (1.35)	38	3 (1.31)	24	4 (1.45)	2	50
TRFK 303/216	7 (1.69)	35	5 (1.49)	23	4 (1.25)	16	5 (1.48)	2	40
TRFK 303/259	10 (2.31)	24	5 (1.67)	19	4 (1.47)	8	6 (1.81)	3	50
TRFK 303/577	6 (1.54)	40	4 (1.29)	36	3 (1.15)	38	4 (1.33)	2	50
TRFK 31/8	6 (1.75)	36	3 (1.21)	44	3 (1.24)	29	4 (1.4)	2	50
TRFK 371/3	19 (2.39)	6	5 (1.49)	23	7 (1.33)	21	9 (1.74)	6	67
TRFK 371/6	6 (1.66)	37	4 (1.43)	30	3 (1.34)	14	5 (1.48)	1	20
TRFK 371/8	12 (2.24)	18	6 (1.64)	17	3 (1.39)	11	7 (1.76)	4	57
TRFK 381/5	10 (2.00)	26	5 (1.53)	27	3 (1.33)	21	6 (1.62)	4	67
TRFK 400/10	14 (2.31)	14	6 (1.91)	12	2 (1.34)	20	8 (1.85)	6	75
TRFK 400/4	11 (2.10)	22	5 (1.48)	25	7 (1.68)	1	8 (1.75)	3	38
TRFK 430/5	8 (1.97)	29	8 (1.61)	6	4 (1.33)	21	6(1.57)	3	50
TRFK 430/61	12 (2.18)	20	5 (1.62)	21	3 (1.17)	36	7(1.66)	5	71
TRFK 430/63	13 (2.01)	16	5 (1.63)	20	3 (1.20)	32	7 (1.76)	5	71
TRFK 430/7	11 (2.01)	23	6 (1.68)	16	2 (1.13)	42	6 (1.61)	5	23
TRFK 480/378	9 (2.06)	28	4 (1.50)	28	3 (1.27)	26	5 (1.61)	3	60
TRFK 480/90	17 (2.25)	9	7 (1.67)	18	4 (1.24)	17	9 (1.67)	7	78
TRFK 481/200	14 (2.25)	15	7 (1.76)	8	3 (1.27)	26	8 (1.76)	6	75
TRFK 481/272	10 (2.21)	25	3 (1.28)	40	3 (1.22)	31	5 (1.57)	4	80
TRFK 6/8	5 (1.68)	41	3 (1.25)	43	2 (1.04)	45	3 (1.33)	2	67
TRIT 201/16	35 (2.90)	1	10 (2.05)	3	5 (1.55)	3	16 (2.16)	16	100
TRIT 201/43	7 (1.77)	31	4 (1.43)	30	3 (1.27)	26	2 (1.49)	2	100
TRIT 201/44	5 (1.53)	44	4 (1.42)	32	4 (1.34)	20	5 (1.43)	1	20
TRIT 201/47	18 (2.44)	7	4 (1.53)	27	3 (1.24)	29	4 (1.74)	8	200
TRIT 201/50	5 (1.48)	45	3 (1.19)	45	4 (0.95)	18	8 (1.20)	1	13
TRIT 201/55	7 (1.77)	31	4 (1.38)	35	2 (1.08)	44	4 (1.41)	3	75
TRIT 201/70	12 (2.16)	21	7 (1.77)	7	4 (1.35)	12	9 (1.76)	4	44
TRIT 201/73	7 (1.83)	30	3 (1.26)	42	3 (1.20)	32	4 (1.43)	2	50
TRIT 201/75	14 (2.42)	13	6 (1.60)	18	3 (1.16)	37	8 (1.73)	5	50
TRIT 201/82	5 (1.57)	43	4 (1.40)	34	3 (1.14)	40	4 (1.36)	1	25
Mean site	12 (2.11)		5 (1.56)		3 (1.29)		6 (1.65)	4	67

CV (%)

18.86 (2.94)

LSD (p≤0.05)

0

0

Interactions (p≤0.05)

0

Variations (%), calculated as  $\frac{STDEV}{Mean} * 100$

Monthly number of red crevice / red spider mites per 10 leaves of clone

Note: Figures in parenthesis are Log<sub>e</sub> (x+1) transformation of mite population



Use of resistant tea varieties is one of the components of integrated pest and disease management (Ahmed, 2012; Barthakur, Dutta & Karan, 1992). Indeed, use of resistant tea varieties may offer a solution to farmers and associated stakeholders who face the challenge of growing the beverage crop in a sustainable way to obtain optimal yield while maintaining the biodiversity and soil fertility with least ecological disruption (Lehmann-Danzinger, 2000). The difference in clonal tea mite infestations had been attributed to morphometric and genetic variability that exist among tea cultivars (Banerjee, 1987; Ghosh, 2001). Indeed, mites levels have been claimed to be also influenced by certain biochemical processes that take place within the tea leaves (Danathanarayan & Ranaweera, 1972).

Changes in weather parameters especially rainfall, temperature and humidity influence mites infestation levels (Ahmed, 2012; Ahmed et al., 2012). Variations in the dynamics of mites with clones and months in Kangaita, Kipkebe and Timbilil are presented in Tables 4, 5 and 6, respectively. Monthly mites population varied significantly ( $p \leq 0.05$ ) in the different clones in all the sites (Tables 4, 5 and 6). The seasonal dynamics of monthly variations were much higher in Kangaita (Table 4) compared to Kipkebe (Table 5) and Timbilil (Table 6). Consequently, cultivars that were very susceptible to mite infestations in Kangaita only showed moderate infestation levels in Kipkebe and low infestation in Timbilil. All clones in Timbilil had very low population of mites throughout the period (Table 6), including clones that had high infestation in both Kangaita and Kipkebe. Similar observation had been recorded in previous studies (Sudoj et al., 2011; Sudoj et al., 1994; Sudoj et al., 2001a). These results show that mite infestation was a minor threat to tea production in areas with similar weather factors such as Timbilil. However, it was a major problem in areas with weather factors similar to Kangaita in the east of Rift Valley.

The results demonstrate that for every location of tea production, it is necessary to select cultivars that can withstand mite infestations to reduce production losses. Areas with weather factors similar to Kangaita should only commercially exploit resistant tea cultivars.

Table 4: Changes in the dynamics of red crevice mites with clones and months in Kangaita

Clone	January	February	March	April	May	June	July	August	Mean clone	ST DEV	Variations (%)
AHP SC 12/28	40 (3.72)	27 (3.33)	34 (3.54)	44 (3.81)	27 (3.33)	4 (1.52)	3 (1.46)	3 (1.38)	23 (2.76)	7	74
AHP S 15/10	15 (2.77)	33 (3.53)	59 (4.10)	68 (4.23)	24 (3.21)	7 (2.11)	6 (1.90)	6 (1.93)	27 (2.97)	24	29
AHP SC 31/37	31 (3.46)	16 (2.83)	18 (2.97)	12 (2.57)	8 (2.15)	2 (1.20)	3 (1.25)	2 (1.06)	12 (2.19)	10	83
EPK C12	11 (2.48)	13 (2.61)	14 (2.68)	6 (2.00)	4 (1.55)	1 (0.83)	1 (0.69)	2 (1.06)	7 (1.74)	5	71
EPK TN 15-23	88 (4.49)	47 (3.87)	48 (3.90)	25 (3.26)	18 (2.97)	3 (1.37)	4 (1.60)	3 (1.27)	29 (2.84)	30	103
TRFCA SFS 150	56 (4.04)	27 (3.33)	17 (2.88)	17 (2.87)	8 (2.24)	3 (1.47)	5 (1.83)	3 (1.33)	17 (2.50)	18	106
TRFK 11/4	27 (3.34)	34 (3.54)	26 (3.29)	10 (3.32)	3 (2.40)	3 (1.43)	3 (1.29)	3 (1.50)	14 (2.51)	13	73
TRFK 12/19	33 (3.52)	19 (2.99)	29 (3.39)	12 (2.58)	16 (2.86)	1 (0.83)	3 (1.37)	3 (1.44)	15 (2.37)	12	80
TRFK 18/19	31 (3.46)	16 (2.86)	44 (3.81)	18 (2.95)	8 (2.26)	3 (1.37)	2 (1.13)	1 (0.83)	15 (2.24)	15	100
TRFK 18/22	7 (2.11)	29 (3.42)	30 (3.43)	17 (2.89)	14 (2.73)	3 (1.29)	2 (1.20)	3 (1.43)	13 (2.31)	11	85
TRFK 18/3	44 (3.81)	66 (4.21)	36 (3.60)	34 (3.56)	19 (3.00)	6 (2.00)	2 (1.20)	2 (1.23)	26 (2.82)	23	88
TRFK 301/4	9 (2.29)	11 (2.51)	9 (2.30)	6 (1.99)	3 (1.46)	2 (0.96)	2 (1.06)	1 (0.69)	5 (1.66)	4	80
TRFK 301/5	13 (2.63)	16 (2.86)	7 (2.70)	18 (2.93)	7 (2.07)	3 (1.43)	2 (1.11)	4 (1.52)	9 (2.15)	6	67
TRFK 301/6	7 (2.10)	16 (2.85)	12 (2.56)	2 (1.27)	4 (1.61)	1 (0.83)	2 (0.92)	1 (0.83)	6 (1.62)	6	100
TRFK 303/1199	4 (1.60)	15 (2.75)	13 (2.67)	5 (1.83)	3 (1.30)	2 (0.96)	2 (0.92)	1 (0.83)	6 (1.61)	5	83
TRFK 303/178	8 (2.17)	17 (2.87)	16 (2.85)	2 (1.23)	3 (1.43)	4 (1.56)	2 (1.00)	2 (0.92)	7 (1.70)	6	86
TRFK 303/216	4 (1.52)	17 (2.87)	17 (2.88)	5 (1.79)	2 (1.13)	1 (0.69)	3 (1.37)	3 (1.29)	7 (1.69)	7	100
TRFK 303/259	11 (2.50)	14 (2.74)	7 (2.10)	19 (3.00)	23 (3.17)	4 (1.60)	2 (1.16)	2 (1.20)	10 (2.31)	8	80
TRFK 303/577	7 (2.14)	7 (2.13)	8 (2.90)	17 (1.60)	4 (1.25)	2 (0.83)	1 (0.69)	2 (0.83)	6 (1.54)	5	83
TRFK 31/8	5 (1.73)	12 (2.61)	15 (2.77)	8 (2.19)	6 (1.92)	1 (0.83)	1 (0.83)	2 (1.13)	6 (1.75)	5	83
TRFK 371/3	20 (3.04)	21 (3.07)	23 (3.17)	22 (3.12)	24 (3.21)	2 (1.06)	2 (0.96)	3 (1.46)	19 (2.39)	10	67
TRFK 371/6	4 (1.60)	11 (2.45)	17 (2.91)	5 (1.73)	3 (1.29)	2 (0.96)	3 (1.33)	2 (1.00)	6 (1.66)	5	83
TRFK 371/8	21 (3.11)	26 (3.28)	23 (3.19)	7 (2.11)	11 (2.45)	2 (1.13)	3 (1.34)	3 (1.29)	12 (2.24)	10	33
TRFK 381/5	6 (1.96)	20 (3.05)	24 (3.20)	16 (2.85)	6 (1.94)	2 (1.16)	2 (0.83)	2 (1.00)	10 (2.00)	9	19
TRFK 400/10	23 (3.17)	16 (2.82)	31 (3.48)	24 (3.20)	12 (2.58)	2 (1.06)	2 (1.13)	2 (1.06)	14 (2.31)	11	79
TRFK 400/4	16 (2.84)	22 (3.12)	22 (3.14)	14 (2.87)	8 (2.15)	1 (0.69)	2 (0.92)	2 (1.06)	11 (2.10)	9	82
TRFK 430/5	8 (2.22)	15 (2.75)	14 (2.73)	14 (2.70)	7 (2.11)	2 (0.92)	2 (1.06)	3 (1.29)	8 (1.97)	6	75
TRFK 430/61	14 (2.72)	19 (3.02)	20 (3.03)	26 (3.30)	8 (2.24)	2 (1.16)	2 (1.23)	1 (0.69)	12 (2.18)	10	83

Table 4 cont...

Clone	January	February	March	April	May	June	July	August	Mean clone	ST DEV	Variations (%)
TRFK 430/63	21 (3.11)	33 (3.52)	20 (3.04)	15 (2.79)	7 (2.04)	1 (0.69)	2 (1.06)	1 (0.69)	13 (2.01)	12	93
TRFK 430/7	16 (2.83)	18 (2.95)	20 (3.04)	43 (3.79)	7 (2.04)	1 (0.69)	2 (1.06)	1 (0.69)	11 (2.01)	14	127
TRFK 480/378	9 (2.26)	15 (2.78)	20 (3.06)	10 (2.37)	5 (1.76)	2 (1.06)	5 (1.72)	3 (1.43)	9 (2.06)	6	67
TRFK 480/90	36 (3.61)	32 (3.49)	29 (3.39)	22 (3.13)	7 (2.11)	2 (1.06)	2 (1.00)	2 (1.13)	17 (2.25)	15	88
TRFK 481/200	14 (2.72)	32 (3.49)	29 (3.39)	22 (3.13)	7 (2.11)	2 (1.06)	2 (1.00)	2 (1.13)	14 (2.25)	12	86
TRFK 481/272	10 (2.42)	24 (3.20)	18 (2.94)	12 (2.57)	9 (2.31)	3 (1.48)	3 (1.34)	3 (1.37)	10 (2.21)	8	80
TRFK 6/8	4 (1.67)	7 (2.06)	8 (2.91)	9 (2.26)	4 (1.67)	1 (0.69)	2 (1.06)	2 (1.16)	5 (1.68)	5	100
TRIT 201/16	26 (3.29)	55 (4.02)	74 (4.32)	98 (4.40)	19 (2.99)	3 (1.43)	3 (1.37)	3 (1.37)	35 (2.90)	36	103
TRIT 201/43	6 (1.98)	17 (2.91)	20 (3.03)	8 (2.19)	4 (1.52)	2 (0.96)	1 (0.69)	1 (0.83)	7 (1.77)	7	100
TRIT 201/44	6 (1.94)	9 (2.29)	15 (2.77)	3 (1.43)	2 (1.06)	1 (0.83)	1 (0.69)	2 (1.20)	5 (1.53)	5	100
TRIT 201/47	22 (3.12)	25 (3.07)	23 (3.16)	21 (3.11)	45 (3.83)	3 (1.27)	4 (1.69)	3 (1.25)	8 (2.44)	15	83
TRIT 201/50	6 (1.90)	9 (2.29)	9 (2.29)	5 (1.73)	2 (0.96)	2 (0.96)	1 (0.69)	2 (0.96)	5 (1.48)	3	60
TRIT 201/55	11 (2.49)	17 (2.88)	16 (2.84)	5 (1.73)	2 (1.23)	1 (0.83)	2 (1.16)	2 (1.00)	7 (1.77)	7	100
TRIT 201/70	4 (2.58)	29 (3.41)	30 (3.43)	18 (2.95)	8 (2.15)	1 (1.06)	1 (0.83)	1 (0.83)	12 (2.16)	12	100
TRIT 201/73	5 (1.83)	12 (2.60)	17 (2.87)	10 (2.40)	3 (1.45)	2 (1.06)	3 (1.29)	2 (1.13)	7 (1.83)	6	86
TRIT 201/75	33 (3.54)	17 (2.90)	19 (2.98)	15 (2.80)	15 (2.79)	2 (0.92)	3 (1.37)	7 (2.07)	14 (2.42)	10	71
TRIT 201/82	4 (1.60)	10 (2.40)	14 (2.72)	7 (2.07)	4 (1.54)	1 (0.83)	1 (0.69)	1 (0.69)	5 (1.57)	5	100
Mean month	18 (2.65)	21 (2.98)	23 (3.09)	18 (2.61)	10 (2.10)	2 (1.11)	2 (1.15)	2 (1.15)	12 (2.11)	8	20
CV (%)					15.71 (2.75)						
LSD (p≤0.05)					0				0		
Interactions (p≤0.05)					0						

Monthly number of red crevice / red spider mites per 10 leaves of clone

Note: Figures in parenthesis are  $\text{Log}_e(x+1)$  transformation of mite population

Table 5: Changes in the dynamics of red spider mites with clones and months in Kipkebe

Clone	January	February	March	April	May	June	July	August	Mean lone	ST DEV	Variations (%)
AHP SC 12/28	14 (2.70)	16 (2.85)	12 (2.60)	23 (3.17)	4 (1.52)	2 (1.13)	2 (1.13)	2 (0.963)	9 (2.01)	8	89
AHP S 15/10	5 (1.71)	12 (2.53)	27 (3.34)	21 (3.10)	10 (2.42)	3 (1.37)	3 (1.37)	3 (1.27)	11(2.14)	9	82
AHP SC 31/37	3 (1.50)	7 (2.06)	8 (2.25)	7 (2.10)	3 (1.42)	2 (1.10)	2 (1.06)	2 (0.96)	4 (1.56)	3	75
EPK C12	5 (1.80)	7 (2.09)	10 (2.41)	5 (1.78)	2 (1.00)	1 (0.83)	1 (0.83)	1 (0.83)	7 (1.44)	3	43
EPK TN 15-23	16 (2.85)	17 (2.88)	10 (2.42)	3 (1.34)	4 (1.61)	1 (0.69)	2 (1.11)	2 (1.06)	7 (1.75)	7	100
TRFCA SFS 150	24 (3.20)	16 (2.81)	12 (2.54)	5 (1.83)	4 (1.60)	1 (0.69)	3 (1.39)	1 (0.83)	8 (1.86)	8	100
TRFK 11/4	7 (2.11)	13 (2.61)	14 (2.73)	6 (1.94)	6 (1.96)	1 (0.83)	1 (0.80)	1 (0.69)	6 (1.71)	5	83
TRFK 12/19	3 (1.27)	9 (2.29)	4 (1.69)	5 (1.71)	4 (1.52)	1 (0.83)	1 (0.83)	2 (1.06)	4 (1.40)	3	75
TRFK 18/19	2 (1.16)	4 (1.52)	5 (1.76)	5 (1.71)	6 (1.90)	3 (1.37)	2 (1.06)	1 (0.83)	4 (1.41)	2	50
TRFK 18/22	3 (1.50)	13 (2.61)	10 (2.39)	14 (2.69)	5 (1.78)	2 (1.16)	2 (1.13)	1 (0.83)	6 (1.76)	5	33
TRFK 18/3	22 (3.13)	23 (3.18)	16 (2.80)	10 (2.37)	7 (2.02)	2 (1.13)	2 (0.92)	2 (0.96)	11 (2.07)	9	82
TRFK 301/4	3 (1.37)	4 (1.54)	5 (1.71)	4 (1.60)	2 (0.96)	1 (0.83)	3 (1.27)	2 (0.96)	3 (1.28)	1	33
TRFK 301/5	8 (2.18)	11 (2.48)	13 (2.61)	6 (1.96)	6 (1.97)	2 (1.23)	2 (1.13)	2 (0.92)	6 (1.81)	4	67
TRFK 301/6	2 (1.06)	7 (2.03)	10 (2.37)	2 (0.92)	2 (0.96)	1 (0.69)	3 (1.50)	1 (0.83)	4 (1.29)	3	75
TRFK 303/1199	2 (1.20)	8 (2.18)	2 (0.96)	4 (1.66)	3 (1.43)	2 (1.20)	1 (0.83)	2 (1.10)	3 (1.32)	2	67
TRFK 303/178	3 (1.37)	6 (1.98)	10 (2.42)	2 (1.20)	2 (1.19)	1 (0.83)	1 (0.83)	2 (1.06)	3 (1.35)	3	100
TRFK 303/216	3 (1.44)	12 (2.59)	13 (2.61)	4 (1.66)	2 (1.00)	2 (1.06)	1 (0.69)	1 (0.83)	5 (1.49)	5	100
TRFK 303/259	6 (1.88)	5 (1.78)	9 (2.33)	12 (2.53)	4 (1.66)	2 (1.19)	2 (1.20)	1 (0.83)	5 (1.67)	4	80
TRFK 303/577	7 (2.04)	2 (0.94)	12 (2.53)	3 (1.27)	1 (0.83)	2 (0.96)	2 (0.92)	1 (0.83)	4 (1.29)	4	100
TRFK 31/8	3 (1.29)	2 (1.20)	9 (2.33)	2 (1.13)	2(1.13)	2 (0.96)	1 (0.69)	2 (0.96)	3 (1.21)	3	100
TRFK 371/3	5 (1.83)	10 (2.42)	14 (2.68)	3 (1.37)	1 (0.69)	1 (0.83)	2 (1.06)	2 (1.06)	5 (1.49)	5	100
TRFK 371/6	3 (1.48)	6 (2.01)	10 (2.38)	4 (1.67)	2 (1.06)	1 (0.83)	2 (1.20)	1 (0.83)	4 (1.43)	3	75
TRFK 371/8	11 (2.50)	13 (2.63)	14 (2.73)	3 (1.29)	2 (1.13)	2 (1.06)	2 (0.96)	1 (0.83)	6 (1.64)	6	100
TRFK 381/5	3 (1.50)	3 (1.37)	12 (2.58)	13 (2.65)	2 (1.13)	2 (1.00)	2 (1.06)	2 (0.96)	5 (1.53)	5	100
TRFK 400/10	6 (1.94)	8 (2.23)	5 (2.79)	16 (2.83)	7 (2.07)	2 (1.06)	3 (1.29)	2 (1.06)	6 (1.91)	5	83

Table 5 cont...

Clone	January	February	March	April	May	June	July	August	Mean clone	ST DEV	Variations (%)
TRFK 400/4	5 (1.83)	6 (1.74)	12 (2.57)	5 (1.75)	2 (1.20)	2 (1.06)	3 (1.06)	2 (1.00)	5 (1.48)	3	60
TRFK 430/5	3 (1.37)	7 (2.03)	25 (3.26)	19 (3.00)	2 (1.20)	6 (0.96)	1 (0.83)	2 (0.96)	8 (1.61)	9	113
TRFK 430/61	5 (1.78)	8 (2.18)	14 (2.73)	7 (2.03)	3 (1.29)	2 (0.96)	3 (1.23)	1 (0.83)	5 (1.62)	4	80
TRFK 430/63	6 (1.90)	7 (2.09)	13 (2.67)	4 (1.67)	4 (1.60)	2 (1.06)	2 (0.92)	2 (1.10)	5 (1.63)	4	80
TRFK 430/7	7 (2.03)	11 (2.51)	19 (2.95)	7 (2.04)	4 (1.52)	1 (0.83)	1 (0.83)	1 (0.69)	6 (1.68)	6	100
TRFK 480/378	5 (1.78)	2 (1.20)	11 (2.51)	4 (1.51)	4 (1.60)	2 (1.10)	3 (1.27)	2 (1.06)	4 (1.50)	3	75
TRFK 480/90	12 (2.53)	13 (2.65)	16 (2.81)	5 (1.78)	2 (1.20)	1 (0.69)	2 (0.92)	1 (0.82)	7 (1.67)	6	86
TRFK 481/200	4 (1.65)	10 (2.41)	16 (2.82)	20 (3.06)	2 (1.06)	2 (1.16)	2 (0.96)	2 (0.92)	7 (1.76)	7	100
TRFK 481/272	3 (1.46)	6 (1.94)	11 (2.47)	3 (1.50)	1 (0.83)	1 (0.69)	1 (0.69)	1 (0.69)	3 (1.28)	4	133
TRFK 6/8	2 (1.20)	3 (1.37)	8 (2.18)	3 (1.46)	3 (1.37)	1 (0.69)	2 (0.92)	1 (0.83)	3 (1.25)	2	67
TRIT 201/16	14 (2.71)	20 (3.03)	24 (3.21)	12 (2.53)	6 (1.93)	2 (1.23)	1 (0.83)	2 (0.93)	10 (2.05)	9	90
TRIT 201/43	3 (1.37)	9 (2.28)	8 (2.15)	4 (1.69)	2 (1.06)	2 (1.13)	1 (0.83)	2 (0.92)	4 (1.43)	3	75
TRIT 201/44	5 (1.72)	6 (1.88)	10 (2.42)	3 (1.37)	3 (1.37)	1 (0.83)	2 (0.92)	1 (0.83)	4 (1.42)	3	75
TRIT 201/47	4 (1.67)	6 (1.98)	12 (2.56)	6 (1.98)	2 (1.23)	1 (0.83)	2 (1.13)	1 (0.83)	4 (1.53)	4	100
TRIT 201/50	2 (1.20)	3 (1.43)	8 (2.15)	3 (1.29)	2 (0.96)	2 (0.96)	1 (0.69)	1 (0.83)	3 (1.19)	2	67
TRIT 201/55	5 (1.84)	9 (2.26)	9 (2.33)	2 (1.23)	1 (0.83)	1 (0.83)	2 (0.92)	1 (0.83)	4 (1.38)	3	75
TRIT 201/70	10 (2.36)	13 (2.61)	11 (2.51)	17 (2.91)	2 (1.06)	2 (1.06)	1 (0.69)	1 (0.69)	7 (1.77)	6	86
TRIT 201/73	3 (1.37)	4 (1.55)	7 (2.12)	3 (1.37)	2 (0.92)	2 (0.96)	2 (1.06)	1 (0.69)	3 (1.26)	2	67
TRIT 201/75	10 (2.38)	11 (2.50)	13 (2.65)	3 (1.29)	2 (1.13)	2 (1.06)	2 (0.96)	1 (0.83)	6 (1.60)	5	83
TRIT 201/82	2 (1.20)	6 (1.98)	11 (2.50)	5 (1.84)	2 (1.05)	2 (1.06)	1 (0.69)	1 (0.83)	4 (1.40)	3	75
Mean month	6 (1.81)	9 (2.14)	12 (2.48)	7 (1.87)	3 (1.34)	2 (0.98)	2 (0.99)	1 (0.90)	5 (1.56)	8	60
CV (%)	19.84 (2.99)										
LSD (p≤0.05)	0										
Interactions p≤0.05)	1										

Monthly number of red red spider mites per 10 leaves of clone

Note: Figures in parenthesis are  $\text{Log}_e (x+1)$  transformation of mite population

Table 6: Changes in the dynamics of red spider mites with clones and months in Timbilil

Clone	January	February	March	April	May	June	July	August	Mean clone	ST DEV	Variations (%)
AHP SC 12/28	6 (1.92)	3 (1.37)	4 (1.65)	13 (2.63)	2 (1.06)	2 (1.10)	2 (1.2)	1 (0.83)	4 (1.51)	4	100
AHP S 15/10	3 (1.46)	5 (1.78)	7 (2.07)	17 (2.91)	6 (1.92)	2 (1.20)	1 (0.83)	1 (0.69)	5 (1.61)	5	100
AHP SC 31/37	3 (1.37)	4 (1.65)	8 (2.21)	5 (1.71)	3 (1.27)	1 (0.83)	2 (1.06)	0 (0.16)	3 (1.38)	2	87
EPK C12	4 (1.60)	5 (1.84)	8 (2.24)	1 (0.83)	1 (0.83)	1 (0.69)	1 (0.83)	1 (0.69)	3 (1.19)	3	100
EPK TN 15-23	6 (1.93)	3 (1.29)	6 (1.92)	2 (1.16)	1 (0.69)	2 (0.96)	1 (0.69)	1 (0.83)	3 (1.18)	2	67
TRFCA SFS 150	13 (2.66)	5 (1.77)	6 (1.94)	3 (1.27)	1 (0.83)	2 (1.06)	1 (0.69)	2 (1.00)	4 (1.40)	4	100
TRFK 11/4	6 (1.92)	7 (2.10)	8 (2.21)	5 (1.78)	4 (1.66)	2 (1.10)	2 (1.06)	1 (0.83)	4 (1.58)	3	75
TRFK 12/19	2 (0.96)	6 (1.93)	11 (2.47)	4 (1.65)	3 (1.27)	1 (0.83)	1 (0.69)	2 (0.96)	4 (1.35)	3	75
TRFK 18/19	3 (1.46)	4 (1.66)	10 (2.38)	4 (1.66)	5 (1.75)	3 (1.27)	2 (0.96)	1 (0.83)	4 (1.50)	3	75
TRFK 18/22	2 (0.96)	4 (1.52)	5 (1.81)	2 (1.19)	3 (1.37)	1 (0.83)	1 (0.83)	2 (1.16)	3 (1.33)	1	33
TRFK 18/3	11 (2.47)	6 (2.01)	7 (2.07)	1 (0.83)	3 (1.33)	1 (0.69)	1 (0.69)	6 (1.13)	5 (1.41)	4	80
TRFK 301/4	2 (0.96)	9 (2.29)	5 (1.78)	2 (1.10)	1 (0.69)	1 (0.83)	1 (0.69)	1 (0.83)	3 (1.15)	3	100
TRFK 301/5	5 (1.78)	5 (1.77)	9 (2.29)	2 (1.20)	3 (1.44)	2 (1.06)	2 (0.96)	1 (0.83)	4 (1.42)	3	75
TRFK 301/6	1 (0.82)	7 (2.03)	6 (1.98)	1 (0.83)	2 (0.92)	1 (0.69)	1 (0.69)	1 (0.83)	3 (1.10)	3	100
TRFK 303/1199	2 (0.92)	5 (1.83)	6 (1.88)	1 (0.83)	1 (0.69)	2 (1.10)	1 (0.83)	1 (0.83)	2 (1.11)	2	100
TRFK 303/178	3 (1.29)	5 (1.85)	7 (2.14)	3 (1.27)	2 (1.06)	2 (0.96)	2 (0.96)	2 (0.92)	3 (1.31)	2	67
TRFK 303/216	2 (1.20)	7 (2.02)	10 (2.40)	2 (1.23)	2 (1.06)	1 (0.69)	2 (0.96)	2 (0.96)	4 (1.25)	3	75
TRFK 303/259	2 (1.10)	5 (1.77)	7 (2.05)	9 (2.29)	3 (1.46)	2 (1.06)	2 (1.06)	2 (0.96)	4 (1.47)	3	75
TRFK 303/577	2 (1.06)	4 (1.54)	10 (2.38)	2 (0.92)	1 (0.83)	1 (0.82)	1 (0.83)	1 (0.83)	3 (1.15)	3	100
TRFK 31/8	1 (0.69)	3 (1.50)	8 (2.24)	2 (1.06)	2 (0.96)	4 (1.65)	1 (0.83)	2 (0.96)	3 (1.24)	2	67
TRFK 371/3	5 (1.76)	2 (1.23)	10 (2.40)	2 (1.06)	3 (1.46)	2 (1.06)	1 (0.83)	1 (0.83)	3 (1.33)	3	100
TRFK 371/6	3 (1.46)	7 (2.12)	10 (2.42)	3 (1.37)	2 (1.16)	1 (0.69)	1 (0.83)	1 (0.69)	4 (1.34)	3	75
TRFK 371/8	6 (1.94)	10 (2.40)	9 (2.29)	2 (1.06)	1 (0.83)	1 (0.83)	1 (0.83)	1 (0.96)	3 (1.39)	3	100
TRFK 381/5	1 (0.83)	6 (1.89)	7 (2.11)	5 (1.78)	3 (1.43)	2 (1.20)	1 (0.69)	1 (0.69)	3 (1.33)	2	67
TRFK 400/10	3 (1.27)	3 (1.33)	6 (1.95)	6 (1.95)	4 (1.55)	2 (0.96)	1 (0.76)	2 (0.96)	3 (1.34)	2	67

Table 6 cont....

Clone	January	February	March	April	May	June	July	August	Mean clone	ST DEV	Variations (%)
TRFK 400/4	3 (1.37)	4 (1.65)	11 (2.48)	10 (2.41)	17 (2.87)	4 (1.52)	1 (0.83)	3 (1.29)	4 (1.68)	5	71
TRFK 430/5	2 (0.96)	5 (1.77)	4 (1.60)	2 (1.10)	3 (1.29)	2 (0.96)	1 (0.69)	1 (0.69)	4 (1.33)	1	33
TRFK 430/61	3 (1.46)	3 (1.37)	7 (2.12)	2 (1.23)	2 (0.96)	1 (0.69)	1 (0.69)	1 (0.83)	3 (1.17)	2	67
TRFK 430/63	3 (1.37)	3 (1.48)	9 (2.35)	2 (1.06)	1 (0.83)	2 (0.96)	1 (0.83)	1 (0.69)	4 (1.20)	4	100
TRFK 430/7	1 (0.83)	3 (1.48)	7 (2.07)	1 (0.83)	3 (1.34)	2 (0.96)	1 (0.83)	1 (0.69)	3 (1.13)	3	100
TRFK 480/378	3 (1.29)	2 (0.92)	10 (2.43)	4 (1.70)	2 (0.92)	2 (0.96)	2 (0.92)	2 (0.96)	3 (1.27)	3	100
TRFK 480/90	4 (1.60)	3 (1.33)	13 (2.63)	3 (1.29)	2 (0.95)	1 (0.69)	1 (0.69)	1 (0.69)	4 (1.24)	4	100
TRFK 481/200	2 (1.20)	7 (2.12)	10 (2.41)	2 (1.20)	1 (0.69)	2 (0.96)	1 (0.69)	2 (0.92)	3 (1.27)	3	100
TRFK 481/272	2 (1.16)	5 (1.78)	10 (2.40)	2 (1.06)	2 (1.11)	1 (0.83)	1 (0.69)	1 (0.69)	3 (1.22)	3	100
TRFK 6/8	1 (0.83)	3 (1.37)	5 (1.71)	2 (1.00)	2 (1.06)	1 (0.69)	1 (0.69)	2 (0.96)	2 (1.04)	1	50
TRIT 201/16	8 (2.20)	4 (1.66)	11 (2.49)	2 (1.13)	4 (1.55)	3 (1.29)	1 (0.69)	3 (1.37)	5 (1.55)	3	60
TRIT 201/43	1 (0.83)	9 (2.34)	7 (2.02)	2 (0.92)	2 (1.06)	2 (1.10)	1 (0.83)	2 (1.06)	3 (1.27)	3	100
TRIT 201/44	4 (1.60)	6 (1.98)	12 (2.59)	2 (0.96)	1 (0.83)	2 (0.96)	2 (0.96)	1 (0.83)	4 (1.34)	4	100
TRIT 201/47	2 (1.20)	5 (1.80)	8 (2.19)	2 (1.29)	2 (0.96)	1 (0.69)	1 (0.83)	2 (0.96)	2 (1.24)	2	67
TRIT 201/50	1 (0.83)	3 (1.34)	3 (1.46)	2 (0.96)	1 (0.62)	1 (0.83)	1 (0.69)	1 (0.83)	4 (0.95)	1	25
TRIT 201/55	2 (0.96)	3 (1.37)	7 (2.07)	2 (1.23)	2 (0.92)	1 (0.69)	1 (0.69)	1 (0.69)	2 (1.08)	2	100
TRIT 201/70	8 (2.18)	5 (1.79)	7 (2.10)	3 (1.37)	2 (1.06)	2 (0.92)	1 (0.69)	1 (0.69)	4 (1.35)	3	75
TRIT 201/73	2 (1.20)	5 (1.83)	6 (2.00)	3 (1.27)	2 (0.96)	1 (0.69)	2 (0.96)	1 (0.69)	3 (1.20)	2	67
TRIT 201/75	4 (1.54)	4 (1.54)	5 (1.72)	1 (0.83)	2 (1.06)	2 (1.06)	1 (0.83)	1 (0.69)	3 (1.16)	2	67
TRIT 201/82	2 (0.96)	2 (1.13)	7 (2.14)	2 (1.23)	2 (0.96)	2 (0.96)	1 (0.69)	2 (0.96)	3 (1.14)	2	67
Mean month	3 (1.36)	5 (1.70)	8 (2.15)	3 (1.35)	3 (1.12)	1 (0.95)	1 (0.81)	1 (0.87)	3 (1.29)	4	67
CV (%)						22.68 (3.12)					
LSD (p≤0.05)						0		0			
Interactions (p≤0.05)						1					

Monthly number of red red spider mites per 10 leaves of clone  
 Note: Figures in parenthesis are Log<sub>e</sub> (x+1) transformation of mite population

The results demonstrate that for every location of tea production, it is necessary to select cultivars that can withstand mite infestations to reduce production losses. Areas with weather factors similar to Kangaita should only commercially exploit resistant tea cultivars. But with increase in climate change factors (Stephens et al., 1992), for a perennial crop like tea, it is necessary to promote commercial exploitation of resistant cultivars to mitigate future production losses.

In previous studies (Ahmed, 2012; Ahmed et al., 2012; Sudoi et al., 2001a), using different clones, mite numbers were low during the rainy seasons and high during dry periods. Similar patterns were observed in the present study. The mites dynamics changed with clones and sites (Tables 4 to 6). The first three months of the study were the dry season with low monthly rainfall, relatively high monthly maximum temperatures and low humidity in all the sites (Table 2). The population of mites increased progressively from January reaching peak in March. This was the driest season in all the three sites (Table 2). There was a sharp decline in infestations levels between April to June, which was the rainy season with high relative humidity and low maximum temperatures. The population reached minimum in July – August, a period characterized with relatively low monthly rainfall, low monthly maximum temperatures and high humidity. These results demonstrate that mite dynamics are to large extent controlled by weather parameters.

The weather conditions between January and March were suitable for mite development (Haque et al., 2007) leading to increase in population in all sites. Unless resistant clones are used in Kangaita, mitigation measures may be necessary in dry months. However levels of mite infestations in Timbilil and Kipkebe were not high enough to warrant mitigation treatment even in clones considered susceptible in any season. Use of resistant clones like those established in this study, remain a viable method to ensure reduced yield losses due to mite infestations in the season in mites prone areas. There were significant



( $p \leq 0.05$ ) interactions effects in the mite infestation levels between clones and months. These observations demonstrated that mite infestation levels in the different clones varied from month to month and the extent of the variation changed with clones and months. Susceptible tea clones showed high mites dynamics while tolerant/resistant clones showed low mites dynamics. The extent of the variations in different clones (Tables 3-6) was a fair measure of their tolerance/resistance or susceptibility.

The mites infestation levels highly correlated with maximum temperatures (Table 7) (Kangaita,  $r = 0.88$ , Kipkebe, ( $r = 0.81$ ) and Timbilil, ( $r = 0.77$ ), but not with minimum temperatures ( $r = -0.49$ ,  $-0.46$  and  $-0.51$  respectively) implying that changes in maximum temperatures were critical in mites dynamics in tea fields. High monthly maximum temperatures encouraged mite infestations levels. The high temperature has been established to accelerate the developmental rate and reduce the duration of developmental stages (Haque et al., 2007). The mite infestations levels showed significant inverse correlation with relative humidity at Kangaita, ( $r = -0.73$ ), and Timbilil ( $r = -0.72$ ) but insignificant inverse correlation in Kipkebe ( $r = -0.53$ ).

Table 7: Correlation coefficients ( $r$ ) between weather parameters and mite population in the three sites

Site	Weather parameter				
	Temperature ( $^{\circ}\text{C}$ )			Relative humidity	Rainfall
	Maximum	Minimum	Mean	(%)	(mm)
Kangaita	0.88**	-0.49	0.74*	-0.73*	-0.184
Kipkebe	0.81*	-0.46	0.54	-0.53	-0.181
Timbilil	0.77*	-0.51	0.77*	-0.72*	-0.24

\*, \*\* significant at  $p \leq 0.05$  and  $0.01$ , respectively

The results confirmed observations in Bangladesh (Ahmed et al., 2012) that hot and dry weather with low humidity are suitable conditions for high mites infestations. The fluctuation in mite populations showed inverse relationship with rainfall in the three sites, similar to the Bangladesh study (Ahmed et al., 2012). However, the relationships were insignificant in all sites.

Of the clones evaluated for the first time, TRFK 303/216, TRFK 371/6, TRIT 201/43, TRIT 201/44, TRIT 201/50, TRIT 201/55, TRIT 201/73 and TRIT 201/82 were identified to be resistant to mites attack while TRFK 18/3 and TRIT 201/16 were susceptible. Cultivation of susceptible clones in mite prone areas should be avoided. During the high maximum monthly temperatures and low relative humidity when mite infestations levels are high, mitigation measures are necessary to reduce yield losses in locations like Kangaita. Use of resistant/tolerant clone may be a practical way of mitigating yield losses without use of pesticides that degrade the environment and cause residue contaminations in made tea. Since mites infestation levels on clones varied with regions and seasons, there is need for the development of region specific suitable clones. Due to climate change, making incidences of droughts and high temperatures frequent, it is necessary for the Kenya tea industry to consider use of resistant/tolerant cultivars even in areas presently with low mite infestations levels as an insurance against future mite infestations.

## **4.2 Determination of the contents and variations in the overhead volatile organic compounds (OVOCs) in selected susceptible and tolerant clones and their relationship with mites attack**

### **4.2.1 Susceptibility/resistance of the selected tea cultivars to mites**

Nine clones were selected from the 45 clones screened in Table 3 together with positive control clones STC 5/3 and TRFK 54/40 (sampling and screening for mites done at

the same time) for further investigations. Analyses of their mites infestations levels are presented in Table 8. The mites population varied significantly ( $p \leq 0.05$ ) with clones (Tables 8). Five clones (TRFK 18/3, TRIT 201/16, AHP S15/10, STC 5/3 and TRFK 54/40) had high ( $p \leq 0.05$ ) mites infestations levels ranging from 36 for TRFK 18/3 to 112 for TRFK 54/40 in the driest month (March) in Kangaita (Tables 8). These clones were classified as susceptible to mites attack. Clones TRFK 6/8, TRIT 201/50, TRFK 303/1199 and TRFK 301/4 had very low levels of mites, ranging from 8-13 in March and were considered resistant/tolerant to mites. However, two clones TRFCA SFS150 and TRFK 31/8 had levels of mites that were between the two extremes. Mites infestations were much lower in Kipkebe and Timbilil during March (Tables 8). Previously, clones TRFK 6/8, TRFK 303/1199, TRFCA SFS150 and TRFK 301/4 exhibited resistance against *B. phoenicis*; STC 5/3 and TRFK 54/40 were susceptible while AHP S15/10 was moderately susceptible (Sudoj, 1989; Sudoj, 1997; Sudoj et al., 2011; Sudoj et al., 2001a). The present study corroborates those observations except on clones TRFCA SFS150 and AHP S15/10 that were moderately resistant and susceptible respectively. The resistant cultivars are recommended for commercial exploitation in the areas prone to mites infestations. TRIT 201/50, TRIT 201/16 and TRFK 18/3 were screened for susceptibility/resistance to *B. phoenicis* for the first time. Both TRIT 201/16 and TRFK 18/3 were susceptible to *B. phoenicis* infestation. The two cultivars may not be suitable for commercial exploitation in geographical regions prone to mites infestation.

Table: 8 Changes in the dynamics of mites with selected clones and months in the three sites

Site	Month	TRFK 6/8	TRFK 18/3	TRFK 31/8	TRFK 54/40	TRIT 201/16	TRIT 201/50	TRFK 301/4	TRFK 303/1199	AHP S15/10	TRFCA SFS 150	STC 5/3	Mean month	Mean site
Kangaita	January	4	44	5	61	26	6	9	4	15	56	39	25	
	February	7	66	12	92	55	9	11	15	33	27	42	34	
	March	8	36	15	112	74	9	9	13	59	17	49	36	
	April	9	34	8	71	98	5	6	5	68	17	35	32	
	May	4	19	6	53	19	2	3	3	24	8	21	15	
	June	1	6	1	12	3	2	2	2	7	3	6	4	
	July	2	2	1	9	3	1	2	2	6	5	3	3	
	August	2	2	2	8	3	2	1	1	6	3	4	3	19
	Mean	5	27	6	52	35	4	5	5	27	17	25		
	Clone CV (%)									24.27				
LSD $p \leq 0.05$									2				1	
Kipkebe	January	2	22	3	28	14	2	3	2	5	24	32	12	
	February	3	23	2	33	20	3	4	8	12	16	37	16	
	March	8	16	9	28	24	8	5	2	27	12	29	15	
	April	3	10	2	24	12	3	4	4	21	5	26	10	
	May	3	7	2	16	6	2	2	4	10	4	20	7	
	June	1	2	2	3	2	2	1	2	3	1	7	2	8
	July	2	2	1	3	1	1	3	1	3	3	4	2	
	August	1	2	2	3	2	1	2	2	3	1	3	2	
	Mean	3	11	3	17	10	3	3	3	11	9	20		
	Clone CV (%)									25.16				
LSD $p \leq 0.05$									1				1	

Table: 8 cont...

Site	Month	TRFK 6/8	TRFK 18/3	TRFK 31/8	TRFK 54/40	TRIT 201/16	TRIT 201/50	TRFK 301/4	TRFK 303/1199	AHP S15/10	TRFCA SFS 150	STC 5/3	Mean month	Mean site	
Timbilil	January	1	11	1	13	8	1	2	2	3	13	28	7		
	February	3	6	3	18	4	3	8	5	6	5	34	9		
	March	5	7	8	16	11	3	5	6	5	5	23	8		
	April	2	1	2	11	2	2	2	1	17	3	10	5		
	May	2	3	2	5	4	1	1	1	6	1	6	3		
	June	1	1	4	2	3	1	1	1	2	2	2	5	2	4
	July	1	1	1	2	1	1	1	1	2	1	1	3	1	
	August	1	6	2	1	3	1	1	1	1	1	2	1	2	
	Mean	2	4	3	9	4	1	2	2	5	4	4	14		
	Clone														
CV (%)								32.52							
	LSD $p \leq 0.05$							1					1		
Mean clone	January	2	26	3	34	16	3	5	2	8	31	33	15		
	February	4	32	6	48	26	5	8	9	19	18	38	19		
	March	7	19	11	52	36	7	6	7	30	11	34	20		
	April	5	15	4	35	37	3	4	3	35	8	23	16		
	May	3	11	3	25	10	1	2	2	13	4	16	8	10	
	June	1	3	2	6	2	1	1	2	4	2	6	3		
	July	1	1	1	5	1	1	2	1	3	3	3	2		
	August	1	3	2	4	2	1	1	2	3	1	3	2		
	Mean	3	14	4	26	16	3	4	4	14	10	19			
	Clone														
CV (%)								28.79							
	LSD $p \leq 0.05$							1				1			
Interactions															

Sites x Months=1.611, Sites x Clones= 1.837, Clones x Months=2.877, Sites x Clones x Months= 4.943

#### 4.2.2 Identification of volatile organic compounds emitted by the cultivars using GC-MS

The compounds eluted from the GC at specific retention times (Table 9). The compounds were identified by comparing the fragmentation pattern with mass spectral data in mass spectral library (NIST/EPA/NIH., 2008) and literature. For example cedrol (**109**) ((1*S*,2*R*,5*S*,7*R*,8*R*)-2,6,6,8-tetramethyltricyclo[5.3.1.0]undecan-8-ol) had a retention time of 19.733 (minutes). The MS spectra of the peak scan (Figure 12) gave fragments with high diagnostic value at  $m/z$  222, 207, 204, 150 and 95. The molecular ion [ $M^+$ ] peak at  $m/z = 222$  corresponding to molecular formula, [ $C_{15}H_{26}O$ ] pointing out a sesquiterpene structure.

(%)

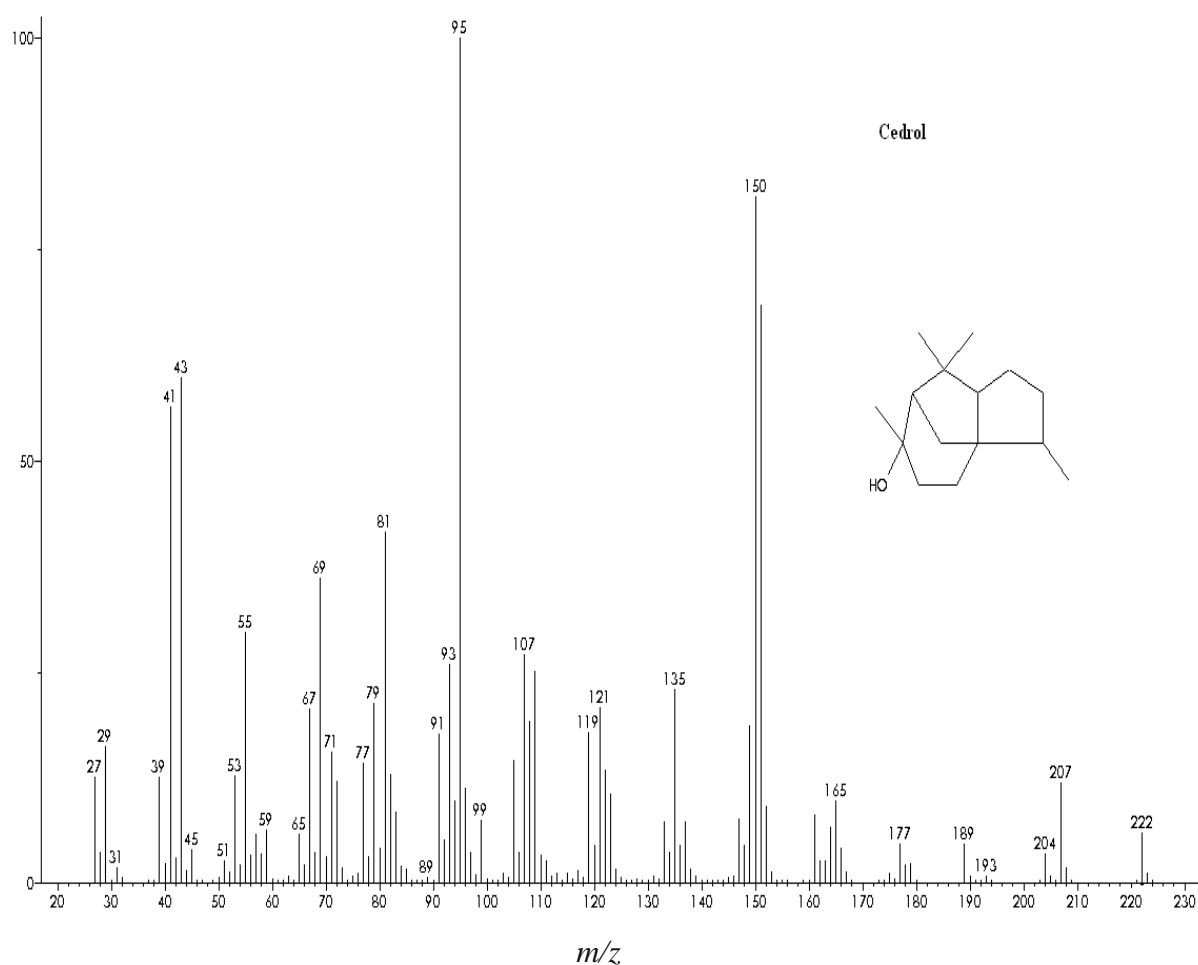


Figure 12: Mass spectrum of cedrol in the headspace of *Camellia sinensis*

The peaks at  $m/z = 204$  [ $C_{15}H_{24}$ ] and  $m/z = 207$  [ $C_{14}H_{23}$ ] are due to loss of water [ $H_2O$ ] and methyl [ $CH_3$ ] group from the molecular ion, respectively. Loss of a methyl [ $-CH_3$ ] group from [ $C_{15}H_{24}$ ] gives the peak at  $m/z = 189$  [ $C_{14}H_{21}^+$ ]. When  $C_{15}H_{24}$  loses [ $C_6H_{13}$ ], [ $C_9H_{11}^+$ ] is formed which corresponds to the peak at  $m/z = 119$ , further loss of  $C_2H_2$  gives the peak at  $m/z = 93$  [ $C_7H_9^+$ ]. This compound undergoes rearrangement to form the base peak at  $m/z = 95$  [ $C_7H_{11}^+$ ] (Kinyanjui, Gitu & Kamau, 2000).

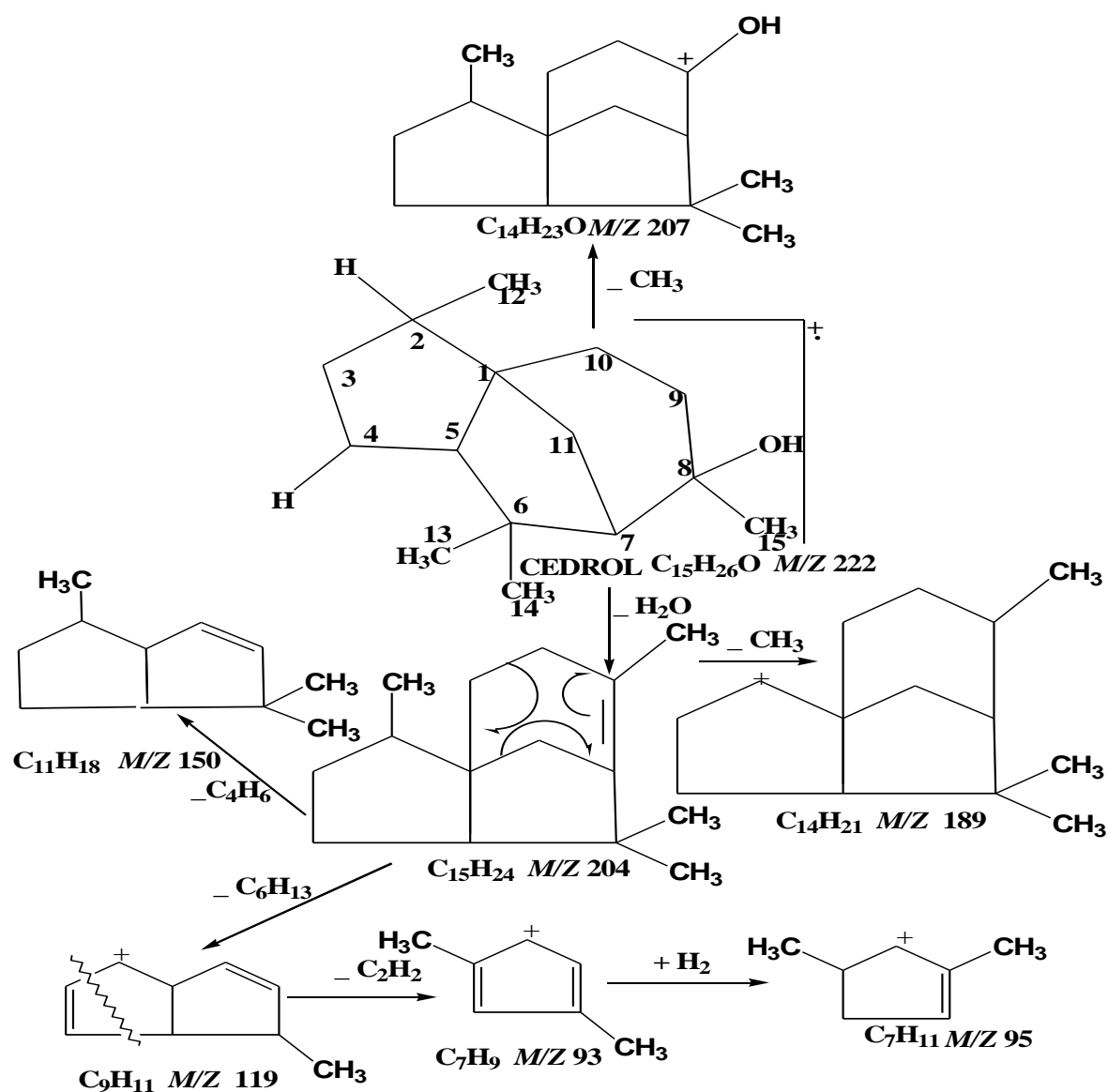


Figure 13: Fragmentation pattern of cedrol

The main peak at  $m/z = 150$  [ $C_{11}H_{18}$ ] is formed when [ $C_{15}H_{24}$ ] loses [ $-C_4H_6$ ] group. The peaks at  $m/z = 41$  and  $m/z = 43$  are due to [ $C_3H_7^+$ ] and [ $C_3H_5^+$ ], respectively which may form from the molecular ion. Species [ $C_{10}H_{15}^+$ ], [ $C_8H_{11}^+$ ], [ $C_{12}H_{17}^+$ ] and [ $C_6H_9^+$ ] have been reported to form during fragmentation of cedrol (Shieh & Sumimoto, 1992). These correspond to peaks at  $m/z = 135$ , 107, 161 and 81, respectively. The formation of the various fragments is outlined in Figure 13. Injection of the sample by cedrol authentic standard led to the enhancement of the peak at retention time 19.733 (minutes). A peak with the same retention time and fragmentation pattern was observed when cedrol standard was analysed. The same procedure used to identify cedrol was followed for all the compounds identified (Table 9).

#### **4.2.3 Variations in volatile organic compounds emitted by the selected cultivars and their relationship with mites infestations**

A total of 60 compounds were identified in this study (Table 9). Tea has produced over 600 VOCs (Robinson et al., 1992) although none of the identified compounds was novel. All chemical groups (GLVs, aromatics, homoterpenes, mono terpenes, and sesquiterpenes) were simultaneously emitted by the eleven tea cultivars thereby giving rise to unique pattern of OVOCs (Tables 10a-d). Such emission of a complex OVOCs had been reported in plants, and implicated in plant-herbivore interactions (Penafior et al., 2011). A total of 8 GLVs were released by the tea varieties (Table 10a). The major GLVs identified were *E*-2-hexenal (**7**), *Z*-3-hexenal (**6**), *Z*-3-hexenol (**9**) and *Z*-3-hexenyl acetate (**12**). All GLVs were positively correlated ( $p \leq 0.05$ ) with mites levels ( $r = 0.698$  to  $0.924$ ) except nonanal (**19**) and *Z*-2-penten1ol (**17**) (Table 10a). (*Z*-3-hexenyl acetate (**12**) ( $r=0.772$  ( $p \leq 0.01$ )) was released in the highest quantities. High amounts of GLVs were emitted by clones STC 5/3, TRFCA SFS150, TRIT 201/16, TRFK 18/3, AHP S15/10 and TRFK 54/40



while TRIT 201/50, TRFK 303/1199, TRFK 301/4, TRFK 6/8 and TRFK 31/8 released relatively lower amounts. Z-3-hexenal (**6**) levels had the highest relationship with mite infestations ( $r = 0.924$ ,  $p \leq 0.001$ ). However it was not detected in TRFK 201/50, TRFK 303/1199 and TRFK 301/4. The total amounts of the GLVs compounds emitted by the cultivars were in the following ascending order TRIT 201/50 < TRFK 303/1199 < TRFK 301/4 < TRFK6/8 < TRFK 31/8 < TRFCA SFS150 < STC 5/3 < TRFK 18/3 < TRIT 201/16 < AHP S15/10 < TRFK 54/40. Cultivars TRFK 54/40, TRIT 201/16, AHP S15/10, STC 5/3 and TRFK 18/3 that released high amounts of GLVs had high levels of mites infestations. In contrast clones TRIT 201/50, TRFK 6/8, TRFK 301/4 and TRFK 303/1199 that emitted low amounts of GLVs had low mites infestations. These results are not unique. Winged tea aphids (Han et al., 2012) and other insect pests (Khan et al., 2008) were attracted to either single or mixture of the GLVs. Insect pests prefer plant cultivars that emit large amounts of GLVs. For example, flea beetles (*Epitrix hirtipennis*) were more abundant on GLV-producing wild type plants compared to plants with reduced hydroperoxide lyase activity (Halitschke et al., 2008). Hydroperoxide lyases catalyse the cleavage of fatty acid hydroperoxides to aldehydes and oxo acids (Vancanneyt et al., 2001) the unsaturated fatty acids being precursors of the GLVS (Robinson et al., 1992). Similarly, *Uschistus heros* preferred soybean pods that released high amounts of GLVs for feeding and oviposition over deficient cultivars (Silva et al., 2013). The GLVs serve as feeding stimulants to pests (Meldau, Wu & Baldwin 2009). Tea cultivars that release low amounts of GLVs are therefore likely to suffer less of mites and can be commercially exploited in mites prone areas.

Unlike the GLVs, the aromatic compounds (Table 10b) were released in smaller quantities ranging from below detection limit to 0.421 (Table 10b). Except indole (**33**), phenyl acetaldehyde (**34**) and methyl salicylate (**32**), the levels of the aromatic compounds

inversely correlated with mites levels. The correlation between the mites infestations and indole (**33**) level was significant ( $r=0.650$ ,  $p\leq 0.05$ ).

The direct relationship between indole (**33**) and mites levels corroborated earlier observation (Erb et al., 2015; Pare et al., 1999) that indole (**33**) induced herbivore attack of plants. Methyl salicylate (**32**) (James et al., 2004; Zhu et al., 2005) and phenyl acetaldehyde (**34**) (Honda et al., 1998; Reddy et al., 2004) have also been associated with attraction of natural enemies of insect pests in plants. Tea plants that emit high levels of overhead indole (**33**) are therefore likely to be susceptible to mites infestations. The level of indole (**33**) was high in clones STC 5/3, TRFK 18/3, AHP S15/10 and TRFK 54/40 that had high mites numbers. However, cultivars that emitted the aromatic compounds in large quantities, e.g. TRIT 201/50, were resistant while clones releasing low quantities of the aromatic compounds, e.g. TRIT 201/16 were susceptible to mites attack. Indeed, there was inverse relationship between most of the aromatic compounds, although the relationships were insignificant. A number of aromatic compounds have been implicated in plant defence against insect pests which may contribute to resistance observed. For example acetophenone (**46**) causes acute toxic insecticidal activities (Mohsen, Ali & Al-Chalabi, 1995), anethole (**38**) is an effective insect repellent while benzothiazole exhibit a wide range of biological properties including antimicrobial activities (Fujita et al., 2007). TRIT 201/50 produced the highest amounts of total aromatic compounds, dominated by acetophenone and had the least mites infestations while TRIT 201/16 produced the least amount of aromatic compounds and was one of the clones with the highest population of mites. These results suggest that the amounts of aromatic compounds released/emitted by the tea cultivars could be a good indicator for selection for resistance/susceptibility to mites infestations.

Table 9: Compounds identified, their retention time, formulae and major fragments

SN	Compound	RT (min)	Formular	Major fragments
1	1-penten-3-ol (16)	3.400	C <sub>5</sub> H <sub>10</sub> O	m/z 86[2% M <sup>+</sup> ], 57 [100 %, M-43], 41[7%, M-59]
2	Phenyl ethyl alcohol (35)	5.419	C <sub>8</sub> H <sub>10</sub> O	m/z 122[22% M <sup>+</sup> ], 91[100 %, M-31], 65[22%, M-57]
3	Z-2-pentenol (17)	5.752	C <sub>5</sub> H <sub>10</sub> O	m/z 86[2% M <sup>+</sup> ], 57 [100 %, M-43], 41[7%, M-59]
4	Hexanal (18)	5.887	C <sub>6</sub> H <sub>12</sub> O	m/z 100[2% M <sup>+</sup> ], 41 [73%, M-59], 56[82%, M-44]
5	Nonanal (19)	6.626	C <sub>9</sub> H <sub>18</sub> O	m/z 142[1% M <sup>+</sup> ], 57[100 %, M-43], 41[89%, M-59]
6	E-2-hexenal (7)	7.992	C <sub>6</sub> H <sub>10</sub> O	m/z 98[24% M <sup>+</sup> ], 41[100 %, M-31], 69[35%, M-29]
7	Ethyl benzene (37)	8.010	C <sub>8</sub> H <sub>10</sub>	m/z 106[28% M <sup>+</sup> ], 91[100 %, M-15], 77[9%, M-29]
8	Z-3-hexenal (6)	8.050	C <sub>6</sub> H <sub>10</sub> O	m/z 98[24% M <sup>+</sup> ], 41[100 %, M-31], 69[35%, M-29]
9	Z-3-hexenol (9)	8.059	C <sub>6</sub> H <sub>12</sub> O	m/z 100[2% M <sup>+</sup> ], 67[100 %, M-33], 41[75%, M-59]
10	p-xylene (41)	8.305	C <sub>8</sub> H <sub>10</sub>	m/z 106[65% M <sup>+</sup> ], 91[100 %, M-15], 77[11%, M-29]
11	Indole (33)	8.460	C <sub>8</sub> H <sub>7</sub> N	m/z 117[100% M <sup>+</sup> ], 90[43 %, M-27], 63[9%, M-54]
12	o-xylene (42)	8.888	C <sub>8</sub> H <sub>10</sub>	m/z 106[65% M <sup>+</sup> ], 91[100 %, M-15], 77[11%, M-29]
13	Heptanal (18)	9.188	C <sub>7</sub> H <sub>14</sub> O	m/z 114[1% M <sup>+</sup> ], 70[93 %, M-44], 44[100 %, M-70]
14	Cumene (112)	9.813	C <sub>9</sub> H <sub>12</sub>	m/z 120[25% M <sup>+</sup> ], 105[100 %, M-15], 77[25%, M-43]
15	α-pinene (81)	9.829	C <sub>10</sub> H <sub>16</sub>	m/z 136[25% M <sup>+</sup> ], 93[100%, M-43], 77[27%, M-59]
16	Myrcene (72)	10.282	C <sub>10</sub> H <sub>16</sub>	m/z 136[5% M <sup>+</sup> ], 93[85%, M-43], 69[79%, M-67]
17	α-phellandrene (78)	11.284	C <sub>10</sub> H <sub>16</sub>	m/z 136[25% M <sup>+</sup> ], 93[100%, M-43], 77[27%, M-59]
18	Z-3-hexenyl acetate (12)	11.329	C <sub>8</sub> H <sub>14</sub> O <sub>2</sub>	m/z 142[22% M <sup>+</sup> ], 67[100 %, M-28], 82[54%, M-59]
19	Benzaldehyde (30)	11.374	C <sub>7</sub> H <sub>6</sub> O	m/z 106[1% M <sup>+</sup> ], 77 [100 %, M-29], 51[38%, M-55]
20	Phenylacetaldehyde (34)	11.461	C <sub>8</sub> H <sub>8</sub> O	m/z 120[28% M <sup>+</sup> ], 91 [100 %, M-29], 65[58%, M-55]
21	Geraniol (65)	11.486	C <sub>10</sub> H <sub>18</sub> O	m/z 154[5% M <sup>+</sup> ], 69[100%, M-85], 41[73%, M-113]
22	Sabinene (85)	11.525	C <sub>10</sub> H <sub>16</sub>	m/z 136[15% M <sup>+</sup> ], 121[5%, M-15], 93[100%, M-43],
23	o-cymene (88)	11.645	C <sub>10</sub> H <sub>14</sub>	m/z 134[40% M <sup>+</sup> ], 119[100%, M-15], 91[35%, M-43]
24	p-cymene (87)	11.675	C <sub>10</sub> H <sub>14</sub>	m/z 134[40% M <sup>+</sup> ], 119[100%, M-15], 91[35%, M-43]
25	β-phellandrene (79)	11.730	C <sub>10</sub> H <sub>16</sub>	m/z 136[25% M <sup>+</sup> ], 93[100%, M-43], 77[27%, M-59]
26	Limonene (76)	11.744	C <sub>10</sub> H <sub>16</sub>	m/z 136[15% M <sup>+</sup> ], 121[10%, M-15], 93[47%, M-43]
27	Phenol (49)	11.846	C <sub>6</sub> H <sub>6</sub> O	m/z 94[100% M <sup>+</sup> ], 66[38% ,M-28], 65[25% ,M-43]
28	Z-β-ocimene (74)	11.910	C <sub>10</sub> H <sub>16</sub>	m/z 136[5% M <sup>+</sup> ], 121[20%, M-15], 93[100%, M-43]
29	Benzyl alcohol (36)	11.934	C <sub>7</sub> H <sub>8</sub> O	m/z 106[1% M <sup>+</sup> ], 77 [100 %, M-29], 51[38%, M-55]
30	Indene (43)	12.068	C <sub>9</sub> H <sub>8</sub>	m/z 116[100% M <sup>+</sup> ], 89[15%, M-27], 63(10%, M-43]
31	E-β-ocimene (73)	12.098	C <sub>10</sub> H <sub>16</sub>	m/z 136[1% M <sup>+</sup> ], 121[20%, M-15], 93[100%, M-43]
32	Acetophenone (46)	12.494	C <sub>8</sub> H <sub>8</sub> O	m/z 120[30% M <sup>+</sup> ], 105[100 % , M-15], 77[85%, M-43]

Table 9 cont.....

SN	Compound	RT (min)	Formular	Major fragments
33	Linalool oxide (cis) furanoid (68)	12.800	C <sub>10</sub> H <sub>18</sub> O <sub>2</sub>	m/z 170[1% M <sup>+</sup> ], 94[59%, M-76], 59[100%, M-111]
34	Linalool oxide (trans) furanoid (67)	12.821	C <sub>10</sub> H <sub>18</sub> O <sub>2</sub>	m/z 170[1% M <sup>+</sup> ], 111[20%, M-59], 94[59%, M-76],
35	Linalool (66)	12.986	C <sub>10</sub> H <sub>18</sub> O	m/z 154[1% M <sup>+</sup> ], 71[100%, M-83], 93[73%, M-69],
37	4,8-dimethyl-1,3(E),7-nontriene (DMNT) (89)	13.101	C <sub>11</sub> H <sub>18</sub>	m/z 150[30% M <sup>+</sup> ], 69[100 %, M-45], 41[48%, M-71]
36	p-mentha-1,3,8-triene (86)	13.278	C <sub>10</sub> H <sub>14</sub>	m/z 134[30% M <sup>+</sup> ], 119[100% , M-15], 91[35%, M-43]
38	Naphthalene (44)	13.994	C <sub>10</sub> H <sub>8</sub>	m/z 128[100% M <sup>+</sup> ], 109[10%, M-29], 96[97%, M-42]
39	Diphenyl ether (48)	14.209	C <sub>12</sub> H <sub>10</sub> O	m/z 170[100% M <sup>+</sup> ], 141[39%, M-29], 115[12%, M-55]
40	Terpinen-4-ol (83)	14.285	C <sub>10</sub> H <sub>18</sub> O	m/z 154[20% M <sup>+</sup> ], 71[100%, M-83], 111[59%, M-43]
41	$\alpha$ -methyl styrene (47)	14.493	C <sub>9</sub> H <sub>10</sub>	m/z 118[100% M <sup>+</sup> ], 103[45%, M-15], 78 [28 %, M-40]
42	Azulene (45)	14.622	C <sub>10</sub> H <sub>8</sub>	m/z 128[100 % M <sup>+</sup> ], 102[11%, M-26], 51[5%, M-42]
43	Z-anethole (38)	14.689	C <sub>10</sub> H <sub>12</sub> O	m/z 148[100% M <sup>+</sup> ], 133 [24 %, M-15], 117[28%, M-31]
44	Methyl salicylate (32)	14.711	C <sub>8</sub> H <sub>8</sub> O <sub>3</sub>	m/z 152[44% M <sup>+</sup> ], 120[100 % ,M-32], 92[65%, M-60]
45	Benzothiozole (39)	15.136	C <sub>7</sub> H <sub>5</sub> NS	135[100% M <sup>+</sup> ], 108[46%, M-105], 69[25%, M-32]
46	Z-jasmone (15)	15.921	C <sub>11</sub> H <sub>16</sub> O	m/z 164[100% M <sup>+</sup> ], 122 [64 %, M-42], 110[75%, M-54]
47	$\alpha$ -copaene (106)	17.184	C <sub>15</sub> H <sub>24</sub>	m/z 204[5% M <sup>+</sup> ], 147[10%, M-57], 93[100%, M-111]
51	<i>E</i> - $\beta$ -caryophyllene (94)	17.191	C <sub>15</sub> H <sub>24</sub>	m/z 204[5% M <sup>+</sup> ], 93[100 %, M-111], 133[92%, M-71]
48	Longifolene (101)	17.623	C <sub>15</sub> H <sub>24</sub>	m/z 204[50% M <sup>+</sup> ], 161[100%, M-43], 94[95%, M-110]
49	Italicene (105)	17.735	C <sub>15</sub> H <sub>24</sub>	m/z 204[50% M <sup>+</sup> ], 161[100%, M-43], 94[95%, M-110]
50	$\alpha$ -cedrene (107)	17.712	C <sub>15</sub> H <sub>24</sub>	m/z 204 [50% M <sup>+</sup> ], 161[100%, M-43], 119[75%, M-85]
52	<i>E</i> - $\beta$ -fernesene (96)	17.780	C <sub>15</sub> H <sub>24</sub>	m/z 204[1% M <sup>+</sup> ], 93[100 %, M-111], 119[30%, M-85]
53	<i>E</i> - $\gamma$ -muurolene (102)	17.800	C <sub>15</sub> H <sub>24</sub>	m/z 204[5% M <sup>+</sup> ], 147[10%, M-57], 93[100%, M-111]
54	$\beta$ -cedrene (108)	17.822	C <sub>15</sub> H <sub>24</sub>	m/z 204 [50% M <sup>+</sup> ], 161[100%, M-43], 119[75%, M-85]
55	Humulene (100)	18.227	C <sub>15</sub> H <sub>24</sub>	m/z 204[5% M <sup>+</sup> ], 147[10%, M-57], 93[100%, M-111]
56	Germacrene D (95)	18.944	C <sub>15</sub> H <sub>24</sub>	m/z 204[23% M <sup>+</sup> ], 161[100%, M-43], 105[77%, M-99]
57	Calamenene (103)	19.056	C <sub>15</sub> H <sub>24</sub>	m/z 204[1% M <sup>+</sup> ], 159 [100 %, M-43], 130[18%, M-57]
58	Nerolidol (99)	19.432	C <sub>15</sub> H <sub>26</sub> O	m/z 222[1% M <sup>+</sup> ], 69[100 %, M-135], 161[30%, M-43]
59	Longicamphenylone (104)	19.638	C <sub>15</sub> H <sub>24</sub> O	m/z 206[100% M <sup>+</sup> ], 175[35%, M-31], 145[50%, M-59]
60	Cedrol (109)	19.733	C <sub>15</sub> H <sub>26</sub> O	m/z 222[5% M <sup>+</sup> ], 150[76%, M-72], 95[100%, M-127]
61	Benzophenone	20.486	C <sub>13</sub> H <sub>10</sub> O	m/z 182[76% M <sup>+</sup> ], 105[100%, M-77], 77[61%, M-105]

The monoterpenes (Table 10c) were produced in the largest number compared to the other groups of compounds. The predominant monoterpenes were *Z*- $\beta$ -ocimene (**74**), *E*- $\beta$ -ocimene (**73**), and linalool (**66**). The relationships between the mono and homo terpenes levels and mites infestations were, however, variable. Large differences in the terpenoid compounds emissions had been reported among 15 susceptible and resistant mango cultivars (Aluja et al., 2014). Similar variations were observed in the 11 tea cultivars. The low emission of mono terpenoids by susceptible tea clones compared to those that were resistant was in agreement with findings amongst 6 peach cultivars (Staudt et al., 2010). The responses of the mites to the mono terpenes varied. Whereas high levels of sabinene (**85**),  $\beta$ -phellandrene (**79**), limonene (**76**), linalool oxides (cis (**68**) and trans (**67**) furanoids), geraniol (**65**) and jasmone (**15**) reduced the mites infestations, there was increase in the infestation with increase in *p*-mentha-1,3,8-triene (**86**), *Z*- $\beta$ -ocimene (**74**), *E*- $\beta$ -ocimene (**73**), linalool (**66**), terpine-4-ol (**83**) and 4,8-dimethyl-1,3(*Z*),7-nonatriene (**50**). However, only *E*- $\beta$ -ocimene (**73**) ( $r=0.626$ ,  $p\leq 0.05$ ) and linalool (**66**) ( $r=0.785$ ,  $p\leq 0.01$ ) levels were significantly correlated with mites levels. In chilli (Saad et al., 2014) and tomato (Bleeker et al., 2009) high levels of monoterpenes repelled insects. The repellency was attributed to the mono terpenes being toxins and feeding deterrents (Mazid et al., 2011) that interfered with acetyl cholinesterase enzyme activity in insects (Zapata & Smagghe, 2010). The dominance of the monoterpenes complex with OVOCs that repel the mites could therefore be selection criteria for selecting mites resistant tea cultivars. The significant attraction of mites by *E*- $\beta$ -ocimene (**73**) and linalool (**66**) were similar to previous observations where *E*- $\beta$ -ocimene (**73**) exhibited attractant effects to insects in wheat (Buttery et al., 1985) and oats (Buttery et al., 1982). Contrary to results in this study, linalool (**66**) was repellent to insects (Ayvaz et al., 2010; Bowers et al., 1993). However, linalool (**66**) has also been reported as a male pheromone attractant to bee *Colletes cunicularius* (Borg-Karlson et al., 2003) and aphid (Pare

et al., 1999). Thus, the ability of linalool (**66**) to attract or repel insects maybe species dependent. The levels of *E*- $\beta$ -ocimene (**73**) and linalool (**66**) were elevated in most of the clones that were susceptible to mites (TRFK 18/3, TRFK 54/40, TRIT 201/16, AHP S15/10 and TRFCA SFS150) compared to those with low mites levels (TRFK 6/8, TRFK 301/4, TRFK 303/1199 and TRIT 201/50).

Clones TRFK 6/8, TRFK 301/4, TRFK 303/1199 and TRIT 201/50 released the highest amounts of total monoterpenes and had low levels of mites while clones TRFK 18/3, TRFK 54/40, TRIT 201/16, AHP S15/10, TRFCA SFS150 and STC 5/3 released lower total monoterpenes levels and had high mites levels. These results show that high levels of monoterpenes defend the cultivars from the mites infestations.

The levels of the overhead volatile sesquiterpenes emitted are presented in Table 10d. High levels of sesquiterpenes observed in this study were in agreement with earlier findings on African grass (Bruce et al., 2010) and other plants (Wason, Agrawal & Hunter, 2013). The levels ranged from below detection limit to 1.90. Cedrol (**109**),  $\alpha$ -cedrene (**107**), and *E*- $\beta$ -fernesene (**96**) were released in large quantities especially by susceptible clones (TRFK 54/40, TRFK 18/3, TRIT 201/16 and AHP S15/10). In contrast *E*- $\beta$ -caryophyllene (**94**) was released in large amounts by all resistant clones (TRFK 6/8, TRIT 201/50, TRFK 301/4 and 303/1199) and small amounts by most susceptible clones. There were mixed responses of the mites to the sesquiterpenes. Mites infestation levels declined with increase in  $\alpha$ -copaene (**106**), *E*- $\beta$ -fernesene (**96**) and calamenene (**103**), but the levels of  $\alpha$ -cedrene (**107**), *E*- $\beta$ -caryophellene (**94**), humulene (**100**), germacrene D (**95**), nerolidol (**99**) and cedrol (**109**) increased as the infestations increased. The relationship was significant for germacrene D (**95**) ( $r = 0.742$ ,  $p \leq 0.01$ ).

Table 10 a: Changes in mite infestations and overhead green leaf volatile compounds (GLVs) and their relationships in tea cultivars in Kangaita during dry season

Clone	Overhead green leaf volatile compounds									Mites
	1-penten-3-ol	Z-2-pentenol	Hexanal	E-2-hexenal	Z-3-hexenal	Z-3-hexenol	Z-3-hexenyl acetate	Nonanal	Sum of GLVS	
TRFK 6/8	0.135	0.080	0.267	0.758	0.029	0.575	4.195	ND	6.039	8
TRFK 18/3	0.250	0.112	0.353	0.792	0.115	1.611	6.558	0.062	9.853	36
TRFK 31/8	0.225	0.112	0.260	0.684	0.019	0.675	4.905	ND	6.880	15
TRFK 54/40	0.242	0.189	0.480	1.466	0.308	1.954	8.509	0.065	13.213	112
TRIT 201/16	0.254	0.059	0.373	1.050	0.106	2.025	6.272	0.099	10.238	74
TRIT 201/50	0.020	ND	0.050	0.163	ND	0.269	0.869	ND	1.371	9
TRFK 301/4	0.059	0.053	0.153	0.149	ND	0.543	3.283	ND	4.240	9
TRFK 303/1199	0.067	0.054	0.140	0.142	ND	0.475	1.464	ND	2.342	13
AHP S15/10	0.270	0.105	0.293	1.084	0.171	2.000	6.965	0.115	11.000	59
TRFCA SFS150	0.142	0.132	0.379	0.138	0.093	1.438	6.051	0.147	8.520	17
STC 5/3	0.229	0.043	0.457	0.849	0.128	1.188	5.745	0.142	8.781	49
Mean	0.172	0.085	0.291	0.661	0.088	1.159	4.983	0.057	7.498	36
STDEV	0.091	0.052	0.135	0.458	0.095	0.677	2.346	0.061	3.881	
CV (%) #	52.907	61.176	46.392	69.289	107.955	58.412	47.080	107.018	51.760	
$r_{\text{mite}}$	0.698*	0.537	0.720*	0.864***	0.924***	0.831**	0.772**	0.492	0.828**	
Variations (%) ##										24.27
LSD, ( $p \leq 0.05$ )										2

ND = not detected, a value of 0.000 has been used in the statistical calculations

\*, \*\*, \*\*\* Significant at  $p \leq 0.05$ , 0.01 and 0.001, respectively

# As  $\frac{STDEV}{Mean} * 100$

## From ANOVA

Table 10b: Clonal variations and the relationships between mites infestations and overhead volatile aromatic compounds in Kangaita during dry season

Clone	Aromatic compounds												Mites	
	Phenyl ethyl alcohol	Methyl salicylate	Ethyl benzene	P-xylene	Indole	Benzaldehyde	Phenyl acetaldehyde	Benzyl alcohol	Acetophenone	Z-anethole	Benzothiozole	Benzophenone		Sum aroma
TRFK 6/8	0.145	0.194	0.268	0.168	0.236	0.161	0.050	0.079	0.103	0.242	0.074	0.072	1.792	8
TRFK 18/3	0.093	0.139	0.149	0.073	0.421	0.054	0.038	0.089	0.115	0.182	ND	ND	1.353	36
TRFK 31/8	0.153	0.153	0.197	0.210	0.260	0.087	0.059	0.094	0.146	0.137	ND	ND	1.496	15
TRFK 54/40	0.112	0.238	0.158	0.202	0.395	0.150	0.136	0.150	0.110	0.112	ND	ND	1.763	112
TRIT 201/16	0.057	0.001	0.061	ND	0.306	0.019	0.054	0.059	0.161	0.164	0.050	ND	0.932	74
TRIT 201/50	0.142	0.088	0.261	0.093	0.300	0.144	0.063	0.064	0.368	0.258	0.114	0.192	2.089	9
TRFK 301/4	0.125	0.073	0.135	0.236	0.140	0.128	0.033	ND	0.122	0.190	ND	0.141	1.323	9
TRFK 303/1199	0.262	0.065	0.118	0.266	0.152	0.122	0.074	0.119	0.270	0.213	ND	0.236	1.897	13
AHP S15/10	0.088	0.054	0.124	ND	0.350	0.192	0.025	0.044	0.190	0.189	ND	ND	1.256	59
TRFCA SFS150	0.121	0.114	0.103	ND	0.284	0.21	0.050	0.163	0.109	0.085	ND	ND	1.239	17
STC 5/3	0.099	0.182	0.263	0.069	0.346	0.115	0.099	0.108	0.161	0.091	ND	ND	1.533	49
Mean	0.127	0.118	0.173	0.120	0.290	0.126	0.062	0.088	0.169	0.169	0.022	0.058	1.516	36
STDEV	0.053	0.07	0.069	0.100	0.090	0.056	0.032	0.047	0.082	0.058	0.015	0.090	0.346	
CV (%) #	41.732	59.322	39.884	83.333	31.034	44.444	51.613	53.409	48.66	34.22	68.182	153.77	22.823	
$r_{(mite)}$	-0.477	0.190	-0.302	-0.249	0.650*	-0.161	0.561	-0.243	-0.265	-0.449	-0.248	-0.550	-0.261	
Variations (%) ##														24.27
LSD, ( $p \leq 0.05$ )														2

ND = not detected, a value of 0.000 has been used in the statistical calculations

\*, Significant at  $p \leq 0.05$

# As  $\frac{STDEV}{Mean} * 100$

## From ANOVA



Table 10c: Changes and relationships in clonal overhead monoterpenes and mites levels in Kangaita during dry season

Clone	Monoterpene compounds														Mites
	P-mentha-1,3,8-triene	Sabinene	$\beta$ -phellandrene	Limonene	Z- $\beta$ -ocimene	E- $\beta$ -ocimene	Linalool oxide (cis) furanoid	Linalool oxide (trans) furanoid	Linalool	Terpinen-4-ol	Geraniol	4,8-dimethyl-1,3(E),7-nontriene	Jasmonene	Sum mono	
TRFK 6/8	0.194	0.066	0.4	0.097	0.548	0.884	0.100	0.047	0.377	0.103	0.100	0.154	0.009	3.079	8
TRFK 18/3	0.139	ND	ND	0.113	0.312	0.75	0.054	0.018	0.563	0.159	ND	0.109	ND	2.217	36
TRFK 31/8	0.153	0.047	0.308	0.165	0.474	0.741	0.114	0.037	0.412	0.233	0.009	0.138	0.004	2.835	15
TRFK 54/40	0.238	0.035	0.467	0.104	0.586	1.186	0.106	0.043	0.719	0.181	ND	0.181	0.013	3.859	112
TRIT 201/16	0.001	ND	ND	0.093	0.361	0.99	0.094	0.026	0.552	0.132	ND	0.137	0.00	2.386	74
TRIT 201/50	0.088	0.071	0.393	0.136	0.439	0.754	0.107	0.046	0.258	0.041	0.025	0.127	0.013	2.498	9
TRFK 301/4	0.073	0.052	0.327	0.122	0.44	0.875	0.102	0.025	0.316	0.07	0.087	0.112	ND	2.601	9
TRFK 303/1199	0.065	0.082	0.433	0.125	0.502	0.765	0.1	0.037	0.304	0.103	0.018	0.113	0.047	2.694	13
S15/10	0.054	ND	ND	0.039	0.346	0.658	0.053	0.024	0.602	0.072	0.07	0.124	ND	2.042	59
TRFCA SFS150	0.114	ND	ND	0.134	0.388	0.91	0.089	0.028	0.615	0.124	ND	0.168	ND	2.57	17
STC 5/3	0.182	0.035	0.205	0.178	0.398	0.903	0.111	0.048	0.468	0.185	0.005	0.179	0.011	2.908	49
Mean	0.118	0.035	0.230	0.119	0.856	0.094	0.034	0.471	0.187	0.029	0.140	0.009	0.088	2.699	36
STDEV	0.070	0.031	0.195	0.037	0.147	0.021	0.011	0.150	0.066	0.038	0.026	0.014	1.090	0.497	
CV (%) #	59.322	88.571	84.783	31.092	17.172	22.340	32.353	31.847	35.294	131.034	18.571	155.556	23.62	18.414	
$r$ (mite)	0.190	-0.481	-0.152	-0.356	0.045	0.626*	-0.141	-0.032	0.785**	0.342	-0.378	0.442	-0.145	0.369	
Variations (%) ##															24.27
LSD, (p $\leq$ 0.05)															2

ND = not detected, a value of 0.000 has been used in the statistical calculations

\*, \*\* Significant at p $\leq$ 0.05 and 0.01, respectively

# As  $\frac{STDEV}{Mean} * 100$

## From ANOVA

Table 10d: The variations and relationship between sesquiterpenes released by 11 tea varieties and mite infestations levels in Kangaita during dry season

Clone	Sesquiterpenes											Mites
	$\alpha$ -copaene	$\alpha$ -cedrene	<i>E</i> - $\beta$ -Caryophyllene	( <i>E</i> )- $\beta$ -farnesene	<i>E</i> - $\gamma$ -muurolene	Humulene	Germacr ene D	Calame nene	Neroli dol	Cedrol	Sum of sesquiterpenes	
TRFK 6/8	0.225	0.798	0.568	0.955	ND	0.034	0.121	0.323	0.116	1.968	5.108	8
TRFK 18/3	ND	0.809	0.346	1.183	ND	0.124	0.233	ND	0.193	1.844	4.372	36
TRFK 31/8	0.210	0.844	0.546	0.735	ND	0.255	0.147	ND	0.126	2.041	4.904	15
TRFK 54/40	0.151	1.269	0.655	1.066	ND	0.341	0.277	0.047	0.320	3.384	7.510	112
TRIT 201/16	ND	0.588	0.416	1.051	ND	0.112	0.271	ND	0.240	1.315	3.993	74
TRIT 201/50	0.284	0.474	0.479	0.578	0.036	0.377	ND	ND	0.067	1.347	3.642	9
TRFK 301/4	ND	0.553	0.514	0.730	ND	0.199	ND	0.223	ND	1.501	3.720	9
TRFK 303/1199	0.193	0.573	0.503	0.670	ND	0.304	ND	ND	0.105	1.654	4.002	13
AHP S15/10	ND	0.564	0.359	1.043	ND	0.132	0.229	ND	ND	1.581	3.908	59
TRFCA SFS150	ND	0.755	0.299	0.632	ND	0.132	0.229	ND	0.287	2.375	4.709	17
STC 5/3	0.062	0.750	0.681	0.934	0.019	0.259	0.217	ND	0.113	1.908	4.943	49
Mean	0.102	0.725	0.488	0.871	0.005	0.206	0.157	0.054	0.142	1.902	4.619	36
STDE	0.111	0.220	0.124	0.207	0.012	0.109	0.111	0.111	0.107	0.585	1.091	
CV (%) #	108.82	30.345	25.410	23.766	240.000	52.91	70.701	205.55	75.35	30.757	23.620	
	4					3		6	2			
$r_{(mite)}$	-0.292	0.570	0.210	-0.266	-0.187	0.114	0.742**	-0.311	0.515	0.505	0.534	
Variations (%) ##												24.27
LSD, ( $p \leq 0.05$ )												2

ND = not detected, a value of 0.000 has been used in the statistical calculations

\*\* Significant at  $p \leq 0.01$

# As  $\frac{STDEV}{Mean} * 100$

## From ANOVA

Overall, total levels of sesquiterpenes directly correlated ( $r = 0.534$ ) with mites infestation levels, however the relationship was insignificant. The result concurred with increased attraction of heliothine moths by germacrene D (**95**) (Mozuraitis, Strandén, Ramirez, Borg-Karlson & Mustaparta, 2002; Strandén, Liblikas, König, Borg-Karlson & Mustaparta, 2003) and the terpene is a useful marker for insect attraction (Buttery, Flath, Mon & Ling, 1986). Indeed sesquiterpenes have been associated with attraction of insects to plants (Metcalf & Kogon, 1987), especially the natural enemies of herbivores although some sesquiterpenes are repellents (Russell, Hunt, Bowers & Blunt, 1994). Sesquiterpenes not only defend plants against pest attack by attracting natural enemies but also possess repellency and toxicity properties (Eller, Vander, Behle, Flor & Palmquist, 2014). The release of high levels of sesquiterpenes during infestations could be the response of the cultivars to repel or kill the mites (Eller et al., 2014).

Generally *E*-2-hexenal (**7**), *Z*-3-hexenol (**9**), *E*- $\beta$ -farnesene (**96**), cedrol (**109**),  $\alpha$ -cedrene (**107**), *E*- $\beta$ -caryophyllene (**94**), linalool (**66**), *E*- $\beta$ -ocimene (**73**), *Z*- $\beta$ -ocimene (**74**) were released in high but different amounts by the different tea cultivars. These VOCs had been identified as volatile semiochemicals involved in plant defence against insect pests (Scala et al., 2013). Identifying the variations of these defence VOCs can form the basis of breeding and clonal selection programmes. Cultivars that released high amounts of *E*-2-hexenal (**7**), *Z*-3-hexenal (**6**), linalool (**66**), germacrene D (**95**), *Z*-3-hexenol (**9**), *Z*-3-hexenyl acetate (**12**), 1-penten-3-ol (**16**), hexanal (**8**) and *E*- $\beta$ -ocimene (**73**) were susceptible to mites infestations. High levels of total GLVs increased while that of aromatic compounds reduced mites infestations.

In previous studies, the ratio of linalool (**66**) to linalool (**66**) plus geraniol (**65**) (terpene index) in macerated tea leaves was shown to be cultivar specific (Takeo, 1981; Takeo, 1983). The ratios were confirmed to be maintained in processed black tea (Owuor,

1989; Owuor, Takeo, Tsushida, Horita & Murai, 1987e) in which clones TRFK 6/8 and AHP S15/10 were shown to have low terpene indices. Although these cultivars expressed high linalool (**66**) levels in the OVOCs composition, the levels of geraniol (**65**) were very low. The results demonstrate that there are volatile compounds in tea leaves which are not easily released to the atmosphere. The released volatile compounds could be responses to environmental stresses and are released as a mechanism of responding to or overcoming such stresses. Only necessary volatiles are released for the defense purposes. Cultivars TRIT 201/50, TRFK 301/4, TRFK 303/1199 and TRFK 6/8 were resistant to the mites while TRFK 54/40, TRFK 18/3, TRIT 201/16, AHP S15/10, and STC 5/3 were susceptible. Susceptibility of the cultivars was correlated ( $p \leq 0.05$ ) with the quantities of the GLVs released. In contrast, resistance was determined by the low amounts of GLVs and the high amounts of both terpenes and aromatics the cultivar emitted. This implies that breeding efforts should be focused on cultivars that produce low levels of overhead GLVs and high levels of both terpenes and aromatics in order to develop tea cultivars that resist mites attack.

#### **4.3 Influence of seasons and region of production of tea varieties on the composition or levels of the volatile organic compounds in relation to mites attack.**

The seasonal variations in the OVOCs at different sites are presented in Tables 10-18. There were variations in the levels and composition of OVOCs released by the cultivars in the different seasons in Kangaita (Tables 10-12). The highest levels and composition of OVOCs were registered in the dry season (Tables 10a-d). The emissions of the GLVs were the greatest (7.50) (Table 10a) dominated by *E*-2-hexenal (**7**), *Z*-3-hexenal (**8**), *Z*-3-hexenol (**9**) and *Z*-3-hexenyl acetate (**12**) which were significantly and linearly correlated to mites levels. Consequently clones such as TRFK 54/40, TRIT 201/16, AHP S15/10, STC 5/3 and TRFK 18/3 that released high levels of these GLVs also recorded high numbers of mites and

were considered susceptible. Similarly clones TRIT 201/50, TRFK 301/4, TRFK 303/1199 and TRFK 6/8 emitted low amounts of the GLVs and had low mites numbers. The levels of GLVs decreased from 7.5 to 1.65 in rainy season (Table 11a) and hexanal (**8**) and *E*-2-hexenal (**7**) were the only GLV compounds that had positive and significant ( $p \leq 0.05$ ) correlation to mites levels. There was a further decrease in the levels of GLVs in cold season to 0.45 (Table 12a) and even total loss of a number of the GLV compounds *E*-2-hexenal (**7**), Penten-3-ol (**16**), *Z*-2-pentenol (**17**) and *Z*-3-hexenol (**6**). The mites levels were directly related to that of GLVs, the number decreased from 36 in dry season to 24 and 4 in rainy and cold seasons respectively. These results demonstrate that GLVs are mainly released in high levels and composition in dry season and that these levels positively affect mites infestations in tea cultivars that emit them in large quantities. Terpenoids function as photoprotectants by dissipating energy and/or scavenging reactive oxygen species in photosynthetic membranes thereby improving lipid membrane stability (Penuelas & Munne-Bosch, 2005). During dry season/drought, the combined effect of high leaf temperatures and water deficits can result in a strong decrease in the emissions of volatile terpenoids, the antioxidant system can be overwhelmed and/or is no longer active and result in membrane peroxidation and the increased emissions of GLVs (Jardine et al., 2015). In another study, Wenda-Piesik (Wenda-Piesik, 2011) demonstrated that the emissions of GLVs by plants increased with water deficit. Similarly lipoxygenase activity responsible for production of green leaf volatiles increases during water deficit periods (Sofa et al., 2004). Insect pests prefer plant cultivars that emit large amounts of GLVs (Halitschke et al., 2008; Meldau et al., 2009; Silva et al., 2013). These findings explain in part why mites infestations occur during drought (Ahmed et al., 2012; Kumara et al., 2011; Sudoi et al., 2011; Sudoi et al., 1994) compared to other seasons. Therefore cultivars that emit high levels and amounts of overhead GLVs are likely to suffer mites attack especially during prolonged drought.

The volatile aromatic compounds emitted by the tea plants were dominated by methyl salicylate (**32**), ethyl benzene (**37**), P-xylene (**41**), indole (**33**), acetophenone (**46**) and Z-anethole (**38**) (Table 10b). The compounds were all inversely correlated to mites levels except methyl salicylate, indole and phenyl acetaldehyde although the relationships were insignificant. The levels decreased from 1.516 in dry to 1.073 in rainy season (Table 11b). Similar to dry season, most of the aromatics were inversely correlated to mites infestations. Mites levels were significantly and inversely correlated to phenyl ethyl alcohol (**17**), ethyl benzene (**37**) and Z- anethole (**38**). The levels of aromatic compounds increased slightly to 1.20 in cold season (Tables 12b) with all the compounds negatively correlated to mites levels except phenyl acetaldehyde and benzyl alcohol but the relationship was insignificant. The results show that cultivars that release aromatic OVOCs may not suffer mites attack.

The most predominant monoterpenes were *E*- $\beta$ -ocimene (**73**), linalool (**66**), *Z*- $\beta$ -ocimene (**74**) and  $\beta$ -phellandrene (**79**) (Table 10c). Both *E*- $\beta$ -ocimene (**73**) ( $r = 0.626^*$ ,  $p \leq 0.01$ ) and linalool (**66**) ( $r = 0.785^{**}$ ,  $p \leq 0.01$ ) were linearly and significantly ( $p \leq 0.05$ ) correlated to infestations of mites. The two monoterpenes were released in elevated levels in cultivars that registered high number of mites (TRFK 54/40, TRIT 201/16, AHP S15/10, STC 5/3 and TRFK 18/3). However more than half the numbers of monoterpenes were negatively correlated to mites levels. The levels of monoterpenes decreased from 2.699 (Table 10c) in dry season to 1.971 (Table 11c) in rainy season and further decreased to 1.961 (Table 12c) in cold season. Sesquiterpenes were released in high levels, second after the GLVs. They were dominated by cedrol (**109**), *E*- $\beta$ - farnesene (**96**),  $\alpha$ -cedrene (**107**) and *E*- $\beta$ - caryophyllene (**94**) (Table 10d). Some sesquiterpenes were negatively while others positively correlated to mites levels. All the correlations were insignificant except that of Germacrene D (**17**) ( $r=0.742$ ,  $p \leq 0.01$ ). The levels of  $\alpha$ -cedrene (**107**), *E*- $\beta$ - farnesene (**96**) and cedrol (**109**) were elevated in clones that registered high numbers of mites. Sesquiterpenes levels dropped from 4.619 in

dry season to 2.273 (Table 11d) during rainy season but slightly increased to 2.785 (Table 12d) in cold season. Unlike GLV compounds that were linearly and significantly ( $p \leq 0.05$ ) correlated to mites levels, the majority of the compounds belonging to aromatics, monoterpenes and sesquiterpenes were negatively correlated to mites levels. The results suggest that these classes of compounds (except GLVs) defend the tea cultivars from the attack by the two mites species. Except for TRFK 54/40 that registered unique results in this study, the other cultivars that released high levels and composition of aromatics, monoterpenes and sesquiterpenes were indeed resistant to mites attack. Although TRFK 54/40 released high levels of aromatic compounds, monoterpenes and sesquiterpenes, it emitted at the same time exceptionally high levels and amounts of GLVs that possibly made it more vulnerable to mites attack, further emphasising the fact that GLVs predispose cultivars to pest infestations (Halitschke et al., 2008; Silva et al., 2013). The terpenes and aromatic OVOCs make cultivars resistant/tolerant while overhead GLVs lead to susceptibility to mites.

The highest amounts and levels of OVOCs were released during dry season. There was reduction in OVOCs emissions in rainy/wet season. Generally the levels of aromatics, monoterpenes and sesquiterpenes slightly increased in cold season while that of GLVs decreased. Dry season is characterized by low soil moisture content, irradiation effects of high sunshine hours and increased leaf temperatures that reduce photosynthesis and growth of plants (Coley, 1998) accompanied with high dry matter accumulation (Muthumani et al., 2013; Rawat et al., 2008)). Warm weather conditions in rainy/wet season favour faster growth accompanied with low dry matter accumulation per shoot volume (Lin et al., 2008) that reduce the biosynthesis of secondary metabolites. Cold season is marked with scanty rainfall, low temperatures and moderate humidity. The conditions cause slow shoots growth rates and favour longer accumulation of secondary metabolites (Rawat et al., 2008). Several

studies have demonstrated seasonal variations in secondary metabolites in tea both in Kenya (Owuor, 1992a; Owuor, 1992c; Owuor, 1999; Owuor et al., 1991) and other parts of the world (Chen et al., 2011; Gulati et al., 1996; Rawat et al., 2008; Wang, Wei, Jiang, Cheng & Zhou, 2011). Contrary to the earlier reports that the VOCs including GLVs amounts increase during cold season, although different method was used (Muthumani et al., 2013; Rawat et al., 2008), there was a decline in the levels of GLVs and even total loss of compounds (*Z*-3-hexenol (**9**), *Z*-3-hexenal (**6**), *E*-2-hexenal (**7**), *Z*-2-pentenol (**17**) and 1-penten-3-ol (**16**). This is in concurrence with Wenda-Piesik (Wenda-Piesik, 2011) that water deficit increases the emissions of GLVs. Consequently the GLVs levels and composition are expected to decrease in wet/rainy seasons. The variations in the OVOCs between rainy and cold season was small. This is in agreement with the observation that in Kenya, tea is grown near the equator with little seasonal variations in temperatures and sunshine hours compared to countries further away from the equator where variations in yields and VOCs are likely to be larger since the environmental conditions controlling growth and hence VOCs are more variable (Owuor, Kamau & Jondiko, 2010a). Generally, the pattern of mites infestations and release of GLVs were closely related in the sites and seasons. GLVs levels and composition were exceptionally high in dry season in Kangaita; similarly mites infestation levels were high in Kangaita in the same season. Seasonal variations in the OVOCs emitted by the cultivars in Kipkebe are presented in Tables 13-15. All GLVs were significantly ( $p \leq 0.05$ ) correlated to mites levels in dry season (Table 13a) except *Z*-2-pentenol (**17**). Clones TRFK 54/40, AHP S15/10 and STC 5/3 not only recorded high levels and composition of GLVs but high numbers of mites as well. On the contrary, clones such as TRIT 201/50, TRFK 301/4 and TRFK 303/1199 that recorded low levels and composition of GLVs had the least number of mites. During rainy season (Table 14a) hexanal (**8**) and nonanal (**19**) were the only GLV compounds that were significantly ( $p \leq 0.05$ ) and linearly correlated to mites levels.



Table 11a: Changes in mite infestations and overhead green leaf volatile compounds (GLVs) and their relationships in tea cultivars in Kangaita during rainy season

Clone	Overhead green leaf volatile compounds (GLVs)								Mites
	1-penten-3-ol	Z-2-pentenol	Hexanal	E-2-hexenal	Z-3-hexenol	Z-3-hexenyl acetate	Nonanal	Sum GLVS	
TRFK 6/8	0.052	ND	0.075	0.326	0.250	1.128	ND	3.662	7
TRFK 18/3	0.062	ND	0.115	0.161	0.221	1.118	ND	3.354	27
TRFK 31/8	0.043	0.007	0.065	0.249	0.287	0.746	ND	2.794	7
TRFK 54/40	0.037	ND	0.402	0.337	0.396	1.386	0.031	2.629	62
TRIT 201/16	0.042	ND	0.117	0.360	0.287	1.085	ND	3.782	59
TRIT 201/50	ND	ND	ND	0.069	0.091	0.262	0.056	0.478	3
TRFK 301/4	ND	ND	0.119	0.065	0.073	0.699	ND	1.912	5
TRFK 303/1199	ND	ND	0.098	0.102	0.196	0.645	ND	1.041	4
AHP S15/10	0.039	ND	0.104	0.338	0.313	1.042	ND	3.672	46
TRFCA SFS 150	0.018	0.011	0.079	0.088	0.516	1.008	0.056	1.776	13
STC 5/3	0.022	ND	0.182	0.356	0.290	1.749	0.047	2.646	28
Mean	0.029	0.002	0.123	0.223	0.265	0.988	0.017	1.647	24
STDEV	0.022	0.001	0.103	0.126	0.126	0.397	0.025	1.342	
CV (%) #	75.862	50.00	83.740	56.502	47.547	40.182	147.059	81.481	
r (mite)	0.459	0.277	0.676*	0.700*	0.443	0.584	0.266	0.563	
Variations (%) ##									24.27
LSD, (p≤0.05)									2

ND = not detected, a value of 0.000 has been used in the statistical calculations

\* Significant at p≤0.05

# As  $\frac{STDEV}{Mean} * 100$

## From ANOVA

Table 11b: Clonal variations and the relationships between mites infestations and overhead volatile aromatic compounds in Kangaita during rainy season

Clone	Aromatic compounds												Mites	
	Phenyl ethyl alcohol	Methyl salicylate	Ethyl benzen e	P-xylene	Indole	Benzaldehyde	Phenyl acetaldehyde	Benzyl alcohol	acetophenone	Benzothiazole	Z-anethole	Benzophenone		Sum aroma
TRFK 6/8	0.159	0.119	0.195	0.088	0.194	0.147	0.036	0.065	0.093	0.046	0.220	0.037	1.399	7
TRFK 18/3	0.054	ND	0.126	0.066	0.346	0.053	ND	ND	0.063	ND	0.145	ND	0.853	27
TRFK 31/8	0.136	ND	0.207	0.218	0.245	0.062	0.067	0.094	0.151	ND	0.135	ND	1.135	7
TRFK 54/40	0.056	ND	0.150	0.212	ND	0.153	0.107	0.153	0.109	ND	0.043	ND	0.983	62
TRIT 201/16	0.060	ND	0.114	ND	ND	0.168	ND	0.028	0.163	ND	0.133	ND	0.666	59
TRIT 201/50	0.147	ND	0.233	0.062	0.211	0.332	ND	0.057	0.302	0.072	0.243	0.120	1.279	3
TRFK 301/4	0.131	ND	0.163	0.245	ND	0.136	ND	ND	0.117	ND	0.216	0.127	1.125	5
TRFK 303/1199	0.223	ND	0.183	0.245	ND	0.122	ND	0.120	0.232	ND	0.205	0.209	1.539	4
AHP S15/10	0.063	ND	0.093	ND	ND	0.152	ND	0.048	0.148	ND	0.148	ND	0.652	46
TRFCA SFS 150	0.063	ND	0.101	ND	0.215	0.149	0.048	0.117	0.113	ND	0.073	ND	0.879	13
STC 5/3	0.099	ND	0.095	0.047	0.022	0.119	0.057	0.092	0.147	ND	0.069	ND	0.655	28
Mean	0.108	0.011	0.151	0.108	0.112	0.145	0.029	0.070	0.149	0.011	0.148	0.045	1.073	24
STDEV	0.056	0.036	0.049	0.102	0.131	0.072	0.037	0.050	0.067	0.024	0.067	0.073	0.741	
CV (%) #	51.852	81.821	32.450	94.444	116.964	49.655	48.276	71.429	44.966	36.364	45.270	64.44	69.059	
$r_{(mite)}$	-0.740*	-0.245	-0.602*	-0.318	-0.345	-0.063	0.251	0.032	-0.237	-0.407	-0.622*	-0.536	-0.394	

Variations (%) ##

24.27

LSD, (p≤0.05)

2

ND = not detected, a value of 0.000 has been used in the statistical calculations

# As  $\frac{STDEV}{Mean} * 100$ ,

\* Significant at p≤ 0.05,

## From ANOVA

Table 11c: Changes and relationships in clonal overhead monoterpenes and mites levels in Kangaita during rainy season

Clone	Monoterpene compounds													Mites	
	P-menthan-1,3,8-triene	Sabienene	$\beta$ -phellandren	limonene	Z- $\beta$ -ocimene	E- $\beta$ -ocimene	Linalool(oxide) (cis) furanoid	Linalool(oxide) (trans) furanoid	Linalool	Terpinen-4-ol	Geraniol	4,8-dimethyl-1,3(E),7-notriene	Jasmonene		Sum mono
TRFK 6/8	0.135	0.056	0.315	0.098	0.518	0.850	0.121	0.026	0.329	0.120	0.093	0.155	0.005	2.821	7
TRFK 18/3	0.154	ND	ND	0.099	0.212	0.684	ND	ND	ND	0.152	ND	0.109	ND	1.410	27
TRFK 31/8	0.202	0.043	0.301	0.163	0.399	0.691	0.113	ND	0.204	0.244	ND	0.128	ND	2.488	7
TRFK 54/40	0.251	0.019	0.393	0.047	0.435	0.598	0.061	ND	0.281	0.154	ND	0.161	ND	2.400	62
TRIT 201/16	0.145	ND	ND	0.035	0.300	0.549	ND	ND	ND	0.030	ND	0.098	ND	1.157	59
TRIT 201/50	0.092	0.038	0.351	0.253	0.427	0.636	0.041	ND	0.098	ND	ND	0.124	0.277	2.377	3
TRFK 301/4	0.088	0.138	0.313	0.097	0.437	0.823	ND	ND	0.194	0.052	ND	0.089	ND	2.231	5
TRFK 303/1199	0.204	0.039	0.420	0.102	0.458	0.659	0.092	ND	0.287	0.106	ND	0.111	0.158	2.636	4
AHP S15/10	0.150	ND	ND	0.049	0.324	0.557	ND	ND	ND	0.041	ND	0.104	ND	1.225	46
TRFCA SFS 150	0.143	ND	ND	0.114	0.338	0.551	ND	ND	ND	0.143	ND	0.124	ND	1.413	13
STC 5/3	0.205	0.022	0.168	0.149	0.255	0.455	ND	ND	ND	0.150	ND	0.161	ND	1.565	28
Mean	0.161	0.032	0.206	0.110	0.373	0.641	0.039	0.002	0.127	0.108	0.008	0.124	0.040	1.971	24
STDE	0.050	0.040	0.175	0.062	0.094	0.119	0.021	0.001	0.135	0.071	0.006	0.025	0.092	0.949	
CV (%) #	31.056	125.000	84.951	56.364	25.201	18.565	53.846	450.00	106.300	65.741	75.000	20.161	72.500	46.148	
$r_{(mite)}$	0.415	-0.545	-0.405	0.683*	0.232	-0.539	0.110	-0.245	-0.311	-0.1	-0.245	0.084	-0.432	0.541	
Variations (%) ##															24.27
LSD, $p \leq 0.05$ ) 2															2

ND = not detected, a value of 0.000 has been used in the statistical calculations

# As  $\frac{STDEV}{Mean} * 100$ ,

\* Significant at  $p \leq 0.05$ ,

## From ANOVA

Table 11d: The variations and relationship between sesquiterpenes released by 11 tea varieties and mite infestations levels in Kangaita during rainy season

Clone	Sesquiterpenes compound										Mites
	$\alpha$ -copaene	$\alpha$ -cedrene	<i>E</i> - $\beta$ -caryophyllene	<i>E</i> - $\beta$ -farnesene	<i>E</i> - $\gamma$ -muurolene	Humulene	Germacrene D	Calamene	Cedrol	Sum sesqui	
TRFK 6/8	0.152	0.592	0.381	0.608	ND	0.019	0.073	0.245	1.009	1.752	7
TRFK 18/3	ND	0.656	0.339	0.508	ND	0.104	ND	ND	0.811	2.418	27
TRFK 31/8	0.180	0.703	0.324	0.757	ND	0.239	0.110	ND	0.940	2.313	7
TRFK 54/40	0.131	0.635	0.583	0.510	ND	0.273	ND	0.022	0.955	3.109	62
TRIT 201/16	ND	0.441	0.222	0.476	ND	0.114	ND	ND	0.822	2.075	59
TRIT 201/50	0.448	0.374	0.416	0.124	ND	0.000	ND	ND	0.793	2.155	3
TRFK 301/4	ND	0.443	0.347	0.405	ND	0.168	ND	0.114	0.673	2.15	5
TRFK 303/1199	ND	0.313	0.530	0.246	ND	ND	ND	ND	0.673	1.762	4
AHP S15/10	ND	0.429	0.302	0.777	ND	0.116	ND	ND	0.820	2.444	46
TRFCA SFS 150	ND	0.465	0.215	0.444	ND	0.090	0.126	ND	0.919	2.259	13
STC 5/3	0.023	0.568	0.531	0.662	0.010	0.117	ND	ND	0.653	2.83	28
Mean	0.085	0.511	0.381	0.502	0.001	0.113	0.028	0.035	0.824	2.273	24
STDEV	0.139	0.126	0.123	0.200	0.003	0.089	0.050	0.078	0.122	1.096	
CV (%) #	37.647	24.658	32.283	39.841	300.00	78.762	60.714	54.286	14.806	48.218	
$r_{(mite)}$	0.308	0.179	0.0	0.344	0.063	0.470	0.295	0.164	0.492	0.596	
Variations (%) ##											24.27
LSD, (p $\leq$ 0.05)											2

ND = not detected, a value of 0.000 has been used in the statistical calculations

\* Significant at p $\leq$ 0.05

# As  $\frac{STDEV}{Mean} * 100$

## From ANOVA

Table12a: Changes in mite infestations and overhead green leaf volatile compounds (GLVs) and their relationships in tea cultivars in Kangaita during cold season

Clone	Overhead green leaf volatile organic compounds			Sum GLVS	Mites
	Hexanal	Z-3-hexenyl acetate	Nonanal		
TRFK 6/8	ND	0.536	ND	0.536	2
TRFK 18/3	0.190	0.346	ND	0.536	2
TRFK 31/8	ND	0.284	ND	0.284	2
TRFK 54/40	0.213	0.525	0.022	0.760	9
TRIT 201/16	0.045	0.483	ND	0.528	2
TRIT 201/50	ND	ND	ND	0.000	1
TRFK 301/4	ND	0.285	ND	0.285	2
TRFK 303/1199	ND	0.323	ND	0.323	2
AHP S15/10	ND	0.520	ND	0.520	6
TRFCA SFS 150	0.113	0.438	ND	0.551	4
STC 5/3	0.195	0.433	ND	0.628	4
Mean	0.069	0.379	0.022	0.450	3
STDEV	0.032	0.158	0.006	0.218	
CV (%) #	46.377	41.689	27.273	48.444	
r <sub>(mite)</sub>	0.430	0.559	0.351	0.496	
Variations (%) ##					24.27
LSD, (p≤0.05)					2

ND = not detected, a value of 0.000 has been used in the statistical calculations

# As  $\frac{STDEV}{Mean} * 100$

## From ANOVA

Table 12b: Clonal variations and the relationships between mites infestations and overhead volatile aromatic compounds in Kangaita during cold season

Clone	Aromatic compounds												Mites
	Phenyl ethyl alcohol	Ethyl benzene	P-xylene	Indole	Benzaldehyde	Phenyl acetaldehyde	Benzyl alcohol	Acetophenone	Z-anethole	Benzothiazole	Benzophenone	Sum aroma	
TRFK 6/8	0.163	0.291	0.135	0.222	0.151	0.033	0.081	ND	0.246	0.057	0.059	1.438	2
TRFK 18/3	0.119	0.105	0.083	ND	0.127	ND	ND	0.179	0.084	ND	ND	0.697	2
TRFK 31/8	0.143	0.221	0.218	ND	0.089	0.068	0.125	0.153	0.153	ND	ND	1.170	2
TRFK 54/40	0.073	0.163	0.216	ND	0.182	0.134	0.169	0.134	0.072	ND	ND	1.143	9
TRIT 201/16	0.073	0.124	ND	ND	ND	ND	0.183	0.183	0.154	ND	ND	0.717	2
TRIT 201/50	0.144	0.232	0.074	0.232	0.358	ND	ND	0.306	0.266	0.086	0.118	1.742	1
TRFK 301/4	0.138	0.176	0.269	ND	0.146	ND	ND	0.131	0.073	ND	0.144	1.029	2
TRFK 303/1199	0.251	0.209	0.272	ND	0.132	ND	0.1323	0.243	0.116	ND	0.221	1.688	2
AHP S15/10	0.084	0.116	ND	ND	0.183	ND	0.054	0.163	0.158	ND	ND	0.758	6
TRFCA SFS 150	0.076	0.129	ND	0.246	0.183	0.059	0.127	0.124	0.089	ND	ND	1.033	4
STC 5/3	0.113	0.108	0.072	0.032	0.132	0.079	0.097	0.154	0.067	ND	ND	0.782	4
MEAN	0.125	0.170	0.122	0.066	0.153	0.034	0.076	0.161	0.120	0.0130	0.049	1.200	4
STDEV	0.053	0.061	0.106	0.012	0.086	0.046	0.059	0.076	0.074	0.011	0.024	0.777	
CV (%) #	42.400	35.882	86.885	18.182	56.209	135.294	77.632	47.205	61.667	84.615	82.759	64.750	
$r_{(mite)}$	-0.535	-0.369	-0.195	-0.237	0.063	0.682*	0.379	-0.228	-0.374	-0.379	-0.437	-0.283	
	Variations (%) ##												24.27
LSD, (p≤0.05)													2

ND = not detected, a value of 0.000 has been used in the statistical calculations

\* Significant at p≤0.05

# As  $\frac{STDEV}{Mean} * 100$

## From ANOVA

Table 12c: Changes and relationships in clonal overhead monoterpenes and mites levels in Kangaita during cold season

Clone	Compound											Mites	
	P-menthan-1,3,8-triene	Sabienene	$\beta$ -phellandren	Limone	Z- $\beta$ -ocimene	E- $\beta$ -ocimene	Linalool (oxide) (cis) furanoid	Linalool (oxide) (trans) furanoid	linalool	Terpinen-4-ol	4,8-dimethyl-1,3(E),7-notriene		Sum
TRFK 6/8	0.137	0.068	0.327	0.124	0.364	0.423	0.121	0.042	0.382	0.131	0.156	2.275	2
TRFK 18/3	0.225	ND	ND	0.159	0.299	0.329	ND	ND	ND	0.160	0.169	1.341	2
TRFK 31/8	0.216	0.054	0.314	0.167	0.433	0.429	0.123	ND	0.325	0.257	0.148	2.320	2
TRFK 54/40	0.319	0.031	0.433	0.057	0.514	0.385	0.072	ND	0.325	0.166	0.174	2.447	9
TRIT 201/16	0.164	ND	ND	0.052	0.326	0.409	0.000	ND	ND	0.042	0.111	1.188	2
TRIT 201/50	0.124	0.042	0.363	0.271	0.426	0.680	0.049	ND	0.123	ND	0.137	2.338	1
TRFK 301/4	0.112	0.046	0.327	0.11	0.452	0.516	ND	ND	0.337	0.056	0.126	2.150	2
TRFK 303/1199	0.208	0.047	0.427	0.122	0.460	0.664	0.112	ND	0.315	0.116	0.123	2.614	2
AHP S15/10	0.157	ND	ND	0.057	0.350	0.437	ND	ND	ND	0.053	0.124	1.297	6
TRFCA SFS 150	0.158	ND	ND	0.127	0.345	0.438	ND	ND	ND	0.143	0.136	1.910	4
STC 5/3	0.223	0.032	0.193	0.163	0.291	0.462	ND	ND	ND	0.156	0.172	1.692	4
Mean	0.186	0.029	0.217	0.128	0.387	0.470	0.043	0.004	0.163	0.116	0.143	1.961	4
STDEV	0.060	0.025	0.183	0.063	0.073	0.110	0.054	0.013	0.169	0.073	0.022	0.073	
CV (%) #	32.258	86.207	84.332	49.219	18.863	23.404	12.558	75.000	42.945	62.931	15.385	3.723	
r(mite)	0.631*	-0.281	0.004	-0.567	0.241	-0.390	-0.118	-0.176	-0.032	0.195	0.356	-0.312	
Variations (%) ##													24.27
LSD, (p $\leq$ 0.05)													2

ND = not detected, a value of 0.000 has been used in the statistical calculations

\* Significant at p $\leq$ 0.05

# As  $\frac{STDEV}{Mean} * 100$

## From ANOVA

Table 12d: The variations and relationship between sesquiterpenes released by 11 tea varieties and mite infestations levels in Kangaita during cold season

Clone	Compound										Mites
	$\alpha$ -copaene	$\alpha$ -cedrene	<i>E</i> - $\beta$ -caryophyllene	<i>E</i> - $\beta$ -farnesene	<i>E</i> - $\gamma$ -muurolene	Humulene	Germacrene D	Calamene	Cedrol	Sum sesqui	
TRFK 6/8	0.173	0.650	0.434	0.714	ND	0.063	0.132	0.294	1.126	3.586	2
TRFK 18/3	ND	0.665	0.579	0.434	ND	0.135	ND	ND	0.735	2.538	2
TRFK 31/8	0.231	0.748	0.376	0.835	ND	0.257	ND	ND	0.973	3.420	2
TRFK 54/40	0.155	0.728	0.664	0.529	ND	0.327	ND	0.035	1.100	3.538	9
TRIT 201/16	ND	ND	0.2333	0.543	ND	0.126	ND	ND	0.832	1.734	2
TRIT 201/50	0.282	0.489	0.410	0.438	ND	0.197	ND	ND	0.857	2.673	1
TRFK 301/4	ND	0.427	0.367	0.437	ND	0.235	ND	0.212	0.723	2.401	2
TRFK 303/1199	0.168	0.446	0.324	0.517	ND	0.264	ND	ND	0.788	2.507	2
AHP S15/10	ND	0.524	0.323	0.634	ND	0.123	ND	0.000	0.960	2.564	6
TRFCA SFS 150	ND	0.531	0.273	0.492	ND	0.152	0.142	0.000	1.126	2.716	4
STC 5/3	0.126	0.648	0.563	0.722	0.027	0.134	ND	0.000	0.753	2.973	4
Mean	0.103	0.532	0.413	0.572	0.002	0.183	0.025	0.049	0.906	2.785	4
STDEV	0.107	0.208	0.136	0.135	0.001	0.079	0.056	0.103	0.158	0.990	
CV (%) #	103.883	39.09	32.930	23.601	50.000	43.169	76.000	67.347	17.439	35.548	
$r_{\text{(mite)}}$	0.141	0.239	0.437	0.063	0.100	0.315	0.045	-0.179	0.464	0.355	
Variations (%) ##											24.27
LSD, (p $\leq$ 0.05)											2

ND = not detected, a value of 0.000 has been used in the statistical calculations

# As  $\frac{STDEV}{Mean} * 100$

## From ANOVA



No significant correlation was recorded during cold season (Table 14a). There was decline in GLVs level from 2.738 in dry season to 0.644 and 0.194 in rainy and cold seasons, respectively. Mites levels equally dropped from 15 in dry season to 9 and 2 in rainy and cold seasons, respectively. The results indicate that mites levels are influenced by the overhead GLVs.

The aromatic compounds were inversely correlated to mites levels although the correlations were insignificant. The levels were high in dry season (1.01) (Table 13b) decreased to 0.67 (Table 14b) in rainy season and further decreased to 0.52 (Table 15b) during cold season. TRFK 18/3, TRIT 201/16, AHP S15/10 and STC 5/3 released relatively low levels of aromatic compounds and recorded high levels of mites infestations. Majority of monoterpenes were negatively correlated to mites levels. Sabinene (**85**) significantly ( $p \leq 0.05$ ) and inversely correlated to mites infestations. Their levels decreased from 1.50 in dry season (Table 13c) to 0.80 in rainy season (Table 14c) and increased to 0.89 (Table 15c) during cold season. Most of the sesquiterpenes were negatively correlated to mites levels (Table 13d), however, the relationship was insignificant. The levels decreased from 2.27 in dry season (Table 13d) to 1.24 in rainy season (Table 14d) and slightly increased to 1.25 (Table 15d) in cold season. The results demonstrate the defensive roles of the terpenes and aromatic compounds in plants against pests attack (Eller et al., 2014) since these compounds were inversely correlated to mites infestation levels. The results further suggest that GLVs contribute to susceptibility of tea plants to the two species of mites. Cultivars that release low levels and composition of GLVs and high levels of terpenes and aromatic compounds should be exploited in Kipkebe.

Table 13a: Changes in mites infestations and overhead green leaf volatile compounds (GLVs) and their relationships in tea cultivars in Kipkebe during dry season

Clone	Overhead green leaf volatile compounds								Mites	
	1-Penten-3-ol	Z-2-Pentenol	Hexanal	E-2-hexenal	Z-3-hexenal	Z-3-hexenol	Z-3-hexenyl acetate	Nonanal		Sum GLVS
TRFK 6/8	0.151	0.072	0.284	0.613	0.022	0.544	2.238	ND	3.924	6
TRFK 18/3	0.251	0.064	0.253	0.451	0.064	0.593	0.735	0.032	2.443	20
TRFK 31/8	0.205	0.116	0.081	0.354	0.025	0.547	1.301	ND	2.629	6
TRFK 54/40	0.221	0.146	0.312	0.567	0.225	0.798	1.340	0.052	3.661	31
TRIT 201/16	0.232	0.028	0.243	0.513	0.104	0.635	0.923	0.053	2.731	22
TRIT 201/50	0.021	ND	0.052	0.154	ND	0.303	0.739	ND	1.269	6
TRFK 301/4	0.076	0.427	0.167	0.145	ND	0.543	0.748	ND	2.106	5
TRFK 303/1199	0.105	0.053	0.152	0.014	ND	0.313	0.663	ND	1.300	5
AHP S15/10	0.214	0.107	0.232	0.543	0.058	0.718	1.613	0.118	3.603	20
TRFCA SFS 150	0.195	0.086	0.264	0.152	0.128	0.523	1.515	0.135	2.998	14
STC 5/3	0.232	0.032	0.342	0.480	0.103	0.624	1.519	0.133	3.465	33
Mean	0.173	0.103	0.217	0.362	0.066	0.558	1.212	0.047	2.738	15
STDEV	0.075	0.115	0.093	0.209	0.070	0.149	0.498	0.056	0.868	
CV (%) #	43.353	111.650	42.857	57.735	106.061	26.703	41.089	119.149	31.702	
$r_{(mite)}$	0.723*	0.205	0.755*	0.602*	0.817*	0.742*	0.173	0.684*	0.565	
Variations (%) ##										25.16
LSD, (p≤0.05)										1

ND = not detected, a value of 0.000 has been used in the statistical calculations

\* Significant at p≤0.05

# As  $\frac{STDEV}{Mean} * 100$

## From ANOVA

Table 13b: Changes and relationships in clonal overhead aromatics and mites levels in Kipkebe during dry season

Clone	Phenyl ethyl alcohol	Methyl salicylate	Ethyl benzene	p-Xylene	Azulene	Benzaldehyde	Phenyl acetaldehyde	Benzyl alcohol	Acetophenone	Naphthalene	Z-Anethole	Indene	Sum aroma	Mites
TRFK 6/8	0.126	0.152	0.173	0.063	0.000	0.056	0.000	0.094	0.105	0.000	0.228	0.000	0.997	6
TRFK 18/3	0.045	0.045	0.117	0.042	0.000	0.033	0.022	0.036	0.072	0.000	0.152	0.000	0.564	20
TRFK 31/8	0.114	0.146	0.123	0.175	0.000	0.072	0.051	0.054	0.142	0.542	0.111	0.000	1.53	6
TRFK 54/40	0.044	0.162	0.142	0.182	0.000	0.103	0.102	0.151	0.107	0.000	0.083	0.000	1.076	31
TRIT 201/16	0.028	0.003	0.022	0.000	0.000	0.016	0.014	0.024	0.122	0.000	0.137	0.000	0.366	22
TRIT 201/50	0.117	0.041	0.195	0.064	0.000	0.114	0.042	0.017	0.334	0.000	0.185	0.000	1.109	6
TRFK 301/4	0.104	0.035	0.148	0.223	0.130	0.126	0.022	0.000	0.103	0.000	0.144	0.000	1.035	5
TRFK 303/1199	0.205	0.042	0.148	0.252	0.000	0.107	0.075	0.105	0.244	0.000	0.152	0.000	1.33	5
AHP S15/10	0.038	0.028	0.102	0.000	0.000	0.142	0.012	0.012	0.122	0.000	0.156	0.000	0.612	20
TRFCA SFS 150	0.053	0.010	0.105	0.000	0.000	0.178	0.024	0.123	0.104	0.000	0.044	0.000	0.641	14
STC 5/3	0.103	0.115	0.123	0.023	0.000	0.105	0.053	0.052	0.142	0.000	0.063	0.000	0.779	33
MEAN	0.089	0.071	0.127	0.093	0.012	0.096	0.038	0.061	0.145	0.049	0.132	0.028	0.941	15
STDEV	0.053	0.060	0.045	0.096	0.039	0.048	0.030	0.050	0.076	0.164	0.054	0.091	0.806	
CV (%)	59.77	84.93	35.31	103.09	331.66	50.01	80.06	82.70	52.51	331.66	40.67	331.66	1584.03	
$r_{(mite)}$	-0.664	-0.161	-0.459	-0.176	-0.281	-0.230	0.399	0.266	-0.237	-.0394	-0.003	0.422	-0.664	
Variations (%) ##														25.16
LSD, (p≤0.05)														1

ND = not detected, a value of 0.000 has been used in the statistical calculations

# As  $\frac{STDEV}{Mean} * 100$

## From ANOVA

Table 13c: Changes and relationships in clonal overhead monoterpenes and mites levels in Kipkebe during dry season

Clone	Monoterpenes compound												Mites	
	p-Mentha-1,3,8-triene	Sabinene	$\beta$ -Phellandrene	Limonene	Z- $\beta$ -ocimene	E- $\beta$ -ocimene	Linalool oxide (cis) furanoid	Linalool	Terpene n-4-ol	Geraniol	4,8-dimethyl 1,3(E),7-nontriene	P-cymene		Sum Mono
TRFK 6/8	0.146	0.054	0.306	0.037	0.364	0.809	0.102	0.299	ND	0.008	0.166	0.042	2.291	6
TRFK 18/3	0.145	ND	ND	0.032	0.202	0.452	0.008	0.343	ND	ND	0.063	ND	1.245	20
TRFK 31/8	0.183	0.042	0.235	0.133	0.263	0.431	0.063	0.216	0.542	ND	0.114	ND	2.222	6
TRFK 54/40	0.216	0.023	0.260	0.062	0.272	0.462	0.064	0.296	ND	ND	0.116	ND	1.771	31
TRIT 201/16	0.113	ND	ND	0.033	0.232	0.546	0.048	0.419	ND	ND	0.114	ND	1.505	22
TRIT 201/50	0.106	0.047	0.268	0.105	0.298	0.426	0.032	0.175	ND	0.021	0.113	ND	1.591	6
TRFK 301/4	0.043	0.035	0.214	0.065	0.274	0.488	0.032	0.189	ND	0.036	0.077	ND	1.453	5
TRFK 303/1199	0.145	0.042	0.322	0.101	0.347	0.546	0.075	0.187	ND	0.004	0.056	ND	1.825	5
S15/10	0.122	ND	ND	0.032	0.212	0.482	0.024	0.415	ND	0.016	0.133	ND	1.436	20
SFS 150	0.172	ND	ND	0.083	0.322	0.532	0.027	0.275	ND	ND	0.142	ND	1.553	14
STC 5/3	0.151	0.014	0.159	0.143	0.143	0.659	0.026	0.265	ND	ND	0.133	ND	1.693	33
Mean	0.140	0.023	0.160	0.075	0.075	0.530	0.045	0.280	0.049	0.008	0.112	0.1115	1.497	15
STDEV	0.045	0.021	0.134	0.041	0.041	0.114	0.028	0.086	0.164	0.024	0.005	0.0339	0.72	
CV (%) #	32.143	91.304	83.750	54.667	54.667	21.509	60.81	30.714	30.714	48.980	62.500	30.357	48.096	
$r_{(mite)}$	0.399	-0.663*	-0.429	-0.071	-0.535	0.000	-0.346	0.562	-0.289	-0.479	0.189	-0.109	-0.330	
Variations (%) ##														25.16
LSD, (p $\leq$ 0.05)														1

ND = not detected, a value of 0.000 has been used in the statistical calculations

\* Significant at p $\leq$ 0.05

# As  $\frac{STDEV}{Mean} * 100$

## From ANOVA

Table 13d: The variations and relationship between sesquiterpenes released by 11 tea varieties and mite infestations levels in Kipkebe during dry season

Clone	Sesquiterpene compounds											Mites
	$\alpha$ -copaene	$\alpha$ -cedrene	<i>E</i> - $\beta$ -caryophyllene	<i>E</i> - $\beta$ -farnesene	Humulene	Longicamphenylone	Longifolene	Calamene	Italicene	Cedrol	Sum Sesquit	
TRFK 6/8	0.212	0.572	0.524	0.953	ND	0.123	ND	0.244	ND	0.766	3.394	6
TRFK 18/3	ND	0.549	0.253	0.497	ND	ND	ND	ND	ND	0.849	2.148	20
TRFK 31/8	0.151	0.446	0.273	0.483	ND	ND	ND	ND	ND	1.329	2.682	6
TRFK 54/40	0.132	0.740	0.328	0.435	0.206	ND	ND	0.024	ND	1.025	2.89	31
TRIT 201/16	ND	0.348	0.261	0.623	ND	ND	ND	ND	ND	0.642	1.874	22
TRIT 201/50	0.222	0.333	0.345	0.406	ND	0.102	0.045	ND	ND	0.932	2.385	6
TRFK 301/4	ND	0.291	0.349	0.472	ND	0.123	ND	0.145	0.285	0.830	2.495	5
TRFK 303/1199	0.172	0.298	0.244	0.404	ND	0.012	ND	ND	ND	0.745	1.875	5
AHP S15/10	ND	0.275	0.215	0.521	ND	ND	ND	ND	ND	0.597	1.608	20
TRFCA SFS 150	ND	0.524	0.223	0.345	ND	ND	ND	ND	ND	0.951	2.043	14
STC 5/3	0.025	0.361	0.311	0.488	ND	ND	ND	ND	ND	0.582	1.767	33
Mean	0.083	0.431	0.302	0.511	0.062	0.033	0.004	0.038	0.026	0.841	2.269	15
STDEV	0.041	0.149	0.087	0.163	0.039	0.028	0.003	0.019	0.018	0.218	0.964	
CV (%) #	49.398	34.571	28.808	31.898	62.903	84.848	75.00	50.000	69.231	25.922	48.486	
r(mite)	-0.468	0.318	-0.251	-0.114	0.479	-0.608	-0.289	-0.392	-0.321	-0.341	-0.326	
Variations (%) ##												25.16
LSD <sub>5</sub> (p<0.05)												1

ND = not detected, a value of 0.000 has been used in the statistical calculations

# As  $\frac{STDEV}{Mean} * 100$

## From ANOVA

Table 14a: Changes in mite infestations and overhead green leaf volatile compounds (GLVs) and their relationships in tea cultivars in Kipkebe during rainy season

Clone	Overhead green leaf volatile compounds						Nonanal	Sum GLVs	Mites
	1-Penten-3-ol	Hexanal	<i>E</i> -2- hexenal	<i>Z</i> -3- hexenal	<i>Z</i> -3- hexenol	<i>Z</i> -3- hexenyl acetate			
TRFK 6/8	0.033	0.046	0.173	ND	0.214	0.372	ND	0.838	3
TRFK 18/3	ND	0.037	0.213	ND	0.194	0.331	0.014	0.789	9
TRFK 31/8	0.032	0.193	0.146	ND	0.145	0.222	ND	0.738	2
TRFK 54/40	0.034	0.183	0.152	ND	0.164	0.312	0.021	0.866	20
TRIT 201/16	0.043	0.062	ND	0.173	0.156	0.372	ND	0.806	9
TRIT 201/50	ND	ND	0.045	ND	ND	ND	ND	0.045	3
TRFK 301/4	ND	ND	0.051	ND	ND	0.282	ND	0.333	4
TRFK 303/1199	ND	ND	0.084	ND	ND	0.189	ND	0.273	4
AHP S15/10	0.035	0.261	0.164	ND	0.175	0.301	ND	0.936	17
TRFCA SFS 150	0.016	0.091	0.004	ND	0.171	0.244	0.022	0.548	5
STC 5/3	0.045	0.152	0.191	ND	0.184	0.302	0.041	0.915	23
Mean	0.022	0.093	0.111	0.016	0.127	0.266	0.009	0.644	9
STDEV	0.019	0.091	0.077	0.038	0.084	0.105	0.004	0.310	
CV (%) #	86.364	97.849	69.369	237.500	66.142	39.474	44.444	48.137	
$r_{(mite)}$	0.155	0.620*	0.456	0.000	0.442	0.371	0.685*	0.624*	
Variations (%) ##									25.16
LSD, (p≤0.05)									1

ND = not detected, a value of 0.000 has been used in the statistical calculations

\* Significant at  $p \leq 0.05$

# As  $\frac{STDEV}{Mean} * 100$

## From ANOVA

Table 14b: Clonal variations and the relationships between mites infestations and overhead volatile aromatic compounds in Kipkebe during rainy season

Clone	Aromatic compounds														Mites	
	Phenyl ethyl alcohol	Ethyl benzen e	Phenol	Phenyl acetalde hyde	Azulen e	Napht halene	Indene	Benzot hiozole	P- xylene	Benzal dehyde	Phenyl acetate	Benzyl alcohol	Acetop henone	Z- anethole		Sum aroma
TRFK 6/8	0.021	0.126	ND	ND	ND	ND	ND	0.021	0.024	0.032	ND	0.034	0.052	0.132	0.523	3
TRFK 18/3	0.102	0.046	ND	0.015	ND	ND	ND	ND	0.023	0.022	0.015	0.012	0.026	0.056	0.317	9
TRFK 31/8	0.103	0.102	ND	0.021	ND	0.401	ND	ND	0.135	0.032	0.021	0.032	0.101	0.045	0.993	2
TRFK 54/40	0.023	0.102	ND	0.037	ND	ND	ND	ND	0.143	0.035	0.037	0.123	0.052	0.037	0.598	20
1.TRIT 201/16	0.015	0.049	ND	ND	ND	ND	ND	0.014	ND	0.013	ND	ND	0.084	0.085	0.260	9
TRIT 201/50	0.113	0.173	0.032	ND	ND	ND	ND	0.035	0.035	0.104	ND	0.015	0.232	0.134	0.873	3
TRFK 301/4	0.094	0.123	ND	ND	0.093	ND	ND	ND	0.133	0.124	ND	ND	0.045	0.104	0.721	4
TRFK 303/1199	0.182	0.134	ND	ND	ND	ND	ND	ND	0.205	0.102	ND	0.102	0.174	0.123	1.022	4
AHP S15/10	0.023	0.035	ND	ND	ND	ND	ND	ND	ND	0.102	ND	0.011	0.103	0.122	0.396	17
TRFCA SFS 150	0.023	0.047	ND	0.021	ND	ND	ND	ND	ND	0.042	0.021	0.074	0.102	0.025	0.355	5
STC 5/3	0.063	0.063	ND	0.021	ND	ND	0.192	ND	0.013	0.032	0.021	0.022	0.103	0.022	0.552	23
MEAN	0.069	0.091	0.003	0.011	0.008	0.036	0.017	0.006	0.064	0.058	0.011	0.039	0.098	0.080	0.673	9
STDEV	0.054	0.046	0.001	0.013	0.0005	0.024	0.013	0.003	0.074	0.041	0.013	0.042	0.060	0.044	0.646	
CV (%) #	78.261	50.549	33.333	118.182	62.500	66.667	76.471	50.000	115.625	70.690	118.182	107.692	61.224	55.000	42.051	
r <sub>(mite)</sub>	-0.549	-0.494	0.227	0.476	0.219	0.227	0.438	0.173	-0.219	-0.224	0.475	0.130	-0.237	-0.432	0.2108	

Variations (%) ## 25.16

LSD, (p<0.05)

ND = not detected, a value of 0.000 has been used in the statistical calculations

# As  $\frac{STDEV}{Mean}$

## From ANOVA

Table 14c: Changes and relationships in clonal overhead monoterpenes and mites levels in Kipkebe during rainy season

Clone	Monoterpene compounds											Mites
	P-mentha-1,3,8-triene	Sabinene	P-cymene	$\beta$ -Phellandrene	Limonene	Z- $\beta$ -ocimene	E- $\beta$ -ocimene	Linalol	Terpinen-4-ol	4,8-dimethyl-1,3(E),7-nontriene	Sum Mono	
TRFK 6/8	ND	0.022	0.064	0.166	0.022	0.252	0.279	ND	0.112	ND	0.917	3
TRFK 18/3	0.055	ND	ND	ND	0.022	0.066	0.224	ND	0.023	0.024	0.414	9
TRFK 31/8	0.131	0.018	ND	0.187	0.111	0.202	0.234	ND	0.152	0.066	1.101	2
TRFK 54/40	0.113	0.011	ND	0.201	0.031	0.228	0.255	ND	0.103	0.094	1.036	20
TRIT 201/16	0.062	ND	ND	ND	0.013	0.122	0.313	ND	0.022	0.043	0.575	9
TRIT 201/50	ND	0.021	ND	0.225	0.101	0.236	0.289	ND	ND	ND	0.872	3
TRFK 301/4	ND	0.021	ND	0.176	0.045	0.221	0.316	ND	0.024	ND	0.803	4
TRFK 303/1199	ND	0.024	ND	0.220	0.055	0.253	0.283	ND	0.022	ND	0.857	4
S15/10	0.102	ND	ND	ND	0.012	0.144	0.167	ND	0.012	0.056	0.493	17
SFS 150	0.121	ND	ND	ND	0.035	0.194	0.208	ND	0.063	0.084	0.705	5
STC 5/3	0.102	ND	ND	0.102	0.109	0.124	0.211	0.201	0.112	0.091	1.052	23
Mean	0.062	0.011	0.002	0.116	0.051	0.186	0.253	0.018	0.059	0.042	0.802	9
STDEV	0.054	0.010	0.001	0.098	0.038	0.062	0.048	0.007	0.052	0.039	0.463	
CV (%) #	87.097	90.909	50.000	84.48	74.510	33.333	18.72	38.889	88.136	92.857	57.731	
$r_{(mite)}$	0.510	-0.605	-0.831	-0.270	-0.063	-0.445	-0.493	0.616*	0.145	0.670*	0.045	
Variations(%)##				3								25.16
LSD, (p $\leq$ 0.05)												1

ND = not detected, a value of 0.000 has been used in the statistical calculations

\* Significant at p $\leq$ 0.05

# As  $\frac{STDEV}{Mean} * 100$

## From ANOVA



Table 14d: The variations and relationship between sesquiterpenes released by 11 tea varieties and mite infestations levels in Kipkebe during rainy season

Clone	Sesquiterpene compounds										Mites
	$\alpha$ -copaene	$\alpha$ -cedrene	<i>E</i> - $\beta$ -caryophyllene	<i>E</i> - $\beta$ -farnesene	Longica mphenylone	Longi folene	Calame nene	Itali cene	Cedrol	Sum Sesquit	
TRFK 6/8	0.052	0.352	0.242	0.303	0.044	ND	0.195	ND	0.452	1.64	3
TRFK 18/3	ND	0.272	0.066	0.235	ND	ND	ND	ND	0.323	0.896	9
TRFK 31/8	0.053	0.304	0.207	0.274	ND	ND	ND	ND	0.346	1.045	2
TRFK 54/40	0.113	0.332	0.243	0.311	ND	ND	0.013	ND	0.554	1.634	20
TRIT 201/16	ND	0.212	0.125	0.331	ND	ND	ND	ND	0.463	1.337	9
TRIT 201/50	0.194	0.300	0.203	0.206	0.044	0.032	ND	ND	0.321	1.3	3
TRFK 301/4	ND	0.204	0.286	0.257	ND	ND	0.102	0.201	0.372	1.422	4
TRFK 303/1199	0.104	0.277	0.178	0.286	0.005	ND	ND	ND	0.382	1.232	4
AHP S15/10	ND	0.236	0.142	0.252	ND	ND	ND	ND	0.304	0.934	17
TRFCA SFS 150	ND	0.327	0.122	0.261	ND	ND	ND	ND	0.439	1.149	5
STC 5/3	0.014	0.244	0.242	0.264	ND	ND	ND	ND	0.297	1.061	23
Mean	0.048	0.278	0.187	0.271	0.008	0.003	0.028	0.018	0.387	1.241	9
STDE	0.064	0.050	0.067	0.036	0.003	0.001	0.017	0.004	0.081	0.45	
CV (%) #	55.000	17.986	35.829	13.284	37.500	33.333	60.714	22.222	20.930	36.261	
r <sub>(mite)</sub>	-0.190	-0.21	0.084	0.148	-0.416	-0.263	-0.322	-0.219	0.0	-0.212	
Variations (%) ##											25.16
LSD, (p $\leq$ 0.05)											1

ND = not detected, a value of 0.000 has been used in the statistical calculations

# As  $\frac{STDEV}{Mean} * 100$

## From ANOVA

Table15a: Changes in mite infestations and overhead green leaf volatile compounds and their relationships in tea cultivars in Kipkebe during cold season

Clone	Overhead green leaf volatile organic compounds				Mites
	Hexanal	Z-3-hexenyl acetate	Nonanal	Sum GLVS	
TRFK 6/8	ND	0.203	ND	0.203	1
TRFK 18/3	0.015	0.182	0.012	0.209	2
TRFK 31/8	0.084	0.146	ND	0.230	4
TRFK 54/40	0.133	0.164	0.016	0.313	3
TRIT 201/16	0.026	ND	ND	0.026	1
TRIT 201/50	ND	ND	ND	0.000	2
TRFK 301/4	ND	0.146	ND	0.146	3
TRFK 303/1199	ND	0.124	ND	0.124	2
AHP S15/10	0.016	0.167	ND	0.183	3
TRFCA SFS 150	0.107	0.165	0.024	0.296	2
STC 5/3	0.047	0.211	0.014	0.272	4
Mean	0.039	0.149	0.006	0.194	2
STDEV	0.028	0.056	0.005	0.1050.107	
CV (%) #	71.795	37.584	83.333	54.124	
r <sub>(mite)</sub>	0.381	0.394	0.152	0.471	
Variations (%) ##					25.16
LSD, (p≤0.05)					1

ND = not detected, a value of 0.000 has been used in the statistical calculations

# As  $\frac{STDEV}{Mean} * 100$

## From ANOVA

Table 15b: Clonal variations and the relationships between mites infestations and overhead volatile aromatic compounds in Kipkebe during cold season

Clone	Aromatic compounds													Mites
	Phenyl ethyl alcohol	Diphey l ether	Phenol	Ethyl benzene	P- xylene	Indene	Benzalde hyde	Phenyl acetaldehy de	Benzyl alcohol	Acetoph enone	Z- anethole	Azulene	Sum aroma	
TRFK 6/8	0.102	ND	ND	0.132	0.032	ND	0.034	ND	0.035	0.055	0.132	ND	0.522	1
TRFK 18/3	0.022	ND	ND	0.051	0.022	ND	0.021	0.016	0.013	0.026	0.057	ND	0.228	2
TRFK 31/8	0.123	ND	ND	0.102	0.136	ND	0.033	0.023	0.034	0.151	0.051	ND	0.653	4
TRFK 54/40	0.025	ND	ND	0.111	0.151	ND	0.042	0.052	0.131	0.056	0.041	ND	0.609	3
TRIT 201/16	0.015	ND	ND	0.013	ND	ND	0.013	ND	ND	0.086	0.086	ND	0.212	1
TRIT 201/50	0.114	0.027	0.032	0.181	0.037	ND	0.106	ND	0.015	0.235	0.141	ND	0.774	2
TRFK 301/4	0.101	ND	ND	0.125	0.135	ND	0.126	ND	ND	0.046	0.111	0.105	0.749	3
TRFK 303/1199	0.182	0.023	ND	0.136	0.205	ND	0.103	ND	0.105	0.176	0.128	ND	1.058	2
AHP S15/10	0.023	ND	ND	0.042	ND	ND	0.102	ND	0.012	0.100	0.124	ND	0.403	3
TRFCA SFS 150	0.024	ND	ND	0.051	ND	ND	0.042	0.022	0.077	0.103	0.031	ND	0.350	2
STC 5/3	0.060	ND	ND	0.063	0.013	0.175	0.042	0.021	0.024	0.104	0.023	ND	0.525	4
MEAN	0.072	0.005	0.003	0.091	0.066	0.016	0.060	0.012	0.040	0.103	0.084	0.010	0.520	2
STDEV	0.056	0.002	0.001	0.051	0.075	0.007	0.040	0.009	0.028	0.062	0.045	0.003	0.267	
CV (%) #	77.778	40.000	33.333	55.004	113.636	43.750	66.667	75.000	70.000	60.194	53.571	30.000	51.346	
r(mite)	0.492	0.494	0.1414	0.494	0.625*	0.490	0.539	0.527	0.1	0.476	0.391	0.524	5.904	
Variations (%) ##														25.16
LSD, (p≤0.05)														1

ND = not detected, a value of 0.000 has been used in the statistical calculations

\* Significant at p≤0.05

## From ANOVA

# As  $\frac{STDEV}{Mean} * 100$

Table 15c: Changes and relationships in clonal overhead monoterpenes and mites levels in Kipkebe during cold season

Clone	Monoterpene compounds									Mites
	P-mentha-1,3,8-triene	Sabiene	$\beta$ -phellandrene	limonen	Z- $\beta$ -ocimene	E- $\beta$ -ocimene	Terpinen-4-ol	4,8-dimethyl-1,3(E),7-notriene	Sum Mono	
TRFK 6/8	ND	0.022	0.166	0.023	0.023	0.301	0.113	ND	0.648	1
TRFK 18/3	0.054	ND	ND	0.022	0.022	0.232	0.023	0.026	0.379	2
TRFK 31/8	0.133	0.022	0.192	0.112	0.112	0.235	0.154	0.071	1.031	4
TRFK 54/40	0.116	0.014	0.214	0.042	0.042	0.262	0.113	0.102	2.905	3
TRIT 201/16	0.0664	ND	ND	0.113	0.013	0.313	0.023	0.043	0.571	1
TRIT 201/50	ND	0.023	0.225	0.103	0.241	0.302	ND	ND	0.894	2
TRFK 301/4	ND	0.022	0.182	0.048	0.048	0.318	0.023	ND	0.641	3
TRFK 303/1199	ND	0.024	0.223	0.056	0.254	0.301	0.022	ND	0.880	2
AHP S15/10	0.112	ND	ND	0.012	0.012	0.181	0.014	0.101	0.432	3
TRFCA SFS 150	0.123	ND	ND	0.035	0.035	0.204	0.065	0.068	0.530	2
STC 5/3	0.103	0.012	0.104	0.113	0.113	0.212	0.113	0.101	0.871	4
Mean	0.064	0.013	0.119	0.053	0.053	0.260	0.060	0.047	0.889	2
STDEV	0.056	0.011	0.100	0.039	0.039	0.049	0.053	0.044	0.391	
CV (%) #	87.500	84.615	84.034	73.585	73.585	18.846	88.333	93.617	43.982	
$r_{(mite)}$	0.534	0.161	0.219	0.620*	-0.315	-0.504	0.440	0.593	0.349	
Variations (%) ##										25.16
LSD, (p $\leq$ 0.05)										1

ND = not detected, a value of 0.000 has been used in the statistical calculations

\* Significant at p $\leq$ 0.05

## From ANOVA

# As  $\frac{STDEV}{Mean} * 100$

Table15d: The variations and relationship between sesquiterpenes released by 11 tea varieties and mite infestations levels in Kipkebe during cold season

Clone	Sesquiterpenes compound										Mites
	$\alpha$ -copaene	$\alpha$ -cedrene	<i>E</i> - $\beta$ -caryophyllene	<i>E</i> - $\beta$ -farnesene	Italicene	Longicamphenylene	Longifolene	Calamene	Cedrol	Sum sesqui	
TRFK 6/8	0.053	0.353	0.242	0.311	ND	ND	ND	0.197	0.453	1.609	1
TRFK 18/3	ND	0.246	0.071	0.236	ND	ND	ND	ND	0.332	0.885	2
TRFK 31/8	0.055	0.304	0.205	0.274	ND	ND	ND	ND	0.344	1.182	4
TRFK 54/40	0.117	0.352	0.258	0.333	ND	ND	ND	0.015	0.573	1.648	3
TRIT 201/16	ND	0.212	0.132	0.334	ND	ND	ND	ND	0.471	1.149	1
TRIT 201/50	0.195	0.302	0.203	0.205	ND	0.046	0.035	ND	0.322	1.308	2
TRFK 301/4	ND	0.206	0.287	0.260	0.232	ND	ND	0.104	0.375	1.464	3
TRFK 303/1199	0.106	0.315	0.181	0.296	ND	0.005	ND	ND	0.385	1.288	2
AHP S15/10	ND	0.243	0.158	0.304	ND	ND	ND	ND	0.314	1.174	3
TRFCA SFS 150	ND	0.344	0.125	0.262	ND	ND	ND	ND	0.443	1.173	2
STC 5/3	0.014	0.245	0.245	0.264	ND	ND	ND	ND	0.302	1.069	4
Mean	0.049	0.284	0.191	0.280	0.021	0.014	0.003	0.029	0.392	1.254	2
STDEV	0.039	0.055	0.066	0.040	0.035	0.009	0.002	0.024	0.084	0.487	
Variations (%) #	79.592	19.366	34.555	14.286	166.667	64.286	66.667	82.759	21.429	38.836	
$r_{(mite)}$	-0.055	0.395	-0.148	-0.170	0.173	-0.182	-0.144	-0.502	-0.377	-0.202	
CV (%) ##											25.16
LSD, (p $\leq$ 0.05)											1

ND = not detected, a value of 0.000 has been used in the statistical calculations

\* Significant at p $\leq$ 0.05

## From ANOVA

# As  $\frac{STDEV}{Mean} * 100$

Changes in OVOCs due to cultivars and seasons in Timbilil are presented in Tables 16-18. Z-3-hexenal (**6**) was the only GLV compound that significantly ( $r = 0.633$ ,  $p \leq 0.05$ ) correlated to mites levels in dry season (Table 16a). Two clones, TRFK 54/40 and STC 5/3 recorded both high levels of GLVs and mites. In rainy season, hexanal (**8**) was the only GLV compound that significantly ( $r = 0.738$ ,  $p \leq 0.05$ ) correlated to mites levels. The levels of GLVs decreased from 2.43 in dry season to 0.59 (Table 17a) and 0.218 (Table 18a) in rainy and cold seasons, respectively. All clones recorded low values of mites in rainy season. Aromatic compounds were released in the highest number and were inversely correlated to mites levels although the correlation was insignificant. The levels of aromatic compounds in dry season (1.007) (Table 16b) decreased to 0.6630 (Table 17b) in rainy season and increased to 0.727 (Table 18b) during cold season. A number of monoterpenes had an inverse correlation to mites levels. Their levels decreased from 1.855 in dry season (Table 16c) to 0.935 in rainy season (Table 17c) and increased to 0.956 (Table 18c) in cold season. Most of the sesquiterpenes were inversely correlated to mites levels (Table 16d). Sesquiterpenes levels dropped from 2.651 in dry season to 1.300 (Table 17d) during rainy season but slightly increased to 1.365 (Table 18d) in cold season.

The levels and composition of OVOCs were influenced by location of production, for instance in Kangaita the tea cultivars recorded much higher OVOCs levels than in Kipkebe and Timbilil. The results indicate that there is locational influence on the OVOCs levels and composition. The results also imply that the OVOCs are likely to influence tea cultivars to mites susceptibility/resistance in Kangaita than Kipkebe and Timbilil. These results corroborate earlier findings that VOCs varied with location of production of the tea cultivars (Owuor et al., 2010a; Owuor et al., 2008a; Owuor et al., 2010b). The levels and composition of OVOCs varied with location of production and clones, demonstrating that the extents of the changes varied from location to location in different clones. This means that it is not

possible to produce same levels and composition of OVOCs from cultivars when production sites are varied implying that a susceptible clone in one site due to specific levels/ composition of OVOCs may not be necessarily susceptible in another site. Tea cultivars must therefore be evaluated for OVOCs in the intended areas of production before commercial exploitation to prevent yield losses due to mites infestations.

Table16a: Changes in mite infestations and overhead green leaf volatile compounds (GLVs) and their relationships in tea cultivars in Timbilil during dry season

Clone	overhead green leaf volatile compounds									Mites
	1-Penten-3-ol	Z-2-pentenol	Hexanal	E-2-hexenal	Z-3-hexenal	Z-3-hexenol	Z-3-hexenyl acetate	Nonanal	SumGL VS	
TRFK 6/8	0.114	0.059	0.266	0.646	0.017	0.535	1.196	ND	2.833	4
TRFK 18/3	0.243	0.044	0.246	0.424	ND	0.522	1.371	0.024	2.85	7
TRFK 31/8	0.210	0.114	0.239	0.319	0.023	0.417	0.782	ND	2.104	6
TRFK 54/40	0.210	0.138	0.295	0.549	0.218	0.436	1.466	0.046	3.358	17
TRIT 201/16	0.229	0.027	0.218	0.474	ND	0.476	1.314	0.048	2.786	8
TRIT 201/50	0.017	ND	0.044	0.140	ND	0.298	0.617	ND	1.116	3
TRFK 301/4	0.056	0.036	0.164	0.139	ND	0.462	0.716	ND	1.534	7
TRFK 303/1199	0.103	0.048	0.156	0.134	ND	0.269	0.603	ND	1.266	6
AHP S15/10	0.214	0.098	0.216	0.427	ND	0.523	1.275	0.116	2.869	6
TRFCA SFS 150	0.186	0.076	0.210	0.130	0.126	0.464	1.385	0.134	2.711	6
STC 5/3	0.223	0.025	0.296	0.453	0.117	0.463	1.472	0.122	3.171	29
Mean	0.164	0.060	0.214	0.349	0.046	0.442	1.109	0.045	2.428	9
STDEV	0.078	0.042	0.072	0.187	0.038	0.087	0.352	0.028	0.946	
CV (%) #	47.561	70.000	33.645	53.582	82.609	19.683	31.740	62.222	38.962	
r(mite)	0.407	0.031	0.582	0.336	0.633*	0.122	0.521	0.477	0.524	
Variations (%) ##										32.52
LSD, (p≤0.05)										1

ND = not detected, a value of 0.000 has been used in the statistical calculations

\* Significant at p≤0.05

# As  $\frac{STDEV}{Mean} * 100$

## From ANOVA



Table16b: Clonal variations and the relationships between mites infestations and aromatic OVOCs in Timbilil during dry season

Clone	Aromatic compounds															Mites	
	Phenyl ethyl alcohol	Methyl salicylate	Ethyl benzen e	P- xylene	Phenol	acetop henone	Azulen e	Napht halen e	Lndole	Bezot hiazol e	Benzal dehyd	Pheny lacetal dehyd e	Benzyl alcohol	Acetop henone	Z- anetho le		Sum aromati cs
TRFK 6/8	0.152	0.149	0.208	0.149	ND	0.118	ND	ND	ND	0.112	0.168	0.062	0.102	0.118	0.256	1.364	4
TRFK 18/3	0.052	0.103	0.126	0.042	ND	0.080	ND	ND	0.216	ND	0.034	0.030	0.043	0.080	0.152	0.878	7
TRFK 31/8	0.137	0.162	0.197	0.198	ND	0.137	ND	0.825	ND	ND	0.082	0.054	0.106	0.137	0.127	1.2	6
TRFK 54/40	0.049	0.182	0.140	0.182	ND	0.119	ND	ND	ND	ND	0.106	0.106	0.148	0.119	0.099	1.131	17
TRIT 201/16	0.033	0.002	0.023	ND	ND	0.125	ND	ND	ND	0.031	0.014	0.042	0.026	0.125	0.146	0.411	8
TRIT 201/50	0.123	0.064	0.216	0.086	0.061	0.346	ND	ND	ND	0.165	0.126	0.045	0.022	0.346	0.216	1.244	3
TRFK 301/4	0.116	0.061	0.163	0.225	ND	0.113	0.155	ND	ND	ND	0.125	0.022	ND	0.113	0.147	0.972	7
TRFK 303/1199	0.215	0.045	0.167	0.253	0.089	0.246	ND	ND	ND	ND	0.127	0.118	0.116	0.246	0.195	1.482	6
AHP S15/10	0.050	0.031	0.105	ND	ND	0.122	ND	ND	ND	ND	0.140	0.014	0.021	0.122	0.158	0.641	6
TRFCA SFS 150	0.102	0.107	0.117	ND	ND	0.116	ND	ND	ND	ND	0.201	0.026	0.129	0.116	0.064	0.862	6
STC 5/3	0.103	0.125	0.147	0.027	ND	0.143	ND	ND	ND	ND	0.114	0.110	0.056	0.143	0.074	0.899	29
Mean	0.103	0.094	0.146	0.106	0.0137	0.152	0.014	0.075	0.020	0.028	0.112	0.057	0.070	0.151	0.148	1.007	9
STDEV	0.055	0.058	0.055	0.098	0.031	0.077	0.032	0.02	0.015	0.019	0.054	0.037	0.051	0.077	0.058	0.608	
CV (%) #	53.398	61.702	37.453	92.86	221.43	50.658	228.57	33.33	75.000	67.86	48.214	64.91	72.857	50.993	39.189	60.377	
r <sub>(mite)</sub>	-0.224	0.335	-0.152	-0.155	0.2702	-0.212	0.084	0.130	-0.084	0.369	-0.110	0.594	0.101	-0.212	-0.272	-0.161	
Variations (%) ##																	32.52
LSD, (p≤0.05)																	1

ND = not detected, a value of 0.000 has been used in the statistical calculations

# As  $\frac{STDEV}{Mean} * 100$

## From ANOVA

Table 16c: Changes and relationships in clonal overhead monoterpenes and mites levels in Timbilil during dry season

Clone	Monoterpenes compounds												Mites	
	P-menthan-1,3,8-triene	sabienene	$\beta$ -phellandrene	limonene	Z- $\beta$ -ocimene	E- $\beta$ -ocimene	Linalool oxide (cis) furanoid	Linalool oxide (trans) furanoid	Linalool	Terpine n-4-ol	Geraniol	4,8-dimethyl-1,3(E),7-notriene		Sum Monoterpenes
TRFK 6/8	ND	0.084	0.329	0.046	0.472	0.767	0.099	ND	0.374	0.152	0.103	ND	2.426	4
TRFK 18/3	0.161	ND	ND	0.036	0.223	0.463	0.018	ND	0.325	0.143	ND	0.114	1.483	7
TRFK 31/8	0.214	0.044	0.234	0.134	0.287	0.473	0.113	0.015	0.326	0.234	ND	0.123	2.106	6
TRFK 54/40	0.218	0.012	0.275	0.063	0.298	0.495	0.086	0.024	0.499	0.143	ND	0.118	2.231	17
TRIT 201/16	0.127	ND	ND	0.033	0.249	0.465	0.115	0.009	0.411	0.107	ND	0.111	1.627	8
TRIT 201/50	ND	0.054	0.324	0.113	0.317	0.533	0.062	ND	0.208	0.031	0.021	ND	1.663	3
TRFK 301/4	ND	0.043	0.243	0.103	0.322	0.526	0.057	ND	0.216	0.056	0.051	ND	1.617	7
TRFK 303/1199	ND	0.044	0.352	0.118	0.384	0.628	0.097	ND	0.236	0.104	0.017	ND	1.98	6
AHPS15/10	0.124	ND	ND	0.032	0.214	0.316	0.027	ND	0.397	0.032	0.022	0.121	1.285	6
TRFCA SFS 150	0.165	ND	ND	0.114	0.323	0.519	0.042	ND	0.378	0.116	ND	0.137	1.794	6
STC 5/3	0.216	0.015	0.163	0.145	0.325	0.645	0.036	ND	0.341	0.165	0.003	0.135	2.189	29
Mean	0.111	0.027	0.175	0.085	0.310	0.530	0.068	0.004	0.337	0.117	0.020	0.078	1.855	9
STDEV	0.094	0.028	0.148	0.044	0.073	0.117	0.035	0.003	0.090	0.061	0.002	0.062	0.792	
CV (%) #	84.685	103.70	84.571	51.765	23.548	22.07	51.471	75.000	26.706	52.13	161.82	79.487	42.695	
r <sub>(mite)</sub>	0.660*	0.324	-0.032	0.3	-0.055	0.176	-0.221	0.324	0.221	0.333	-0.332	0.458	0.378	
Variations (%) ##														32.52
LSD, (p $\leq$ 0.05)														1

ND = not detected, a value of 0.000 has been used in the statistical calculations

\* Significant at p $\leq$ 0.05

# As  $\frac{STDEV}{Mean} * 100$

## From ANOVA

Table 16d: The variations and relationship between sesquiterpenes released by 11 tea varieties and mite infestations levels in Timbilil during dry season

Clone	Sesquiterpenes compound							Mites
	$\alpha$ -copaene	$\alpha$ -cedrene	<i>E</i> - $\beta$ -caryophyllen e	<i>E</i> - $\beta$ -farnesene	Calamenene	Cedrol	Sum sesquiterpenes	
TRFK 6/8	0.218	1.339	0.543	0.964	0.264	1.793	5.121	4
TRFK 18/3	ND	0.611	0.286	0.575	ND	1.048	2.52	7
TRFK 31/8	0.153	0.524	0.276	0.549	ND	1.271	2.773	6
TRFK 54/40	0.136	1.128	0.427	0.461	ND	0.628	2.78	17
TRIT 201/16	ND	0.428	0.270	0.524	ND	0.676	1.898	8
TRIT 201/50	0.226	0.369	0.486	0.414	ND	1.157	2.652	3
TRFK 301/4	ND	0.318	0.351	0.512	0.175	1.258	2.614	7
TRFK 303/1199	0.203	0.337	0.329	0.447	ND	1.207	2.523	6
AHP S15/10	ND	0.318	0.247	0.461	ND	0.628	1.654	6
TRFCA SFS 150	ND	0.542	0.216	0.496	ND	1.427	2.681	6
STC 5/3	0.034	0.394	0.335	0.483	ND	0.701	1.947	29
Mean	0.088	0.574	0.342	0.535	0.040	1.072	2.651	9
STDEV	0.099	0.344	0.103	0.150	0.038	0.379	1.166	
CV (%) #	112.500	59.930	30.117	28.037	3.800	22.035	43.983	
r(mite)	-0.232	0.000	0.063	-0.221	-0.243	-0.550	-0.134	
Variations (%) ##								32.52
LSD, (p $\leq$ 0.05)								1

ND = not detected, a value of 0.000 has been used in the statistical calculations

# As  $\frac{STDEV}{Mean} * 100$

## From ANOVA

Table 17a: Changes in mite infestations and overhead green leaf volatile compounds (GLVs) and their relationships in tea cultivars in Timbilil during rainy season

Clone	Overhead green leaf volatile compounds (GLVs)							Mites
	1-penten-3-ol	Hexanal	E-2-hexenal	Z-3-hexenal	Z-3-hexenyl acetate	Nonanal	Sum GLVS	
TRFK 6/8	0.112	0.040	0.158	0.192	0.345	ND	0.848	2
TRFK 18/3	ND	0.101	0.214	0.191	0.316	ND	0.761	2
TRFK 31/8	0.025	0.334	0.133	0.139	0.212	0.013	0.623	2
TRFK 54/40	0.031	0.202	0.145	0.159	0.272	0.019	0.829	10
TRIT 201/16	0.039	0.095	0.159	0.148	0.334	ND	0.761	4
TRIT 201/50	ND	ND	0.042	ND	ND	ND	0.159	2
TRFK 301/4	ND	ND	0.044	ND	0.246	ND	0.246	2
TRFK 303/1199	ND	ND	0.148	ND	0.182	ND	0.226	1
AHP S15/10	0.032	0.098	0.153	0.174	0.288	ND	0.664	12
TRFCA SFS 150	0.014	0.066	0.041	0.158	0.242	0.024	0.544	2
STC 5/3	0.045	0.140	0.180	0.178	0.294	0.029	0.866	8
Mean	0.027	0.098	0.122	0.122	0.248	0.008	0.593	4
STDEV	0.033	0.101	0.061	0.080	0.096	0.012	0.383	
CV (%) #	111.111	102.041	49.180	65.574	40.323	62.500	64.081	
r(mite)	0.190	0.738*	0.071	0.434	0.297	0.303	0.530	
Variations (%) ##								32.52
LSD, (p≤0.05)								1

ND = not detected, a value of 0.000 has been used in the statistical calculations

\* Significant at p≤0.05

# As  $\frac{STDEV}{Mean} * 100$

## From ANOVA

Table 17b: Clonal variations and the relationships between mites infestations and overhead volatile aromatic compounds in Timbilil during rainy season

Clone	Aromatic compounds											Mites
	Phenylethyl alcohol	Ethyl benzene	P-Xylene	Azulene	Benzaldehyde	Phenyl acetaldehyde	Benzyl alcohol	Acetophenone	Z-anethole	Indene	Sum aroma	
TRFK 6/8	0.137	0.174	0.052	ND	0.111	0.020	0.040	0.072	0.158	ND	0.763	2
TRFK 18/3	0.025	0.071	0.024	ND	0.021	0.017	0.012	0.039	0.111	ND	0.321	2
TRFK 31/8	0.091	0.165	0.167	ND	0.040	0.032	0.059	0.121	0.092	ND	0.768	2
TRFK 54/40	0.033	0.123	0.151	ND	0.053	0.064	0.135	0.079	0.044	ND	0.682	10
TRIT 201/16	0.013	0.014	ND	ND	0.123	ND	ND	0.103	0.091	ND	0.344	4
TRIT 201/50	0.118	0.215	0.036	ND	0.124	ND	0.018	0.242	0.158	ND	0.91	2
TRFK 301/4	0.096	0.146	0.160	0.112	0.116	ND	ND	0.062	0.135	ND	0.826	2
TRFK 303/1199	0.188	0.153	0.217	ND	0.113	ND	0.095	0.197	0.147	ND	1.109	1
AHP S15/10	0.023	0.052	ND	ND	0.114	ND	0.014	0.107	0.136	ND	0.445	12
TRFCA SFS 150	0.048	0.058	ND	ND	0.044	0.034	0.088	0.087	0.047	ND	0.330	2
STC 5/3	0.084	0.066	0.013	ND	0.105	0.045	0.030	0.134	0.042	0.283	0.803	8
MEAN	0.078	0.112	0.075	0.010	0.088	0.019	0.045	0.113	0.106	0.026	0.663	5
STDEV	0.055	0.063	0.082	0.034	0.039	0.022	0.043	0.060	0.045	0.085	0.66	
CV (%) #	71.11	56.26	109.80	331.63	44.52	114.91	99.74	53.11	43.07	331.65	1269.21	
r <sub>(mite)</sub>	-0.158	0.424	-0.249	0.084	0.089	0.352	0.217	-0.130	-0.366	0.321	-0.224	
Variations (%) ##												32.52
LSD, (p≤0.05)												1

ND = not detected, a value of 0.000 has been used in the statistical calculations

# As  $\frac{STDEV}{Mean} * 100$

## From ANOVA

Table 17c: Changes and relationships in clonal overhead monoterpenes and mites levels in Timbilil during rainy season

Clone	Monoterpene compounds									Sum monoter	Mites
	P-Mentha-1,3,8-triene	Sabinene	$\beta$ -Phellandrene	Limonene	Z- $\beta$ -ocimene	E- $\beta$ -ocimene	Linalool	Terpinen-4-ol	DMNT		
TRFK 6/8	ND	0.024	0.176	0.027	0.263	0.316	ND	0.106	ND	0.912	2
TRFK 18/3	0.102	ND	0.000	0.022	0.103	0.254	ND	0.047	0.033	0.561	2
TRFK 31/8	0.166	0.025	0.213	0.117	0.214	0.261	ND	0.175	0.126	1.297	2
TRFK 54/40	0.125	0.010	0.212	0.034	0.247	0.272	ND	0.126	0.104	1.130	10
TRIT 201/16	0.106	ND	ND	0.015	0.174	0.356	ND	0.023	0.058	0.731	4
TRIT 201/50	ND	0.018	0.24	0.099	0.248	0.318	ND	ND	ND	0.926	2
TRFK 301/4	ND	0.022	0.205	0.056	0.261	0.338	ND	0.032	ND	0.914	2
TRFK 303/1199	ND	0.025	0.231	0.079	0.275	0.339	ND	0.071	ND	1.019	1
S15/10	0.112	ND	ND	0.013	0.177	0.313	ND	0.014	0.078	0.707	12
SFS 150	0.131	ND	ND	0.095	0.211	0.210	ND	0.103	0.106	0.855	2
STC 5/3	0.113	0.011	0.148	0.123	0.132	0.234	0.2361	0.128	0.105	1.229	8
<b>Mean</b>	0.078	0.012	0.130	0.062	0.210	0.292	0.022	0.075	0.0554	0.935	5
<b>STDEV</b>	0.064	0.0108	0.1059	0.0421	0.0570	0.0479	0.0712	0.0564	0.0506	0.506	
<b>CV (%) #</b>	82.24	89.26	81.60	68.09	27.20	16.42	331.65	75.32	19.34	863.13	
$r_{(mite)}$	0.432	-0.446	-0.219	-0.285	-0.283	-0.114	0.321	0.009	0.497	0.063	
Variations (%) ##											32.52
LSD, (p $\leq$ 0.05)											1

ND = not detected, a value of 0.000 has been used in the statistical calculations

# As  $\frac{STDEV}{Mean} * 100$

## From ANOVA

Table 17d: The variations and relationship between sesquiterpenes released by 11 tea varieties and mite infestations levels in Timbilil during rainy season

Clone	Sesquiterpenes compounds										Mites
	$\alpha$ -copaene	$\alpha$ -cedrene	<i>E</i> - $\beta$ -caryophyllene	<i>E</i> - $\beta$ -farnesene	Italicene	Longifolene	Calamene	Longicampenylone	Cedrol	Sum sesqui	
TRFK 6/8	0.073	0.369	0.269	0.312	ND	ND	0.211	ND	0.562	1.796	2
TRFK 18/3	ND	0.287	0.106	0.264	ND	ND	ND	ND	0.351	1.008	2
TRFK 31/8	0.103	0.335	0.216	0.304	ND	ND	ND	ND	0.404	1.362	2
TRFK 54/40	0.123	0.343	0.268	0.318	ND	ND	ND	ND	0.558	1.609	10
TRIT 201/16	0.234	0.234	0.139	0.338	ND	ND	ND	ND	0.528	1.239	4
TRIT 201/50	0.189	0.189	0.210	0.204	ND	0.035	ND	0.115	0.339	1.390	2
TRFK 301/4	0.209	0.209	0.321	0.277	0.242	ND	0.125	ND	0.460	1.633	2
TRFK 303/1199	0.117	ND	0.202	0.303	ND	ND	ND	0.010	0.413	1.044	1
AHP S15/10	ND	0.252	0.163	0.264	ND	ND	ND	ND	0.318	0.997	12
TRFCA SFS 150	ND	0.359	0.161	0.274	ND	ND	ND	ND	0.463	1.257	2
STC 5/3	0.025	0.287	ND	0.315	ND	ND	ND	ND	0.312	0.939	8
Mean	0.057	0.270	0.187	0.288	0.022	0.003	0.031	0.021	0.391	1.300	5
STDEV	0.067	0.103	0.088	0.037	0.073	0.011	0.071	0.044	0.160	0.582	
CV (%) #	117.08	38.24	47.18	12.85	331.65	331.56	211.06	211.06	40.79	44.77	
$r_{(mite)}$	-0.158	0.158	0.0	0.161	-0.195	-0.164	-0.276	-0.221	-0.155	-0.226	
Variations (%) ##											32.52
LSD, ( $p \leq 0.05$ )											1

ND = not detected, a value of 0.000 has been used in the statistical calculations

# As  $\frac{STDEV}{Mean} * 100$

## From ANOVA

Table 18a: Changes in mite infestations and overhead green leaf volatile compounds (GLVs) and their relationships in tea cultivars in Timbilil during cold season

Clone	Overhead green leaf volatile organic compounds				Mites
	Hexanal	Z-3-hexenyl acetate	Nonanal	Sum GLVS	
TRFK 6/8	ND	0.218	ND	0.218	1
TRFK 18/3	0.124	0.184	0.021	0.329	4
TRFK 31/8	ND	0.145	ND	0.145	2
TRFK 54/40	0.134	0.266	0.021	0.421	2
TRIT 201/16	0.102	0.215	ND	0.317	2
TRIT 201/50	ND	ND	ND	0.000	1
TRFK 301/4	ND	0.144	ND	0.144	1
TRFK 303/1199	ND	0.180	ND	0.180	1
AHP S15/10	ND	0.148	ND	0.148	1
TRFCA SFS 150	0.053	0.153	ND	0.206	2
STC 5/3	0.123	0.163	ND	0.286	2
Mean	0.049	0.165	0.004	0.032	2
STDEV	0.060	0.067	0.008	14.679	
CV (%) #	122.40	40.40	222.43		
$r_{(mite)}$	0.327	0.285	0.363	0.493	
Variations (%) ##					32.52
LSD, (p≤0.05)					1

ND = not detected, a value of 0.000 has been used in the statistical calculations

# As  $\frac{STDEV}{Mean} * 100$

## From ANOVA



Table 18b: Clonal variations and the relationships between mites infestations and overhead volatile aromatic compounds in Timbilil during cold season

Clone	Aromatic compounds														Mites
	Phenyl ethyl alcohol	Ethyl benzene	P-xylene	Phenol	Naphthalene	Diphenyl ether	Azulene	Benzaldehyde	Phenyl acetaldehyde	Benzyl alcohol	Acetophenone	Z-anethole	Indene	Sum aroma	
TRFK 6/8	0.149	0.192	0.063	ND	ND	ND	ND	0.123	0.024	0.044	ND	0.169	ND	0.764	1
TRFK 18/3	0.316	0.074	0.030	ND	ND	ND	ND	0.032	0.022	0.023	0.053	0.121	ND	0.671	4
TRFK 31/8	0.110	0.170	0.176	ND	0.553	ND	ND	0.046	0.035	0.070	0.134	0.109	ND	0.704	2
TRFK 54/40	0.035	0.129	0.146	ND	ND	ND	ND	0.103	0.072	0.147	0.086	0.047	ND	0.765	2
TRIT 201/16	0.021	0.021	ND	ND	ND	ND	ND	ND	ND	ND	0.113	0.111	ND	0.266	2
TRIT 201/50	0.122	0.213	0.040	0.047	ND	0.030	ND	0.122	ND	0.022	0.261	0.181	ND	0.961	1
TRFK 301/4	0.116	0.156	0.179	ND	ND	ND	0.114	0.125	ND	ND	0.070	0.139	ND	0.899	1
TRFK 303/1199	0.201	0.149	0.198	ND	ND	0.027	ND	0.114	ND	0.107	0.209	0.173	ND	1.151	1
AHP S15/10	0.025	0.056	ND	ND	0.136	ND	ND	0.124	ND	0.021	0.112	ND	ND	0.338	1
TRFCA SFS 150	0.074	0.066	ND	ND	ND	ND	ND	0.159	0.040	0.104	0.104	0.072	ND	0.619	2
STC 5/3	0.086	0.085	0.016	ND	ND	ND	ND	0.115	0.062	0.041	0.140	0.051	0.279	0.859	2
MEAN	0.114	0.119	0.077	0.004	0.063	0.005	0.010	0.097	0.023	0.053	0.117	0.107	0.025	0.727	2
STDEV	0.086	0.062	0.081	0.014	0.168	0.012	0.034	0.049	0.027	0.048	0.072	0.059	0.084	0.601	
CV (%) #	75.79	52.13	104.40	331.58	267.67	222.87	331.63	50.23	114.25	91.85	61.35	54.89	331.65	82.67	
$r_{(mite)}$	0.462	0.463	-0.341	0.071	0.0316	0.396	-0.266	-	0.374	-0.217	-0.303	-0.187	0.1	-0.253	
Variations(%)##								0.579							32.52
LSD, (p≤0.05)															1

ND = not detected, a value of 0.000 has been used in the statistical calculations

## From ANOVA,

# As  $\frac{STDEV}{Mean} * 100$

Table 18c: Changes and relationships in clonal overhead monoterpenes and mites levels in Timbilil during cold season

Clone	Monoterpene compounds									Mites
	p-menthan-1,3,8-triene	Sabienene	$\beta$ -phellandrene	limonene	Z- $\beta$ -ocimene	E- $\beta$ -ocimene	Terpine n-4-ol	4,8-dimethyl-1,3(E),7-notriene	Sum Monoterpenene	
TRFK 6/8	ND	0.031	0.184	0.032	0.314	0.219	0.119	ND	0.8997	1
TRFK 18/3	0.089	ND	ND	0.025	0.105	0.258	0.051	0.042	0.5702	4
TRFK 31/8	0.167	0.032	0.221	0.125	0.230	0.242	0.185	0.132	1.3346	2
TRFK 54/40	0.123	0.012	0.022	0.034	0.254	0.281	0.134	0.105	0.964	2
TRIT 201/16	0.111	ND	ND	0.025	0.184	0.365	0.025	0.065	0.7745	2
TRIT 201/50	ND	0.057	0.249	0.114	0.261	0.322	ND	ND	1.0026	1
TRFK 301/4	ND	0.201	0.221	0.066	0.312	0.352	0.035	ND	1.1876	1
TRFK 303/1199	ND	0.030	0.305	0.084	0.315	0.365	0.079	ND	1.1782	1
AHP S15/10	0.117	ND	ND	0.022	0.179	0.262	0.025	0.090	0.6946	1
TRFCA SFS 150	0.134	ND	ND	0.105	0.220	0.232	0.051	0.106	0.848	2
STC 5/3	0.114	0.013	0.171	0.139	0.141	0.242	0.125	0.113	1.058	2
Mean	0.078	0.034	0.125	0.070	0.228	0.285	0.075	0.059	0.956	2
STDE	0.064	0.058	0.120	0.045	0.071	0.055	0.058	0.053	0.526	
CV (%) #	82.78	170.61	96.38	64.38	31.21	19.41	76.70	88.84	55.02	
$r_{(mite)}$	0.505	-0.410	-0.537	-0.138	-0.752	-0.295	0.134	0.359	-0.460	
Variations (%) ##										32.52
LSD, (p $\leq$ 0.05)										1

ND= not detected, a value of 0.000 has been used in the statistical calculations

# As  $\frac{STDEV}{Mean} * 100$

## From ANOVA

Table 18d: The variations and relationship between sesquiterpenes released by 11 tea varieties and mite infestations levels in Timbilil during cold season

Clone	Sesquiterpenes compounds										Mites
	$\alpha$ -copaene	$\alpha$ -cedrene	<i>E</i> - $\beta$ -caryophyllene	longifolene	Italucene	Longichenylene	<i>E</i> - $\beta$ -fernesene	Calamene	Cedrol	Sum sesqui	
TRFK 6/8	0.083	0.412	0.296	ND	ND	0.110	0.323	0.214	0.597	1.925	1
TRFK 18/3	ND	0.309	0.112	ND	ND	ND	0.264	ND	0.352	1.037	4
TRFK 31/8	0.115	0.350	0.226	ND	ND	ND	0.310	ND	0.451	1.452	2
TRFK 54/40	0.124	0.345	0.281	ND	ND	ND	0.330	ND	0.572	1.652	2
TRIT 201/16	ND	0.235	0.147	ND	ND	ND	0.304	ND	0.535	1.221	2
TRIT 201/50	0.202	0.331	0.222	0.042	ND	0.122	0.238	ND	0.416	1.409	1
TRFK 301/4	ND	0.216	0.224	ND	0.247	ND	0.321	0.132	0.517	1.41	1
TRFK 303/1199	0.150	0.325	0.329	ND	ND	0.092	0.325	ND	0.451	1.58	1
AHP S15/10	ND	0.262	0.164	ND	ND	ND	0.304	ND	0.372	1.102	1
TRFCA SFS 150	ND	0.371	0.169	ND	ND	ND	0.254	ND	0.520	1.314	2
STC 5/3	0.034	0.309	0.314	ND	ND	ND	0.245	ND	0.324	1.226	2
Mean	0.064	0.286	0.226	0.004	0.022	0.029	0.294	0.031	0.464	1.365	2
STDE	0.074	0.111	0.073	0.013	0.074	0.051	0.036	0.072	0.091	1.364	
CV (%) #	114.64	38.95	32.14	331.58	331.65	172.78	12.15	230.02	19.70	447.6	
<b>r</b> (mite)	-0.362	0.032	-0.508	0.100	0.302	0.1643	-0.383	-0.310	-0.502	-0.362	
Variations (%) ##											32.52
LSD, (p $\leq$ 0.05)											1

ND = not detected, a value of 0.000 has been used in the statistical calculations,

## From ANOVA,

# As  $\frac{STDEV}{Mean} * 100$

#### **4.4 Effect of nitrogenous fertilizer rates on the overhead volatile organic compounds composition or levels in relation to mites attack.**

The variation of OVOCs separated into classes and red spider mites levels with the rate of nitrogenous fertilizer rates are presented in Tables 19 a-d. There were significant ( $p \leq 0.05$ ) variations in mites population due to nitrogen fertilizer rates. The 300 Kg N/ha/year and control N (0 Kg N/ha/year) resulted in high population of red spider mites. There was decline in the population of mites at 0 to between 150 and 225 Kg N/ha/year followed by a rise at 300 kg N/ha/year. The lowest ( $p \leq 0.05$ ) red spider mites levels were recorded at 150 and 225 kg N/ha/year fertilizer rates. The results are similar to previous results on red spider mites in the west (Sudoj et al., 2001a) and red crevice mites in the east (Sudoj et al., 1996; Sudoj et al., 2001b) of Rift Valley that N-rates between 150 and 200 Kg N/ha/year induced tolerance to mites infestation while high rates encouraged the buildup of mites on tea. Thus the recommended nitrogenous fertilizer rates in Kenya of 100-225 kg N/ha/year (Anonymous, 2002; Othieno, 1988) are not only leading to increased tea production (Msomba et al., 2014; Owuor et al., 1996b; Owuor, Othieno, Kamau, Wanyoko & Ng'etich, 2008b; Owuor & Wanyoko, 1996c) and being a compromise between yields and quality (Owuor et al., 2000; Owuor et al., 1987d; Owuor et al., 1997; Owuor, Wanyoko & Othieno, 1990d) but also helpful in reducing the red spider mites infestations of tea plants. Application of correct nitrogenous fertilizer rate is therefore a viable pest control mechanism in tea, as had also been observed on other plants (Hagg, Zagrobelny & Bak, 2013; Scala et al., 2013).

All the seven green leaf volatiles and the sum of GLVs (Table 19a) increased linearly with increase in rate of nitrogen fertilizer. The pattern of response was similar to that of their precursor fatty acids (Okal et al., 2012b; Owuor et al., 1990a; Owuor et al., 2013c) and levels in

black tea (Owuor et al., 2000; Owuor et al., 1987d; Owuor et al., 1997; Robinson et al., 1992). The levels of GLVs released overhead plants are therefore directly related to the levels of the precursor compounds in the leaf. The correlation coefficients ( $r$ ) of the linear regression between 1-penten-3-ol (**16**) ( $r = 0.983$ ), *Z*-2-pentenol (**17**) ( $r = 0.934$ ), *E*-2-hexenal (**7**) ( $r = 0.971$ ) and *Z*-3-hexenyl acetate (**12**) ( $r = 0.988$ ) and nitrogenous fertilizers were direct and significant ( $p \leq 0.05$ ). High nitrogenous fertilizer rates should not be applied in tea farms as this would make tea cultivars to release high levels of GLVs that may encourage mites attack. Several studies point to the fact that high levels of N-rates application reduce tea qualities by increasing formation of GLVs but decreasing those of terpenoids and aromatics (Owuor, 1989; Owuor, 1992a; Owuor, 1992c; Owuor et al., 2010a). Generally, the total levels of aromatic and terpenoid compounds declined with an increasing nitrogenous fertilizer rates (Tables 19b-d). But the individual compounds (Tables 19 b-d) showed mixed responses to increasing nitrogen application rates, with some directly and others inversely correlating with nitrogen fertilizer rates. None of the direct relationships were significant. However, only phenyl ethyl alcohol (**35**) ( $r = -0.903$ ), ethyl benzene (**37**) ( $r = -0.932$ ),  $\alpha$ -methyl styrene (**47**) ( $r = -0.981$ ), longifolene (**101**) ( $r = -0.915$ ) and  $\beta$ -cedrene (**108**) were significantly ( $p \leq 0.05$ ) inversely correlated with nitrogen fertilizer rates. In previous studies (Owuor et al., 2000; Owuor et al., 1987d; Owuor et al., 1997) the sum of the terpenoid compounds in black tea decreased with increase in nitrogen fertilizer rates, although no regressions were performed to establish if the relationships were significant. However, the compositions of the terpenoid compounds detected in black tea (Owuor et al., 2000; Owuor et al., 1997) were different from those in the overhead composition in live tea reported herein. Phenyl ethyl alcohol (**35**), ethyl benzene (**37**) and  $\alpha$ -methyl styrene (**47**) were demonstrated to decline with increasing nitrogenous fertilizer rates. Most of the terpenoids in the

OVOCs composition were olefins and it is likely they were volatilized/lost during black tea processing. It may be necessary to establish the agronomic and cultural practices that influence the terpenoid compounds composition in the OVOCs, especially longifolene (**101**) and  $\beta$ -cedrene (**108**), as these may be used to deter red spider mites infestations. Similarly, cultural and agronomic factors that influence production of the volatile aromatic overhead compounds have not been established.

Table 19a: Effect of nitrogenous fertilizer rates on the emissions of green leaf volatile organic compounds in relation to mites levels

N-rates	Green leaf volatile organic compounds							Sum GLVS	Mites
	1-penten-3-ol	Z-2- pentenol	Hexanal	E-2- hexenal	Z-3- hexenol	Heptanal	Z-3-hexenyl acetate		
0	0.076	0.032	0.148	0.445	0.826	0.148	1.423	2.677	12
75	0.100	0.037	0.260	0.521	0.394	0.260	1.703	3.275	9
150	0.111	0.060	0.254	0.606	0.516	0.254	1.910	3.711	6
225	0.132	0.131	0.272	0.878	0.471	0.272	2.005	4.161	1
300	0.138	0.214	0.282	0.949	0.527	0.282	2.225	4.647	10
Mean	0.111	0.095	0.243	0.680	0.463	0.243	1.859	3.694	8
STDEV	0.025	0.077	0.016	0.222	0.061	0.053	0.314	0.590	
CV (%) #	22.523	81.053	6.584	32.647	13.175	21.811	16.891	15.972	
r (mite)	0.983**	0.934*	0.815	0.971**	0.826	0.815	0.988***	0.658	
Variations (%) ##									23.83
LSD (p≤0.05)									3

ND = not detected, a value of 0.000 has been used in the statistical calculations,

\*, \*\*, \*\*\* Significant at p≤0.05, 0.01 and 0.001, respectively

## From ANOVA,

# As  $\frac{STDEV}{Mean} * 100$

Table 19b: Influence of nitrogenous fertilizer rates on the emissions of aromatic compounds in relation to mites levels

N- rates	Aromatic compounds											Mites
	Phenyl ethyl alcohol	Ethylbenzene	P-xylene	O-xylene	Benzaldehyde	$\alpha$ -methylstyrene	Phenylacetaldehyde	Benzyl alcohol	Acetophenone	Benzothiazole	Sum Aromatic s	
0	0.151	0.224	ND	ND	0.123	0.315	0.055	0.060	0.071	0.047	1.046	12
75	0.159	0.234	0.135	ND	0.131	0.260	0.077	0.077	0.100	0.073	1.246	10
150	0.141	0.206	0.133	0.133	0.157	0.231	0.056	0.086	0.114	0.071	1.328	6
225	0.116	0.169	0.119	0.027	0.153	0.127	0.202	0.084	ND	0.076	1.073	1
300	0.115	0.156	0.115	0.126	0.144	0.102	0.071	0.072	0.101	0.077	1.079	11
Mean	0.137	0.198	0.125	0.095	0.142	0.207	0.092	0.076	0.097	0.069	1.238	8
STDEV	0.020	0.034	0.010	0.059	0.014	0.090	0.062	0.010	0.018	0.012	0.126	
CV (%) #	14.599	17.172	8.000	62.105	9.859	43.478	67.391	13.158	18.557	17.391	10.178	
r(mite)	-0.903*	-0.932*	0.584	0.638	0.701	-0.981**	0.399	0.469	-0.134	0.802*	-0.126	
Variations (%) ##												23.83
LSD (p $\leq$ 0.05)												3

ND = not detected, a value of 0.000 has been used in the statistical calculations

\*, \*\* Significant at p $\leq$ 0.05 and 0.01 respectively

# As  $\frac{STDEV}{Mean} * 100$ ;

## From ANOVA



Table 19c: Effect of nitrogenous fertilizer rates on the emissions of monoterpenes in relation to mites levels

N- rates	Monoterpenes						Sum monoterpenes	Mites
	O- cymene	$\beta$ - phellandrene	<i>E</i> - $\beta$ - ocimene	Linalool oxide -Z- (furanoid)	Linalool	Terpinen-4-ol		
0	0.138	0.296	0.716	0.076	0.279	0.138	1.643	12
75	0.108	0.276	0.600	0.075	0.329	0.150	1.538	10
150	ND	0.297	0.761	0.113	0.358	0.155	1.684	6
225	0.099	0.299	0.763	0.094	0.346	0.146	1.747	1
300	0.057	0.278	0.756	0.099	0.351	0.144	1.685	11
Mean	0.101	0.289	0.719	0.091	0.333	0.146	1.680	8
STDEV	0.033	0.011	0.069	0.016	0.032	0.006	0.089	
CV (%) #	32.673	3.806	9.597	17.582	9.610	4.110	5.298	
r(mite)	-0.498	-0.182	0.553	0.638	0.800	0.197	0.594	
Variations (%) ##								23.83
LSD (p $\leq$ 0.05)								3

ND = not detected, a value of 0.000 has been used in the statistical calculations,

## From ANOVA,

# As  $\frac{STDEV}{Mean} * 100$

Table 19d: Effect of nitrogenous fertilizer rates on the emissions of sesquiterpenes in relation to mites levels

N- rates	Sesquiterpenes									Mites
	Longifolene	$\alpha$ -cedrene	<i>E</i> - $\beta$ -caryophyllene	<i>E</i> - $\beta$ -farnesene	$\beta$ -cedrene	Calamenene	Longicampenylone	Cedrol	Sum Sesquiterpenes	
0	0.093	1.072	0.441	0.719	0.302	0.241	0.115	1.471	4.454	12
75	0.093	1.198	0.487	0.779	0.299	0.236	0.125	1.587	4.804	10
150	0.065	1.241	0.531	0.828	0.187	0.256	0.097	1.583	4.788	6
225	0.055	1.237	0.528	0.796	0.000	0.247	0.011	1.634	4.508	1
300	0.057	1.159	0.515	0.836	0.000	0.241	0.253	1.504	4.565	11
Mean	0.073	1.181	0.500	0.792	0.263	0.244	0.120	1.556	4.729	8
STDEV	0.019	0.070	0.038	0.047	0.065	0.008	0.087	0.067	0.152	
CV (%) #	26.027	5.927	7.600	5.934	24.715	3.279	72.500	4.306	3.214	
r(mite)	-0.915*	0.484	0.797	0.849	-0.900*	0.226	0.295	0.268	-0.063	
Variations (%) ##										23.83
LSD (p $\leq$ 0.05)										3

ND = not detected, a value of 0.000 has been used in the statistical calculations

\* Significant at p $\leq$ 0.05

# As  $\frac{STDEV}{Mean} * 100$ ;

## From ANOVA

For the first time influence of nitrogen fertilizer rates on the overhead volatile organic compounds in tea are reported. Out of the 24 compounds released belonging to aromatics, monoterpenes and sesquiterpenes, 15 (63%) reached maximum level when 150 and 225 kg N/ha/year fertilizer application rates are used. This was the rate at which minimum mites levels were recorded and by coincidence the recommended fertilizer N-rate in Kenya (Anonymous, 2002; Othieno, 1988). These three classes of OVOCs have been reported to defend plants against insect pests attack, in chilli (Saad et al., 2014) and tomato (Bleeker et al., 2009) where high levels of monoterpenes repelled insects. The repellence was attributed to the monoterpenes being toxic and feeding deterrents (Mazid et al., 2011); the toxins interfere with acetyl cholinesterase enzyme activity in insects (Zapata et al., 2010). These results may explain the low number of mites observed when OVOCs levels were high (between 150 and 225 kg N/ha/year fertilizer rates).

Several volatile compounds have been implicated in plant defence mechanism against insect attack. For example, acetophenone (**46**) causes acute insecticidal activities (Mohsen et al., 1995) while benzothiazole (**40**) exhibit a wide range of biological properties including antimicrobial activities (Fujita et al., 2007). Benzaldehyde has the capacity to kill insects at very low doses (Paulraj, Reegan & Ignacimuthu, 2011). Sesquiterpenes not only defend plants against pest attack by attracting natural enemies but also possess repellency and toxicity properties (Eller et al., 2014). Indeed, aromatic compounds, monoterpenes and sesquiterpenes were repellants to the two species of mites (Table 20). The high population of mites at 0 Kg N/ha/year, could be attributed in part to the low levels of the repellent defense compounds. The high levels of mites at 300 Kg N/ha/year, could be due to the high levels of GLVs which are attractants to the mites and low amounts of aromatics, monoterpenes and sesquiterpenes which repel the mites (Table

20). These findings corroborate earlier findings that mites infestation levels were high in plots receiving 0 and above 400 Kg N/ha/year and application rates between 150 and 200 kg N/ha/year induced tolerance to mites attack (Sudoj, 1997; Sudoj et al., 2001a; Sudoj et al., 2001b). The high levels of GLVs at high nitrogen fertilizer rate may be a contributing factor to the abundance of the mites, while the high levels of mites at 0 kg N/ha/year may be due to the low levels of aromatic and terpenoid compounds. Preferences of insect infestation of plants that emit high levels of GLVs have been reported. For example, flea beetles (*Epitrix hirtipennis*) were more abundant on GLV-producing wild type plants compared to plants with reduced hydroperoxide lyase activity (Halitschke et al., 2008). *Uschistus heros* preferred soybean pods that released high amounts of GLVs for feeding and oviposition over deficient cultivars (Silva et al., 2013). Meldau *et al* (Meldau et al., 2009) reported that GLVs serve as feeding stimulants to pests. Highest yields had been reported in this trial at 225 kg N/ha/year (Msomba et al., 2014).

The low levels of aromatics, monoterpenes and sesquiterpenes at 300 Kg N/ha/year may be due to increased growth rates due to fertilization which trade-off with carbon allocation to secondary metabolites, leading to reduced concentration of chemical defences hence reduced resistance against herbivores (Moreira et al., 2008). This study demonstrated that the tolerance inferred to tea by applying N- rates between 150 and 200 kg N/ha/year is due to high levels of defence compounds; aromatics, monoterpenes and sesquiterpenes as has been observed in previous studies (Erbilgin et al., 2007; Jaenson et al., 2006; Khan et al., 2008; Zhang et al., 2015) and the infestations of mites at increased rates is as a result of high levels of GLVs that are known to attract and act as feeding stimulants to pests (Halitschke et al., 2008; Meldau et al., 2009). A delicate balance between yield, nitrogen fertilizer application and formation of chemical defences in plant can protect plants against pests, a strategy that is superior to chemical

control that sometimes makes tea rejected in international markets due to pesticide residues chemical contamination.

#### **4.5 Responses of red spider mites (RSM) and red crevice mites (RCM) to volatile organic compounds associated with *Camellia cinencis***

In the Y-tube olfactometer set-up, the distribution of red spider mites (RSM) and red crevice mites (RCM) when presented with equal chances of choosing the odour, the control (diclomethane, used as a solvent) and not making choice are presented in Table 20. It was interpreted that choice of control meant that the odour from the volatile organic compound repelled the mite species under test.

The mites species were significantly ( $P \leq 0.05$ ) attracted by all GLVs tested. The strongest attraction was displayed by *Z*-3-hexenyl acetate (**12**) (90%) followed by *E*-2-hexenal (**7**) = *Z*-3-hexenol (**9**) (80%). Hexanal (**8**), 1-penten-3-ol (**16**) and *Z*-2-pentenol (**17**) were comparatively weakly attracted to both species of mites. There was a synergistic effect when blends of GLVs were evaluated. Blend of *E*-2-hexenal (**7**) and *Z*-3-hexenol (**9**) (ratio 1:1) attracted 100% of the mites. Similar to GLVs, linalool (**66**) (80%), *E*- $\beta$ -ocimene (**73**) (75%) and terpinen-4-ol (**83**) (75%) elicited significant ( $P \leq 0.05$ ) attractant response from the mites. This result, suggests that red spider and red crevice mites could use these VOCs to locate suitable hosts for feeding as VOCs are sensory cues that define host specificity for food site (Ajayi *et al.*, 2015) more particularly the GLVs (Scala *et al.*, 2013). These findings have been reported in other studies. GLVs including hexanal (**8**), *E*-2-hexenal (**7**), *Z*-3-hexenol (**9**) and *Z*-3-hexenyl acetate (**12**) attracted stem borer (Khan *et al.*, 2008). *Z*-3-hexenyl acetate (**12**) attracted both *Myloccerinus aurolineatus* (Coleoptera: Curculionidae) (Sun *et al.*, 2010) and *Pantomorus*

*cervinus* (Coleoptera: Curculionidae) in Y-tube olfactometers. In laboratory Y-tube choice assays and in field experiments *Nicotiana attenuate* plants with reduced expression of hydroperoxide lyase were less attractive to the generalist predator *Geocoris* ssp. feeding on eggs and early larval instars of the specialist lepidopteran herbivore *Manduca sexta* (Halitschke et al., 2008). Another study from the same group showed that the *Z/E*-ratio of GLVs released from *Nicotiana attenuate* plants changed when plants were attacked by *M. sexta* caterpillars and that this herbivore-induced change in the *Z/E*-ratio tripled the foraging efficiency of the generalist predators *Geocoris* spp. in nature (Allmann & Baldwin, 2010). Early results from Visser and Ave (Visser & Avé, 1978) showed that the odour from a blend of GLVs *E*-2-hexenol (**10**), *Z*-3-hexenol (**9**) and *E*-2-hexenal (**7**) was repellent to the Colorado potato beetle, *Leptinotarsa decemlineata*, but that individual components were attractants. *E*- $\beta$ -ocimene (**73**) was attractant to insects in wheat (Buttery et al., 1985) and oats (Buttery et al., 1982) and was shown to be one of the most attractive volatiles to *Chlorophorus caragana* (Coleoptera: Cerambycidae) a trunk borer that feeds on *Caragana* shrubs (Zhang et al., 2015). Linalool (**66**) has also been reported as a male pheromone attractant to bee *Colletes cunicularius* (Borg-Karlson et al., 2003) and aphid (Pare et al., 1999). However, repellent properties of linalool (**66**) (Ayvaz et al., 2010; Bowers et al., 1993; Marckovic et al., 1996) and *E*- $\beta$ -ocimene (**73**) (Khan et al., 2008) to insects have been reported. Cultivars that release high levels of *E*- $\beta$ -ocimene (**73**), linalool (**66**), and the GLVs would be vulnerable to attack by both red spider and red crevice mites.

Table 20: Response of red spider mites and red crevice mites to various classes of VOCs

Compound/VOC	Number attracted		Number repelled		Number without choice		Compound classification
	RCM	RSM	RCM	RSM	RCM	RSM	
<b>Green leaf volatiles compounds</b>							
Z-3-hexenol (9)	16	16	0	0	4	4	A
E-2- hexenal (7)	15	16	0	2	5	2	A
Z-3-hexenyl acetate (12)	18	18	0	0	2	2	A
Hexanal (8)	14	14	3	4	3	2	A
1-penten-3-ol (16)	13	14	5	2	2	4	A
Z-2-pentenol (17)	9	11	5	5	6	4	A
Nonanal (19)	15	14	3	2	2	4	A
Blend A	18	20	0	0	2	0	A
Blend B	16	16	1	2	3	2	A
Blend C	19	18	0	0	1	2	A
<b>Aromatic compounds</b>							
Methyl salicylate (32)	0	0	15	15	5	5	R
Benzaldehyde (30)	0	0	16	17	4	3	R
Phenyl ethyl alcohol (35)	NT	1	NT	13	NT	6	R
Ethyl benzene (37)	NT	3	NT	14	NT	3	R
P-xylene (41)	0	0	17	16	3	4	R
Acetophenone (46)	0	0	16	16	4	4	R
Benzophenone (30)	0	0	15	15	5	5	R
Naphthalene (44)	NT	0	NT	17	NT	3	R

A = Attractant

R = Repellent

NT = Not tested

Blend A = Z-3-hexenol + E-2- hexenal

Blend B = Hexanal + 1-penten-3-ol + Z-2-pentenol + Nonanal

Blend C = Hexanal + Penten-3-ol + Z-2-pentenol + Nonanal + Z-3-hexenyl acetate

**Red crevice mites (RCM):** CV = 13%; LSD ( $P \leq .05$ ) Response = 0; Interaction: Volatile organic compound (VOC) X response = 1

**Red spider mites (RSM):** CV = 13.87%; LSD ( $P \leq .05$ ) Response = 0; Interaction: Volatile organic compound X response = 0

Table 20: Cont.....

Compound/VOC	Number attracted		Number repelled		Number without choice		Compound classification
	RCM	RSM	RCM	RSM	RCM	RSM	
<b>Monoterpene compounds</b>							
Linalool ( <b>66</b> )	16	15	0	1	4	4	A
Geraniol ( <b>65</b> )	NT	0	NT	15	NT	5	R
Sabinene ( <b>85</b> )	NT	1	NT	13	NT	6	R
Limonene ( <b>76</b> )	NT	2	NT	15	NT	3	R
<i>E</i> - $\beta$ -ocimene ( <b>73</b> )	14	15	4	1	2	4	A
<i>Z</i> - $\beta$ -ocimene ( <b>74</b> )	NT	3	NT	13	NT	4	R
$\alpha$ - phellandrene ( <b>78</b> )	NT	0	NT	16	NT	4	R
$\beta$ -phellandrene ( <b>79</b> )	NT	3	NT	14	NT	3	R
$\alpha$ -pinene ( <b>81</b> )	0	0	15	17	5	3	R
Myrcene ( <b>72</b> )	0	0	15	15	5	5	R
Terpinen-4-ol ( <b>83</b> )	NT	14	NT	4	NT	2	A
<b>Sesquiterpene compounds</b>							
<i>E</i> - $\beta$ - farnesene ( <b>96</b> )	0	0	17	16	3	4	R
<i>E</i> - $\beta$ - caryophyllene ( <b>94</b> )	0	0	15	16	5	4	R
Murolene ( <b>102</b> )	2	2	16	15	2	3	R
$\alpha$ -humulene ( <b>100</b> )	0	0	14	15	6	5	R
$\alpha$ -copaene ( <b>106</b> )	NT	1	NT	15	NT	4	R
Cedrol ( <b>109</b> )	0	0	15	16	5	4	R

A = Attractant

R = Repellent

NT = Not tested

**RCM:** CV = 13%; LSD ( $P \leq 0.05$ ) Response = 0; Interaction: Volatile organic compound X response = 1

**RSM:** CV = 13.87%; LSD ( $P \leq 0.05$ ) Response = 0; Interaction: Volatile organic compound X response = 0



Regardless of the species, the mites were repelled by the odours released from all the aromatic, monoterpene and sesquiterpene compounds except linalool (**66**), *E*- $\beta$ -ocimene (**73**) and terpinen-4-ol (**83**). Benzaldehyde (**30**), *p*-xylene (**41**), acetophenone (**46**), naphthalene (**44**),  $\alpha$ -phellandrene (**78**),  $\alpha$ -pinene (**81**), *E*- $\beta$ -farnesene (**96**), *E*- $\beta$ -caryophyllene (**94**), muurolene (**102**) and cedrol (**109**) showed highest (85-80%) significant ( $P \leq 0.05$ ) repellency to both mites. The findings demonstrate that these compounds could deter the mites from feeding from the tea cultivars. Aromatics, monoterpenes and sesquiterpenes defend plants against pests (Erbilgin et al., 2007; Jaenson et al., 2006; Khan et al., 2008; Zhang et al., 2015). Erbilgin *et al* (Erbilgin et al., 2007) showed that acetophenone (**46**) had a strong repellent activity on western pine beetles. It also causes acute toxic insecticidal activities (Mohsen et al., 1995). Acetophenone (**46**) and phenyl ethyl alcohol (**35**) deterred *Tribolium castaneum* (Herbst) and *Lasioderma serricorne* (Fabricius) from feeding well (at concentrations of 25-50  $\mu\text{l ml}^{-1}$ ) which subsequently affected the growth of the insects and died after 65 days (Jonfia-Esseen, Alderson, Tucker, Linforth & West, 2007). But when the concentration was increased, the insects became weak and died within a few minutes. This demonstrates that these VOCs not only acted as antifeedants but also as insecticides. The strong repellency of benzaldehyde to these mites is not strange. Benzaldehyde (**30**) is known to protect tea plants against tea aphid *Toxoptera aurantii* by attracting its natural enemies (Han et al., 2002). Benzaldehyde (**30**) not only protects plants against pests indirectly but has the capacity to kill insects at very low doses (Paulraj et al., 2011). Other aromatic VOCs have shown activities against pests. For example, methyl salicylate (**32**) showed antifeedant activity against pine weevils (Borg-Karlson et al., 2006). Anethole (**38**) is an effective insect repellent while benzothiazole (**39**) exhibited a wide range of biological properties including antimicrobial activities (Fujita et al., 2007). Terpenes protect plants against pests by

acting as repellents or toxins (Eller et al., 2014). Several studies have demonstrated this property for both monoterpenes ( $\alpha$ -phellandrene (**78**) (Bleeker et al., 2009), limonene (**76**),  $\alpha$ -Pinene (**81**), (Nerio et al., 2010; Yang et al., 2010),  $\beta$ -phellandrene (**79**), and myrcene (**72**),(Yang et al., 2010) and sesquiterpenes *E*- $\beta$ -caryophyllene (**94**) (Khan et al., 2008; Oluwafemi et al., 2013), humulene (**100**) (Khan et al., 2008), nerolidol (**99**) and cedrol (**109**) (Yatagai et al., 2002) and *E*- $\beta$ -farnesene (**96**) (Oluwafemi et al., 2013). The results of the present investigation clearly indicate that the VOCs associated with *Camellia cinensis* influence the responses of both red spider and red crevice mites and that generally the aromatics, monoterpenes and sesquiterpenes could deter the mites from attacking tea while GLVs make tea plants susceptible to these pests.

## CHAPTER FIVE: SUMMARY, CONCLUSIONS, RECOMMENDATIONS AND SUGGESTIONS FOR FURTHER STUDIES

The study aimed at screening clones (new and imported in relation to some commercial clones) for their susceptibility/resistance to mites and further relating susceptibility /resistance to overhead volatile organic compounds. The study was also expected to determine if there were variations in the overhead volatile organic compounds with cultivars, nitrogen fertilizer rates, seasons and region of production. Further, it was to determine the response of the mites to trapped overhead volatile organic compounds.

### 5.1 Summary

1. There were significant ( $p \leq 0.05$ ) monthly and clonal variations in mite infestations at all sites. Kangaita, near Mount Kenya in the east of Rift Valley recorded higher ( $p \leq 0.05$ ) mites infestations than Kipkebe and Timbilil, in the west of Rift Valley. Sixteen clones were resistant out of which TRFK 6/8, TRFK 301/4, TRFK 303/1199 and TRIT 201/50 were the most resistant. Five clones AHP SC12/28, AHP S 15/10, EPK TN 15-23, TRFK 18 /3 and TRIT 201/16 were susceptible. Of the clones evaluated for the first time, TRFK 303/216, TRFK 371/6, TRIT 201/43, TRIT 201/44, TRIT 201/50, TRIT 201/55, TRIT 201/73 and TRIT 201/82 were resistant to mites attack while TRFK 18/3 and TRIT 201/16 were susceptible. There was significant ( $p \leq 0.05$ ) linear correlation between mites levels and maximum temperature and inverse correlation between minimum temperature, relative humidity and rainfall.
2. The infestations of mites were linearly and significantly ( $p \geq 0.05$ ) correlated with GLVs especially *E*-2-hexenal (**7**), *Z*-3-hexenal (**6**), *Z*-3-hexenol (**9**) and *Z*-3-hexenyl acetate (**12**) and

the monoterpenes, linalool (**66**) and *E*- $\beta$ -ocimene (**73**). Most of the aromatic and terpenoid compounds were inversely correlated with mites infestations. Susceptible varieties to mites emitted high amounts of GLVs, particularly *E*-2-hexenal (**7**), *Z*-3-hexenal (**6**), *Z*-3-hexenol (**9**) and *Z*-3-hexenyl acetate (**12**) as well as linalool (**66**) and *E*- $\beta$ -ocimene (**73**) while resistant varieties released low levels and composition of GLVs but high levels and composition of aromatics and terpenes.

3. OVOCs were released in high levels and composition during dry season and low levels and composition in rainy season. There was a slight increase in the levels during cold season except GLVs that further decreased. Similarly, mites infestations levels were high in dry season, decreased in rainy and further declined in cold seasons. Kangaita registered the highest amounts of OVOCs particularly GLVs and Kipkebe the least amounts.
4. High mites infestation levels were recorded at 0 and 300 kg N/ha/year and minimum between 150 and 225 kg N/ha/year, fertilizer rates. All green leaf volatiles (GLVs) increased while some aromatic and terpenoid compounds decreased with increase in nitrogenous fertilizer rates. When aromatic, monoterpene and sesquiterpene compounds levels maximum at 150 - 225 kg N/ha/year, fertilizer rates mites infestation levels were at minimum.
5. The mites species were significantly ( $P \leq 0.05$ ) attracted by all GLVs tested. There was a synergistic effect in attraction of the mites (up to 100%) when some blends of GLVs were evaluated. All aromatic, monoterpene and sesquiterpene compounds repelled mites except *E*- $\beta$ -ocimene, linalool and terpinen-4-ol. *E*-2-hexenal (**10**), *Z*-3-hexenyl acetate (**12**) and linalool (**66**) displayed the strongest attraction while acetophenone (**46**), *E*- $\beta$ -farnesene (**96**), *E*- $\beta$ -caryophyllene (**94**),  $\alpha$ -pinene (**81**),  $\alpha$ -phellandrene (**78**), benzaldehyde (**30**), *p*-xylene (**41**), naphthalene (**44**) and benzaldehyde (**30**) exhibited the greatest repulsion to mites.

## 5.2 Conclusions

1. Sixteen clones were resistant, five susceptible and the rest moderately resistant. Thus cultivar selection may be a viable option for mites control.
2. Resistant cultivars emitted high number and levels of aromatics and most terpenoids and low levels and number of GLVs while susceptible cultivars released high levels of GLVs, *E*- $\beta$ -ocimene (**73**) and linalool (**66**) and low levels of aromatic and terpenoid compounds. GLVs, *E*- $\beta$ -ocimene (**73**) and linalool (**66**) could be responsible for the susceptibility of the cultivars to mites while aromatic and most terpenoid compounds make tea cultivars resistant to mites attack.
3. High levels and composition of OVOCs especially GLVs were emitted during dry season and low levels and amounts during rainy season. Kangaita registered the highest levels and composition of OVOCs while Kipkebe the least. Mites attack in Kangaita during dry seasons especially on susceptible clones could be due to the high levels of GLVs.
4. Mites infestation levels were high at both low (0 kg N/ha/year) and high (300 kg N/ha/year) fertilizer rates and minimum at rates 150 - 225 kg N/ha/year which were influenced by the levels and composition of the OVOCs. The recommended fertilizer rates in Kenya defend tea plants against red spider mites attack; since maximum levels of defence compounds (aromatics and terpenes) were registered.
5. All aromatics and terpenoids were repellents except *E*- $\beta$ -ocimene (**73**), linalool (**66**) and terpinen-4-ol (**83**) while all GLVs were attractants to both species of mites. GLVs, *E*- $\beta$ -ocimene (**73**) and linalool (**66**) could make tea plants susceptible to mites while aromatic and most terpenoid compounds could deter red spider and red crevice mites from attacking the plants.

### 5.3 Recommendations

1. Resistant cultivars are recommended for commercial exploitation in mites prone areas. For the susceptible clones, mitigation strategies are necessary in mites prone areas during hot seasons with high monthly temperatures and low humidity. However, susceptible cultivars/clones should not be commercially exploited in mites prone areas. Use of resistant/tolerant clone may be a practical way of mitigating yield losses without use of pesticides that degrade the environment. Since mites infestation levels on clones varied with regions and seasons, there is need for the development of region specific suitable clones.
2. Breeding/selection programmes should incorporate OVOCs profiles to develop tea cultivars that resist mites attack. Breeding efforts should be focused on cultivars that produce low levels of GLVs and high levels of both terpenes and aromatics in order to develop tea cultivars that resist mites attack.
3. There was a link between the levels and composition of GLVs and mites infestations. Resistant cultivars that release low levels of GLVs are recommended for commercial exploitation in mites prone areas as an insurance against yield losses due to mite infestations especially during drought.
4. The tea industry should continue to use the recommended fertilizer rates of 100 to 225 kg N/ha/year as it protects the tea plants against the red spider mites attack.
5. Breeding/selection programmes should focus on cultivars that produce low levels of mites attractants (GLVs, *E*- $\beta$ - ocimene (**73**), linalool (**66**) and terpinen-4-ol (**83**) and high levels of repellents (both terpenes and aromatics).

#### 5.4 Suggestions for further studies

1. Being a perennial monoculture crop, tea plant is attacked by many insect pests. There is need to evaluate if the Kenyan recommended fertilizer rates defend tea plants against the many pests that reduce both yield and quality of tea.
2. Response of natural enemies of red spider and red crevice mites to the OVOCs should be investigated to account for the high levels of some OVOCs emitted by some cultivars for example, *E*- $\beta$ - ocimene (**73**) and linalool (**66**) whether they defend tea plants by attracting natural enemies of the mites. Studies have demonstrated that VOC induction may have a double edge, as plant enemies may also be attracted (Kessler & Heil, 2011).

## REFERENCES

- Adhikari, U., Nejadhashemi, A. P., & Woznicki, S. A. (2015). Climate change and eastern Africa: a review of impact on major crops. *Food and Energy Security, 4*, 110-132.
- Agnihotrudu, V. (1999). Potential of using bio control agents in tea. In: N. K. Jain, *Global advances in tea science* (pp. 675-692). New Delhi, India: New Age International Ltd.
- Aharoni, A., Giri, A. P., Deuerlein, S., Griepink, F., De Kogel, W. J., Verstappen, F. W., Verhoeven, H. A., Jongasma, M. A., Schwab, W., & Bouwmeester, H. J. (2003). Terpenoid metabolism in wild-type and transgenic Arabidopsis plants. *Plant Cell., 15*, 2866-2884.
- Ahmed, M. (2012). Ecofriendly pest management of tea in Bangladesh. *Two and a Bud, 59*, 11-26.
- Ahmed, M., Chowdhury, R. S., Haque, M. M., & Mamum, M. (2012). Influence of weather parameters on red spider mites. A major pest of tea in Bangladesh. *SUST Journal of Science and Technology, 19*, 47-53.
- Akio, T., & Hiroshi, A. (2001). Biological control of insect pests in Japan, a control of multiple pests of tea, and spider mites in greenhouses. Matsudo 271-8510, Japan-08-01: Laboratory of Applied Entomology and Zoology, Faculty of Horticulture, Chiba University.
- Allmann, S., & Baldwin, I. T. (2010). Insect betray themselves in nature to predators by rapid isomerization of green leaf volatiles. *Science, 329*, 1075-1078.
- Aluja, M., Arredondo, J., Diaz-Fleischer, F., Birke, A., Rull, J., Niogret, J., & Epsky, N. (2014). Susceptibility of 15 mango (Sapindales: *Anacardiaceae*) cultivars to the attack by *Anastrepha ludens* and *Anastrepha obliqua* (Diptera: *Tephritidae*) and the role of underdeveloped fruit as pest reservoirs: management implications. *Journal of Economic Entomology, 107*(1), 375-388.



- Anjum, S. A., Xie, X., Wang, L., Saleem, M. F., Man, C., & Lei, W. (2011). Morphological, physiological and biochemical responses of plants to drought stress. *African Journal of Agricultural Research*, 6, 2026-2032.
- Anonymous (2002). *The Tea Growers Handbook*. Kericho, Kenya: Tea Research Foundation of Kenya.
- Anonymous (2008). *Tea Research Foundation of Kenya, Annual Technical Report for 2008*. Kericho, Kenya: Tea Research Foundation of Kenya.
- Anonymous (2011). *International Tea Committee; Annual Bulletin of Statistics*. London: International Tea Committee.
- Antonious, G. F. (2004). Residues and half-lives of pyrethrins on field-grown pepper and tomato. *Journal of Environmental Science and Health*, 39, 491–503.
- Atijegbe, S. R., Nuga, B. O., Lale, E. N. S., & Nwanna, R. O. (2013). Growth of cucumber (*Cucumis Sativus* L.) in the humid tropics and the incidence of insect pests as affected by organic and inorganic fertilizers. *Journal of Applied Science and Agriculture*, 8, 1172-1117.
- Ayvaz, A., Sagdic, O., Karaborklu, S., & Ozturk, I. (2010). Insecticidal activity of essential oils from different plants against three store-product insects. *Journal of Insect Science*, 10(1), 2.
- Banerjee, B. (1987). Can leaf aspect affect herbivory? A case study of tea. *Ecology*, 68, 839-843.
- Barthakur, B. K., Dutta, P., & Karan, S. (1992). Clonal susceptibility to red rust. *Two and a Bud*, 39, 52-60.
- Bilham, J. (2011). Climate Change Impacts upon crop yields in Kenya: learning from the past. *Earth and Environment*, 6, 1-45.

- Bleeker, P. M., Diergaarde, P. J., Ament, K., Guerra, J., Weidner, M. S., S., de Both, M. T. J., Haring, M. A., & Schuurink, R. C. (2009). The role of specific tomato volatiles in tomato-whitefly interaction. *Plant Physiology*, *151*, 925-935.
- Bleeker, P. M., Spyropoulou, E. A., Diergaarde, P. J., Volpin, H., De Both, M. T., Zerbe, P., Bohlmann, J., Falara, V., Matsuba, Y., & Pichersky, E. (2011). RNA-seq discovery, functional characterization, and comparison of sesquiterpene synthases from *Solanum lycopersicum* and *Solanum habrochaites* trichomes. *Plant Molecular Biology*, *77*, 323–336.
- Boatright, J., Negre, F., Chen, X., Kish, C. M., Wood, B., Peel, G., Orlova, I., Gang, D., Rhodes, D., & N., D. (2004). Understanding in vivo benzenoid metabolism in petunia petal tissue. *Plant Physiology*, *135*, 1993-2011.
- Borg-Karlson, A. K., Nordlander, G., Mudalige, A., Nordenhem, H., & Unelius, C. R. (2006). Antifeedants in the feces of the pine weevil *Hylobius abietis*: Identification and biological activity. *Journal of Chemical Ecology*, *32*, 943–957.
- Borg-Karlson, A. K., Tengo, J., Valterova, I., Unelius, C. R., Taghizadelh, T., Tolash, T., & Francke, W. (2003). (S)-(+)-Linalool: A mate attractant pheromone compound in bee *Colletes cunicularis*. *Journal of Chemical Ecology*, *29*(1), 1-14.
- Bormann, S., Maria, M. W., Etschmann, M. M., & Schrader, J. (2012). Integrated bioprocess for the stereospecific production of linalool oxides from linalool with *Corynespora cassiicola* DSM 62475. *Journal of Industrial Microbiology and Biotechnology*, *39*, 1761-1769.
- Borthakur, M., Rahman, A. M., Sarmah, M., & Gurusubramanian, G. (2005). Predators of phytophagous mites of tea (*Camellia sinensis*) in North East India. In: *Proceedings of 2005 International Symposium on Innovation in Tea Science and Sustainable Development in Tea Industry* (pp. 749-755). Hangzhou, China.

- Bowers, W. S., Ortega, F., You, X., & Evans, P. H. (1993). Insect repellents from the Chinese prockly ash *Zanthoxylum bungeanum*. *Journal of Natural Products*, *56*(6), 935-938.
- Bruce, T. J. A., Midega, C. A. O., Birkett, M. A., Pickett, J. A., & Khan, Z. R. (2010). Is quality more important than quantity? Insect behavioural responses to changes in a volatile blend after stem borer oviposition on an African grass. *Biology Letters*, *6*, 314–317.
- Buttery, R. G., Flath, R. A., Mon, R. T., & Ling, L. C. (1986). Identification of germacrene D in walnut and fig leaf volatiles. *Journal of Agricultural and Food Chemistry*, *34*(5), 820-822.
- Buttery, R. G., Ling, L. C., & Wellso, S. G. (1982). Oat leaves volatiles: Possible insect attractants. *Journal of Agricultural and Food Chemistry*, *30*(4), 791-792.
- Buttery, R. G., Xu, L., & Ling, L. C. (1985). Volatile components of wheat leaves (and stems): Possible insect attractants. *Journal of Agricultural and Food Chemistry*, *33*(1), 115-117.
- Cai, L. M. S. X., Dong, W. X., Wang, C. G., & Chen, M. Z. (2012). Variability and stability of tea weevil-induced volatile emissions from tea plants with different weevil densities, photoperiod and infestation duration. *Insect Science*, *19*, 507–517.
- Calumpang, S. M. F., Burgonio, G. A. S., Navasero, M. M., & Navasero, M. V. (2013). Behavioral and olfactory responses of rice green leaf hopper, *Nephotettix virescens* (Distant) to volatile cues from Tagbak (*Alpinia elegans* (C. Presl) K. Schum). *Philippine Journal of Science*, *142*, 167-173.
- Chen, Y., Olson, D. M., & Ruberson, J. R. (2010). Effects of nitrogen fertilization on tritrophic interactions. *Arthropod-Plant Interactions*, *4*, 81-94.
- Chen, Y., Whitehill, J. G. A., Bonello, P. Y., & Poland, M. T. (2011). Differential response in foliar chemistry of three ash species to emerald ash borer adult feeding. *Journal of Chemical Ecology*, *37*, 29–39.

- Cheng, Y. J., Cheng, S. S., & Chang, S. T. (2010). Monitoring the emission of volatile organic compounds from the leaves of *Calocedrus macrolepis* var. *formosana* using solid-phase micro-extraction. *Journal of Wood Science*, *56*, 140-147.
- Cherotich, L., Kamunya, S. M., Alakonya, A., Msomba, S. W., Uwimana, M. A., & Owuor, P. O. (2013a). Variation in catechin composition of popularly cultivated tea clones in East Africa (Kenya). *Tea*, *34*, 14-30.
- Cherotich, L., Kamunya, S. M., Alakonya, A., Msomba, S. W., Uwimana, M. A., & Owuor, P. O. (2014). Genotypic stability and adaptability of tea cultivars in relation to catechin levels across four environments. *Tea*, *35*, 8-16.
- Cherotich, L., Kamunya, S. M., Alakonya, A., Msomba, S. W., Uwimana, M. A., Wanyoko, J. K., & Owuor, P. O. (2013b). Variation in catechin composition of popularly cultivated tea clones in East Africa (Kenya). *American Journal of Plant Science*, *4*, 628-640.
- Cherotich, L., Kamunya, S. M., Alakonya, A., Msomba, S. W., Uwimana, M. A., Wanyoko, J. K., & Owuor, P. O. (2013c). Variation in catechin composition of popularly cultivated tea clones in East Africa (Kenya). *American Journal of Plant Science*, *4*, 628-640.
- Coley, P. (1998). Possible effects of climate change on plant/herbivore interactions in moist tropical forests. *Climatic Change*, *39*, 445-472.
- Copolovici, L., Kännaste, A., Pazouki, L., & Niinemets, Ü. (2012). Emissions of green leaf volatiles and terpenoids from *Solanum lycopersicum* are quantitatively related to the severity of cold and heat shock treatments. *Journal of Plant Physiology*, *169*, 664-672.
- Danathanarayan, N., & Ranaweera, D. J. W. (1972). The effect of rainfall and shade on the occurrence of three pests of tea in Ceylon. *Annals of Applied Biology*, *70*, 1-12.

- De Boer, J. G., Snoeren, A. L., & Dicke, M. (2005). Predatory mites learn to discriminate between plant volatile induced prey and nonprey herbivores. *Animal Behaviour*, *69*, 869-879.
- Degenhardt, J., Tobias, G., & Köllne, J. G. (2009). Monoterpene and sesquiterpene synthases and the origin of terpene skeletal diversity in plants. *Phytochemistry*, *70*, 1621–1637.
- Dicke, M. (1986). Volatile spider mite pheromone and host-plant kairomone involved in space out gregariousness in the spider mite. *Physiological Entomology*, *11*, 251-262.
- Dicke, M., & Baldwin, I. T. (2010). The evolutionary context for herbivore-induced plant volatiles: beyond the ‘cry for help’. *Trends Plant Sciences*, *15*, 167-175.
- Dickens, J. C. (2006). Plant volatiles moderate response to aggregation to pheromone in Colorado potato beetle. *Journal of Applied Entomology*, *130*, 26–31.
- Dudareva, N., Negre, F., Nagegowda, D. A., & Orlova, I. (2006). Plant volatiles: Recent advances and future perspectives. *Critical Reviews in Plant Sciences*, *25*(5), 417-440.
- Eller, F. J., Vander, M. R. K., Behle, R. W., Flor, W. L. B., & Palmquist, D. E. (2014). Bioactivity of cedarwood oil and cedrol against arthropods pests. *Environmental Entomology*, PMID:24690252.
- Elmoghazy, M. M. E., El-Saiedy, E. M. A., & Romeih, H. M. A. (2011). Integrated control of the two spotted spider mite *Tetranychus Urticae* Koch (Acari: Tetranychidae) on faba bean, *Vicifaba* (L.) in an open field at Behaira Governorate, Egypt. *International Journal of Emerging of Science and Engineering*, *2*, 93-100.
- Erb, M., Veyrat, N., Robert, C. A., Xu, H., Hey, M., J., T., & Turlings, T. C. J. (2015). Indole is an essential herbivore-induced volatile priming signal in maize. *Nature Communications*, *6*(6273).

- Erbilgin, N., Krokene, P., Kvamme, T., & Christiansen, E. (2007). A host monoterpene influences *Ips typographus* (Coleoptera: Curculionidae, Scolytinae) responses to its aggregation pheromone. *Agricultural and Forest Entomology*, 9, 135–140.
- FAO (2005). Committee on commodity problems: Intergovernmental group on tea. . <http://www.fao.org/docrep/meeting/009/j5602 e.htm>.
- FAO (2014). Committee on MRLs in brew: Intergovernmental group on tea. <http://www.fao.org/fileadmin/templates/est/meetings/tea May14/FAO IGG TEA>.
- Fatouros, N. E., Lucas-Barbosa, D., Weldegergis, B. T., Pashalidou, F. G., van Loon, J. J. A., & Dicke, M. (2012). Plant volatiles induced by herbivore egg deposition affect insects of different trophic levels. *PLoS ONE*, 7, e43607.
- Fujita, K., Fujita, T., & Kubo, I. (2007). Anethole, a potential antimicrobial synergist, converts a fungistatic dodecanol to a fungicidal agent. *Phytotherapy Research*, 21, 47–51.
- Gardner, E. J., Ruxton, C. H., & Leeds, A. R. (2007). Black tea--helpful or harmful? A review of the evidence. *European Journal of Clinical Nutrition*, 61, 3-18.
- Ghosh, H. N. (2001). Advances in selection and breeding of tea. A review. *Journal of Plantation Crops*, 29, 1-17.
- Gotoh, T., & Nagata, T. (2001). Development and reproduction of *Oligonychus coffeae* (Acarina: Tetranychidae) on tea. *International Journal of Acarology*, 27, 293–298.
- Gulati, A., & Ravindranath, S. D. (1996). Seasonal variations in quality of Kangra tea (*Camellia sinensis* (L.) O Kuntze) in Himachal Pradesh. *Journal of the Science of Food and Agriculture*, 71, 231–236.
- Gurusubramanian, G. (2005). Safe and economic pest management in tea, shade trees and green crops. In: A. K. Dutta, Ahmed, N., Kotoky, B., Burugohain, D., *Manual on tea culture for*

- condensed course* (pp. 113-127). Jorhat, Assam, India: Tocklai Experimental Station, TRA, Jorhat Assam Printing Works Private Limited.
- Gutensohn, M., Orlova, I., Nguyen, T. T. H., Davidovich-Rikanati, R., Ferruzzi, M. G., & Sitrit, Y. (2013). Cytosolic monoterpene biosynthesis is supported by plastid-generated geranyl diphosphate substrate in transgenic tomato fruits. *Plant Cell*, *75*, 351–363.
- Hagg, J. F., Zagrobelny, M., & Bak, S. (2013). Plant defense against insect herbivores. *International Journal of Molecular Sciences*, *14*, 10242-10297.
- Halitschke, R., Stenberg, J. A., Kessler, D., Kessler, A., & Baldwin, I. T. (2008). Shared signals - “alarm calls” from plants increase apparency to herbivores and their enemies in nature. *Ecology Letters*, *11*(1), 24–34.
- Han, B., & Chen, Z. (2002). Behavioral and electrophysiological responses of natural enemies to synomones from tea shoots and kairomones from tea aphids, *Toxoptera aurantii*. *Journal of Chemical Ecology*, *28*, 2203-2219.
- Han, B., Zhang, Q. H., & Byers, J. A. (2012). Attraction of the tea aphid, *Toxoptera aurantii*, to combinations of volatiles and colors related to tea plants. *Entomologia Experimentalis et Applicata*, *144*(3), 258–269.
- Haque, M., Begum, A., Naher, N., & Wahab, A. (2007). Developmental stages of red spider mite, *Oligonychus coffeae* Neitner (Acari: Tetranychidae) infesting rose. *University Journal of Zoology, Rajshani University*, *26*, 71-72.
- Hartikainen, K., Riikonen, J., Nerg, A. M., Kivimaenpaa, M., Ahonen, V., Tervahauta, A., Karenlampi, S., Maenpaa, M., Rousi, M., Kontunen-Soppela, S., Oksanen, E., & Holopainen, T. (2012). Impact of elevated temperature and ozone on the emission of

- volatile organic compounds and gas exchange of silver birch (*Betula pendula* Roth). *Environmental and Experimental Botany*, 84, 33-43.
- Hatanaka, A., Kajiwa, T., & Sekiya, J. (1987). Enzymic oxygenative cleavage reaction of linolenic acid in leave-chloroplastic lipoxygenase and fatty acid hydroperoxide lyase in tea leaves. In: P. K. Stumpf, J. B. Mudd, & W. B. Nes, *Metabolism, Structure and Functions of Lipids* (pp. 392-398). New York: Plenum Publishing Corporation.
- Hatanaka, A., Kajiwara, T., Matsui, K., & Toyota, H. (1992). Substrate specificity of tea leaf hydroperoxide lyase. *Zeitschrift für Naturforschung*, 47, 677-679.
- Hatanaka, A., Kajiwara T., Matsui, K. (1995). The biogeneration of green odour by green leaves and physiological functions- past, present and future. *Zeitschrift für Naturforschung*, 50, 467-472.
- Holopainen, J. K., & Gershenzon, J. (2010). Multiple stress factors and the emission of plant VOCs. *Trends in Plant Sciences*, 15, 176-184.
- Honda, K., Omura, H., & Hayashi, N. (1998). Identification of floral volatiles from *Ligustrum japonica* that stimulate flower visting by cabbage butterfly, *Pieris rapae*. *Journal of Chemical Ecology*, 24(2), 2167-2180.
- Horita, H., & Owuor, P. O. (1987). Comparison and characterisation of volatile components of Kenyan clonal black teas and various black teas from other producing areas of the world. *Bulletin of the National Research Institute of Vegetables, Ornamental Plants and Tea, (Japan)*, 1(B), 55-65.
- Hägg , J. F., Zagrobelny, M., & Bak, S. (2013). Plant defense against insect herbivores. *International Journal of Molecular Sciences*, 14(5), 10242-10297.



- Ishiwari, H., Suzuki, T., & Maeda, T. (2007). Essential compounds in herbivore-induced plant volatile that attract mite *Neoseiulus womersleyi*. *Journal of Chemical Ecology*, *33*, 1670-1681.
- Jaenson, T. G., Palsson, K., & Borg-Karlson, A. K. (2006). Evaluation of extracts and oils of mosquito (Diptera: *Culicidae*) repellent plants from Sweden and Guinea-Bissau. *Journal of Medical Entomology*, *43*, 113-119.
- James, D. G., & Price, T. S. (2004). Field testing of methyl salicylate for treatment and retention of beneficial insects in grapes and hops. *Journal of Chemical Ecology*, *30*(8), 1613-1628.
- Jardine, K. J., Chambers, J. Q., Holm, J., Angela, B., Fontes, J. C. G., Zorzanelli, R. F., Kimberly, T., Fernandez de Souza, M. V., Garcia, S., Gimenez, B. O., Piva, L. R. d. O., Higuchi, N., Artaxo, P., Martin, S., & Manzi, A. O. (2015). Green leaf volatile emissions during high temperature and drought stress in a Central Amazon. *Plants*, *4*, 678-690.
- Jeyaramraja, P. R., Pius, P. K., Raj Kumar, R., & Jayakumar, D. (2003). Soil moisture stress-induced alterations in bioconstituents determining tea quality. *Journal of the Science of Food and Agriculture*, *83*, 1187-1191.
- Jonfia-Essean, W. A., Alderson, P. G., Tucker, G., Linforth, R., & West, G. (2007). The growth of *Tibolium castaneum* (Herbst) and *Losioderma serriocorne* (Fabricius) on feed media closed with flavour volatile compounds found in dry cocoa beans. *Pakistan Journal of Biological Sciences*, *10*, 1301-1304.
- Kamunya, S. M., Chalo, R., Korir, R., Kiplang'at, J., & Wachira, F. N. (2010a). Assessment of genetic similarities among popularly cultivated tea clones in Kenya using rapid markers. *Tea*, 40-44.

- Kamunya, S. M., Muoki, R. C., Owuor, P. O., Pathak, R. S., Sharma, R. K., Wachira, F. N., & Wanyoko, J. K. (2010b). Quantitative genetic parameters for yield, drought tolerance and some quality traits of tea (*Camellia sinensis* (L.) O. Kuntze). *Research Journal of Agricultural Sciences*, *1*, 53-65.
- Kamunya, S. M., Wachira, F. N., Pathak, R. S., Korir, R., Sharma, V., Kumar, R., Bhardwaj, P., Muoki, R. C., Ahuja, P. S. B., & Sharma, R. K. (2010c). Genomic mapping and testing for quantitative trait loci in tea (*Camellia sinensis* (L.) O. Kuntze). *Tree Genetics and Genomes*, *6*, 915-929.
- Kannaste, A., Pazouki, L., Suhhorutsenko, M., Copolovici, L., & Niinemets, U. (2013). Highly variable chemical signatures over short spatial distances among Scots pine (*Pinus sylvestris*) populations. *Tree Physiology*, *33*, 374–387.
- Kessler, A., & Heil, M. (2011). The multiple faces of indirect defences and their agents on natural selection. *Functional Ecology*, *25*, 348–357.
- Khan, Z. R., Midega, C. A. O., Amudavi, D. M., Hassanali, A., & Pickett, J. A. (2008). On-farm evaluation of the 'push-pull' technology for the control of stemborers and striga weed on maize in western Kenya. *Field Crops Research*, *106*, 224-233.
- Kinyanjui, T., Gitu, P. M., & Kamau, G. N. (2000). Potential antitermite compounds from *Juniperus procera* extracts. *Chemosphere*, *41*, 1071-1074.
- Krips, O. E., Willems, P. E., Gols, R., Posthumus, M. A., Gort, G., & Dicke, M. (2001). Comparison of cultivars of ornamental crop *Gerbera jamesonii* on production of spider mite-induced volatiles, and their attractiveness to the predator *Phytoseiulus persimilis*. *Journal of Chemical Ecology*, *27*, 1355-1372.

- Kumara, D. V., Babub, A., Rahmana, V. K. J., & Roobakkumara, A. (2011). Impact of temperature and pesticide applications on the prey consumption of *Mallada desjardinsi* (Navas) (Neuroptera: *Chrysopidae*), a predator of red spider mite infesting tea. *Two and a Bud*, 59, 43-48.
- Kumara, R., Bisen, J. S., Choubey, M., Singh, M., & Bera, B. (2015). Studies on effect of altitude and environment on physiological activities and yield of Darjeeling tea (*Camellia sinensis* L.) plantation. *Journal of Crop and Weed*, 11, 71-79.
- Kwach, B. O., Kamau, D. M., Msomba, S. W., Muhoza, C., & Owuor, P. O. (2014). Effects of location of production, nitrogenous fertilizer rates and plucking intervals on clone TRFK 6/8 tea in East Africa: II. Mature leaf nutrients. *International Journal of Tea Science*, 10(3&4), 25-40.
- Kwach, B. O., Kamau, D. M., Owuor, P. O., Wanyoko, J. K., Msomba, S. W., & Muhoza, C. (2011). Effects of location of production, fertilizer rates and plucking intervals on mature leaf nutrients of clone TRFK 6/8 in East Africa. *Tea*, 32(2), 56-68.
- Kwach, B. O., Owuor, P. O., Kamau, D. M., Msomba, S. W., & Uwimana, M. A. (2015). Variations in the precursors of plain black tea quality parameters due to location of production and nitrogen fertilizer rates in Eastern African clonal tea leaves. *Experimental Agriculture*, In press. *Experimental Agriculture*, (In press).
- Kwach, B. O., Owuor, P. O., Kamau, D. M., Msomba, S. W., & Uwimana, M. A. (2016). Variations in the precursors of plain black tea quality parameters due to location of production and nitrogen fertilizer rates in Eastern African clonal tea leaves. *Experimental Agriculture*, 52(2), 266-278.

- Kwach, B. O., Owuor, P. O., Kamau, D. M., Wanyoko, J. K., & Kamunya, S. M. (2013). Influence of location of production, season and genotype on caffeine and flavan-3-ols in young green leaves of tea (*Camellia sinensis*) leaves in Kenya. *Journal of Agricultural Science and Technology*, *3B*(8), 557-574.
- Lange, B. M., & Ahkami, G. W. (2013). Terpenoid biosynthesis in glandular trichomes-current status and future opportunities. *Plant Biotechnology Journal*, *11*, 2–22.
- Lee, J. C. (2010). Effect of methyl salicylate-based lures on beneficial and pest arthropods in strawberry. *Environmental Entomology*, *39*, 635-666.
- Lehmann-Danzinger, H. (2000). Diseases and pests of tea: Overview and possibilities of integrated pest and disease management. *Journal of Agriculture in the Tropics and Subtropics*, *101*, 13=38.
- Lin, L., Chen, P., & Harnly, J. M. (2008). New phenolic components and chromatographic profiles of green and fermented teas. *Journal of Agricultural and Food Chemistry*, *56*, 8130–8140.
- Loreto, F., Barta, C., Brillì, F., & Nogues, I. (2006). On the induction of volatile organic compound emissions by plants as consequence of wounding or fluctuations of light and temperature. *Plant Cell Environment*, *29*, 1820-1828.
- Lucker, J., Schwab, W., Van Hautum, B., Blaas, J., Van der Plas, L. H. W., Bouwmeester, H. J., & Verhoeven, H. A. (2004). Increased and altered fragrance of tobacco plants after metabolic engineering using three monoterpene synthases from lemon. *Plant Physiology*, *134*, 510–519.
- Maeda, H., Shasany, A. K., Schnepf, J., Orlova, I., Taguchi, G., Cooper, B. R., Rhodes, D., Pichersky, E., & Dudareva, N. (2010). RNAi suppression of Arogenate Dehydratase1

- reveals that phenylalanine is synthesized predominantly via the aroenate pathway in petunia petals. *Plant Cell*, 22, 832-849.
- Mahanta, P. K., Baruah, S., Owuor, P. O., Murai, T., Horita, H., & Tsushida, T. (1988). Flavour volatiles of Assam black teas manufactured from different plucking standards and orthodox teas manufactured from different altitudes of Darjeeling. *Journal of the Science of Food and Agriculture*, 45, 317–324.
- Mamati, G. E., Wachira, F. N., & Njuguna, C. K. (2001). The 2001 released clones. *Tea*, 22, 6-7.
- Mamun, M. S. A., & Ahmed, M. (2011). Prospect of indigenous plant extract in tea pest management. *International Journal of Agricultural Research, Innovation and Technology*, 1, 16-23.
- Marckovic, I., Norris, D. M., Phillips, J. K., & Webster, F. X. (1996). Volatiles involved in the non host rejection of *Fraxinus pennsylvanica* by *Lymantria dispar* larvae. *Journal of Agricultural and Food Chemistry*, 44, 929–935.
- Mazid, M., Khan, T. A., & Mohammad, F. (2011). Secondary metabolites in defence mechanisms of plants. *Biology and Medicine*, 3(2), 232-249.
- Meldau, S., Wu, J. Q., & Baldwin, I. T. (2009). Silencing two herbivory-activated MAP kinases, IPK and WIPK, does not increase *Nicotiana attenuata*'s susceptibility to herbivores in the glasshouse and in nature. *New Phytologist*, 181(1), 161–173.
- Metcalf, R. L., & Kogon, M. (1987). Plant volatiles as insect attractants. *Critical Reviews in Plant Sciences*, 5(3), 251-301.
- Mohsen, Z. H., Ali, Y., & Al-Chalabi, B. M. (1995). Insecticidal effects of acetophenone against *Culex quinquefasciatus* (Diptera: Culicidae). *Japanese Journal of Sanitary Zoology*, 46(4), 405-408.

- Moreira, X., Sampedro, L., Zas, R., & A., S. (2008). Alterations of the resin canal system of *Pinus pinaster* seedlings after fertilization of a healthy and of a *Hylobius abietis* attacked stand. *Trees*, 22, 771-777.
- Morley, K., Finch, S., & Collier, R. H. (2005). Companion planting – behaviour of the cabbage root fly on host plants and non-host plants. *Entomologia Experimentalis et Applicata*, 117, 15–25. .
- Mozuraitis, R., Strandén, M., Ramirez, M. I., Borg-Karlson, A. K., & Mustaparta, H. (2002). (-)-Germacrene D increases attraction and oviposition by the tobacco budworm moth *Heliothis virescens*. *Chemical Senses*, 27(6), 505-509.
- Msomba, S. W., Kamau, D. M., Uwimana, M. A., Muhoza, C., & Owuor, P. O. (2014). Effects of location of production, nitrogenous fertilizer rates and plucking intervals on clone TRFK 6/8 tea in East Africa: I. Yields. *International Journal of Tea Science*, 10(3&4), 14-24.
- Muraleedharan, N. (1992). Pest control in Asia. In: K. C. Willson, Clifford, M.N., *Tea: Cultivation to Consumption*. (pp. 375-412). London: Chapman and Hall.
- Muraleedharan, N., & Chen, Z. M. (1997). Pest and disease of tea and their management. *Journal of Plantation Crops*, 25, 15-43.
- Muthumani, T., Verma, D. P., Venkatesan, S., & Senthil Kuman, R. S. (2013). Influence of climatic seasons on quality of south Indian black teas. *Journal of Natural Product and Plant Resources*, 3, 30–39.
- Najar-Rodriguez, A., Bellutti, N., & Dorn, S. (2013). Larval performance of the oriental fruit moth across fruits from primary and secondary hosts. *Physiological Entomology*, 38, 63–70.

- Nerio, L. S., Olivero-Verbel, J., & Stashenko, E. (2010). Repellent activity of essential oils: A review. *Bioresource Technology*, *101*, 372-378.
- Ng'etich, W. K., Stephens, W., & Othieno, C. O. (1995). Clonal tea response to altitude in Kericho. II. Weather, climate analysis and soil water deficits. *Tea*, *16*, 85-98.
- Ng'etich, W. K., Stephens, W., & Othieno, C. O. (2001). Responses of tea to environment in Kenya. 3. Yield and yield distribution. *Experimental Agriculture*, *37*, 361-372.
- Ngetich, W. K., & Stephens, W. (2001). Response of tea to environment in Kenya. 1. Genotype X environment interactions for total dry matter yield. *Experimental Agriculture*, *37*, 333-334.
- Niinemets, U., Kannaste, A., & Copolovici, L. (2013). Quantitative patterns between plant volatile emissions induced by biotic stresses and the degree of damage. *Frontiers in Plant Science*, *4*, 262.
- NIST/EPA/NIH. (2008). *NIST Mass Spectral Library*. Washington D.C.: National Institute of Standard and Technology, US Secretary of Commerce, U.S.A.
- Obaga, S. M. O., Squire, G. R., & Langat, J. K. (1988). Altitude, temperature and the growth rate of tea shoots. *Tea*, *9*, 30-35.
- Obanda, M., & Owuor, P. O. (1995). Impact of shoot maturity on chlorophyll content, composition of volatile flavour compounds and plain black tea chemical quality parameters of clonal leaf. *Journal of the Science of Food Agriculture*, *69*, 529-534.
- Ogola, S. O., & Kibiku, P. N. (2004). Smallholder tea growing enterprise: Productivity and Profitability. Nairobi: Tea Board of Kenya Survey Report.
- Ohgami, S., Ono, E., Horikawa, M., Murata, J., Totsuka, K., Toyonaga, H., Ohba, Y., Dohra, H., Asai, T., Matsui, K., Mizutani, M., Watanabe, N., & Ohnishi, T. (2015). Volatile

- glycosylation in tea plants: sequential glycosylations for the biosynthesis of aroma  $\beta$ -primeverosides are catalyzed by two *Camellia sinensis* glycosyltransferases. *Plant Physiology*, *168*, 464–477.
- Okal, A. W., Owuor, P. O., Kamau, D. M., & Manguro, L. A. O. (2012a). Effect of production location and plucking interval on fatty acids concentration in clonal tea. *Food Science and Technology International, Tokyo*, *18*(351-356).
- Okal, A. W., Owuor, P. O., Kamau, D. M., & Manguro, L. A. O. (2012b). Variations of fatty acids levels in young shoots of clonal tea with location of production and nitrogenous fertilizer rates in the Kenya highlands. *Journal of Agricultural Science Technology*, *14*, 1545-1554.
- Oluwafemi, S., Dewhurst, S. Y., Veyrat, N., Powers, S., & Bruce, T. J. A. (2013). Priming of production in maize of volatile organic defence compounds by the natural plant activator cis-jasmone. *PLoS ONE*, *8*(6), e62299.
- Othieno, C. O. (1988). Summary of recommendations and observations from TRFK. *Tea*, *9*, 50-65.
- Othieno, C. O., Stephens, W., & Carr, M. K. V. (1992). Yield variability at the Tea Research Foundation of Kenya. *Agriculture and Forestry Meteorology*, *61*, 237-252.
- Owuor, P. O. (1989). Differentiation of teas by the variations in the linalools and geraniols Levels. *Bulletin of the Chemical Society of Ethiopia*, *3*, 31-35.
- Owuor, P. O. (1992a). Changes in quality parameters of commercial black seedling tea due to time of the year in the eastern highlands of Kenya. *Food Chemistry*, *45*, 119-124.



- Owuor, P. O. (1992b). A comparison of gas chromatographic volatile profiling methods for assessing the flavour quality of Kenyan black teas. *Journal of the Science of Food and Agriculture*, 59, 189-197.
- Owuor, P. O. (1992c). High rates of nitrogen on tea at high altitude. VI. Changes in clonal black tea quality due to time of the year, rates and splitting nitrogen application. *Tea*, 13, 36-43.
- Owuor, P. O. (1999). Tea in East Africa (Kenya, Uganda, Tanzania). In: N. K. Jain, *Global Advances in Tea Science* (pp. 171-188). New Delhi, India: New Age International.
- Owuor, P. O. (2014). Black tea: Biochemical changes during processing. In: C. W. Bamforth, & R. E. Ward, *The Oxford Handbook of Food Fermentations* (pp. 659-694). New York: Oxford University Press.
- Owuor, P. O., Horita, H., Tsushida, T., & Murai, T. (1987a). Variations in the chemical composition of some Kenyan clonal teas. *Kenya Journal of Sciences*, 8(A), 27-32.
- Owuor, P. O., Kamau, D. M., & Jondiko, E. O. (2010a). The influence of geographical area of production and nitrogenous fertilizer on yields and quality parameters of clonal tea. *Journal of Agriculture, Food and Environment*, 8(2), 682-690.
- Owuor, P. O., Kamau, D. M., Kamunya, S. M., Msomba, S. W., Jondiko, E. O., & Uwimana, M. A. (2013a). The response of clone BBK 35 tea to nitrogen fertilizer rates and harvesting intervals in the Lake Victoria Basin of Kenya. *Journal of Agriculture, Food and Environment*, 11(3&4), 757-763.
- Owuor, P. O., Kamau, D. M., Kamunya, S. M., Msomba, S. W., Jondiko, E. O., & Uwimana, M. A. (2013b). The response of clone BBK 35 tea to nitrogen fertilizer rates and harvesting intervals in the Lake Victoria Basin of Kenya. *Journal of Agriculture, Food and Environment*, 11(3&4), 757-763.

- Owuor, P. O., Kamau, D. M., Kamunya, S. M., Msomba, S. W., Uwimana, M. A., Okal, A. W., & Kwach, B. O. (2011). Effects of genotype, environment and management on yields and quality of black tea. In: E. Lichtfouse, *Genetics, Biofuels and Local Farming Systems: Sustainable Agriculture Reviews*, vol. 7 (pp. 277-307). Heidelberg: Springer.
- Owuor, P. O., Munavu, R. M., & Muritu, J. W. (1990a). Changes in fatty acid levels of young shoots of tea (*Camellia sinensis* L.) due to nitrogenous fertilizers. *Food Chemistry*, 38(3), 211-219.
- Owuor, P. O., Ng'etich, W. K., & Obanda, M. (2000). Quality response of clonal black tea to nitrogen fertilisers, plucking intervals and standards. *Journal of the Science of Food and Agriculture*, 80, 439-446.
- Owuor, P. O., Obaga, S. O., & Othieno, C. O. (1990b). Effects of altitude on the chemical composition of black tea. *Journal of the Science of Food and Agriculture*, 50, 9-17.
- Owuor, P. O., Obanda, A. M., Othieno, C. O., Horita, H., Tsushida, T., & Murai, T. (1987b). Changes in chemical composition and quality of black tea due to plucking standards. *Agricultural and Biological Chemistry*, 51, 3383-3384.
- Owuor, P. O., Obanda, M., Nyirenda, H. E., & Mandala, W. L. (2008a). Influence of region of production on clonal black tea chemical characteristics. *Food Chemistry*, 108, 263-271.
- Owuor, P. O., Obanda, M., Nyirenda, H. E., Mphangwe, N. I. K., Wright, L. P., & Apostolides, Z. (2006). The relationship between some chemical parameters and sensory evaluations for plain black tea (*Camellia sinensis*) produced in Kenya and comparison with similar teas from Malawi and South Africa. *Food Chemistry*, 97, 644-653.
- Owuor, P. O., & Obanda, M. A. (1996a). The impact of withering temperature on black tea quality. *Journal of the Science of Food and Agriculture*, 70, 288-292.

- Owuor, P. O., & Odhiambo, H. O. (1993). The response of quality and yield of black tea of two *Camellia sinensis* varieties to methods and intervals of harvesting. *Journal of the Science of Food and Agriculture*, *62*, 337-343.
- Owuor, P. O., Okal, A. W., Kamau, D. M., Msomba, S. W., Uwimana, M. A., & Kamunya, S. M. (2013c). Influence of nitrogen fertilizer rates and harvesting intervals on clonal tea green leaf fatty acids levels in the Lake Victoria Basin of Kenya. *Journal of Agriculture, Food and Environment*, *11*(3&4), 667- 674.
- Owuor, P. O., & Orchard, J. E. (1990c). Variations of the chemical composition of clonal black tea (*Camellia sinensis*) due to delayed withering. *Journal of the Science of Food and Agriculture*, *52*, 55-61.
- Owuor, P. O., Orchard, J. E., & McDowell, I. J. (1994). Changes in the quality parameters of clonal tea due to fermentation time. *Journal of the Science of Food and Agriculture*, *64*, 319-326.
- Owuor, P. O., & Othieno, C. O. (1996b). Optimising nitrogen fertiliser application rates to different tea cultivars. *Tropical Science*, *36*, 211-223.
- Owuor, P. O., Othieno, C. O., Horita, H., & Tsushida, T. (1987c). Effects nitrogenous fertilizers on the chemical composition of Kenyan black tea. *Agricultural and Biological Chemistry*, *51*, 2665-2670.
- Owuor, P. O., Othieno, C. O., Horita, H., Tsushida, T., & Murai, T. (1987d). Effects of nitrogenous fertilizers on the chemical composition of CTC black tea. *Agricultural, Biological and Chemistry*, *51*(10), 2665-2670.

- Owuor, P. O., Othieno, C. O., Kamau, D. M., Wanyoko, J. K., & Ng'etich, W. K. (2008b). Long term fertilizer use on high yielding clone S15/10: Tea yields. *International Journal of Tea Science*, 7, 19-31.
- Owuor, P. O., Othieno, C. O., Odhiambo, H. O., & Ng'etich, W. K. (1997). Effect of fertiliser levels and plucking intervals of clonal tea *Camellia sinensis* L. O. Kuntze. *Tropical Agriculture, (Trinidad)*, 74, 184-191.
- Owuor, P. O., Othieno, C. O., Robinson, J. M., & Baker, D. M. (1991). Response of tea quality parameters to time of the year and nitrogen fertilisers. *Journal of the Science of Food and Agriculture*, 55, 1-11.
- Owuor, P. O., Takeo, T., Tsushida, T., Horita, H., & Murai, T. (1987e). Differentiation of clonal teas by terpene index. *Journal of the Science of Food and Agriculture*, 40, 341-345.
- Owuor, P. O., Tsushida, T., Horita, H., & Murai, T. (1988). Effects of geographical area of production on the composition of volatile flavour compounds in Kenyan clonal black CTC teas. *Experimental Agriculture*, 24, 227-235.
- Owuor, P. O., Wachira, F. N., & Ng'etich, W. K. (2010b). Influence of region of production on relative clonal plain tea quality parameters in Kenya. *Food Chemistry*, 119, 1168-1174.
- Owuor, P. O., & Wanyoko, J. K. (1996c). Rationalization of nitrogen fertilizer use in tea production. *Tea*, 17, 53-59.
- Owuor, P. O., Wanyoko, J. K., & Othieno, C. O. (1990d). High rates of nitrogen on tea: I. Response and distribution of yield of clonal tea. *Tea*, 11, 78-89.
- Pare, P. W., & Tumlinson, J. H. (1997). De novo biosynthesis of volatiles induced by insect herbivory in cotton plants. *Plant Physiology*, 114, 1161-1167.

- Pare, P. W., & Tumlinson, T. H. (1999). Plant volatiles as defense against insect herbivores. *Plant Physiology*, *121*, 325-311.
- Paulraj, M. G., Reegan, A. D., & Ignacimuthu, S. (2011). Toxicity of benzaldehyde and propionic acid against immature and adult stages of *Aedes aegypti* (Linn.) and *Culex quinquefasciatus* (Say) (Diptera: Culicidae). *Journal of Entomology*, *8*, 539-547.
- Penaflor, M. F. G. V. M., Erb, L. A., Miranda, A. G., Werneburg, J. M. S., & Bent, J. (2011). Herbivore-induced plant volatiles can serve as host location cues for a generalist and a specialist egg parasitoid. *Journal of Chemical Ecology*, *37*(12), 1304–1313.
- Penuelas, J., & Munne-Bosch, S. (2005). Isoprenoids: an evolutionary pool for photoprotection. *Trends in Plant Sciences*, *10*, 166–169.
- Ramakrishna, A., & Ravishankar, G. A. (2011). Influence of abiotic stress signals on secondary metabolites in plants. *Plant Signaling and Behavior*, *6*, 1720–1731.
- Rawat, R., & Gulati, A. (2008). Seasonal and clonal variations in some major glycosidic bound volatiles in Kangra tea (*Camellia sinensis* (L.) O. Kuntze). *European Food Research and Technology*, *226*, 1241–1249.
- Reddy, G. V., & Guenero, A. (2004). Interactions of insect pheromones and plant semiochemicals. *Trends in Plant Sciences*, *9*(5), 253-261.
- Robinson, J. M., & Owuor, P. O. (1992). Tea aroma. In: K. C. Willson, & M. N. Clifford, *Tea: Cultivation to Consumption* (pp. 603-647). London: Chapman and Hall.
- Rodriguez-Saona, C., Vorsa, N., Singh, A. P., Johnson-Cicalese, J., Szendrei, Z., Mescher, M. C., & Frost, C. J. (2011). Tracing history of plants traits under domestication in cranberries: Potential consequences on anti-herbivore defences. *Journal of Experimental Botany*, *62*(8), 2633-2644.

- Roy, S., Muraleedharan, N., & Mukhopadhyay, A. (2014). The red spider mite, *Oligonychus coffeae* (Acari: Tetranychidae): its status, biology, ecology and management in tea plantations. *Experimental and Applied Acarology*, *63*, 431-463.
- Russell, G. B., Hunt, M. B., Bowers, W. S., & Blunt, J. W. (1994). A sesquiterpenoid and repellent from *Dysoxylum spectabile*. *Phytochemistry*, *35*(6), 1455-1456.
- Ruto, J. K., Wanyoko, J. K., & Othieno, C. O. (1994). Economic analysis of seedling tea response to nitrogen fertilizers in west of Rift Valley region: Nandi Hills. *Tea*, *15*, 94-98.
- Saad, K. A., Roff, M. N. M., Shukri, M. A. M., Mirad, R., Mansour, S. A. A., Abuzid, I., Mohd, Y., Hanifah, M., & Idris, A. B. (2014). Artificial damage induction in the leaves of chilli plants leads to the release of volatiles that alter the host plant selection behaviour of *Bemisia tabaci* (Hemiptera: Aleyrodidae). *Journal of Entomology*, *11*, 273-282.
- Saad, K. A., Roff, M.N.M., Hallett, R.H., Idris, A.B. (2015). Aphid-induced defences in Chilli affect preferences of the whitefly, *Bemisia tabaci* (Hemiptera: Aleyrodidae). *Scientific Reports*, 13697.
- Sandrine, P., Gouinguene, T., & Turlings, C. J. (2002). The effects of abiotic factors on induced volatile emissions in corn plants. *Plant Physiology*, *129*, 1296-1307.
- Sanjaya, Y., Virginia, R., Ocampo, V. R., Barbara, L., & Caoili, B. L. (2013). Selection of entomopathogenic fungi against the red spider mite *Tetranychus kanzawai* (Kishida) (Tetranychidae: Acarina). *Arthropods*, *2*, 208-215.
- Sarwar, S., Ahmad, F., Hamid, F. S., Khan, B. M., & Khurshid, F. (2007). Effect of different nitrogenous fertilizers on the growth and yield of three years old tea (*Camellia sinensis*) plants. *Sarhad Journal of Agriculture*, *23*, 907-910.

- Scala, A., Allmann, S., Mirabella, R., Haring, M. A., & Schuurink, R. C. (2013). Green leaf volatiles: A plant's multifunctional weapon against herbivores and pathogens. *International Journal of Molecular Sciences*, *14*(9), 17781-17811.
- Shamoda, T. (2010). A key volatile infochemical that elicits a strong response of predatory mite *Neoseiulus californicus*, an important natural enemy of two spotted spider mite *Tetranychus urticae*. *Experimental Applied Acarology*, *50*, 9-22.
- Shen, J., Tieman, D., Jeffrey, B., Mark, J., Taylor, G., Schmelz, E., Huffaker, A., Bies, D., Chen, K., Harry, J., & Klee, A. (2014). 13-Lipoxygenase, Tomlox C, is essential for synthesis of C<sub>5</sub> flavour volatiles in tomato. *Journal of Experimental Botany*, *65*, 419-428.
- Shieh, J. C., & Sumimoto, M. (1992). Identification of the volatile components in the leaves and wood of *Cunninghamia lanceolata*. *Journal of Faculty of Agriculture, Kyushu University*, *36*, 301-310.
- Silva, F. A. C., O-Panizzi, M. C. C., Blassioli-Moraes, M. C., & Panizzi, A. R. (2013). Influence of volatile and nonvolatile secondary metabolites from soybean pods on feeding and on oviposition behavior of *Urchistus heros* (Hemiptera: Heteroptera: *Pentatomidae*). *Environmental Entomology*, *42*(6), 1375-1382.
- Simmons, A. M., Gurr, G., & Trichomes, M. (2005). Trichomes of *lycopersicon* species and their hybrids: Effects on pests and natural enemies. *Agricultural and Forest Entomology*, *7*, 265-276.
- Singh, B., & Sharma, R. A. (2015). Plant terpenes: Defense responses, phylogenetic analysis, regulation and clinical applications. *Biotechnology*, *5*, 129-151.

- Sofo, A., Dichio, B., Xiloyannis, C., & Massia, A. (2004). Lipoxygenase activity and proline accumulation in leaves and roots of olive trees in response to drought stress. *Physiologia Plantarum*, *121*, 58-65.
- Squire, G. R. (1978). Stomatal behavior of tea in relation to environment. *Journal of Applied Ecology*, *15*, 287-301.
- Squire, G. R. (1979). Weather, physiology and seasonality of tea (*Camellia sinensis*) yields in Malawi. *Experimental Agriculture*, *15*, 321-330.
- Squire, G. R., Obaga, S. M. O., & Othieno, C. O. (1993). Altitude, temperature and shoot production of tea in the Kenyan Highlands. *Experimental Agriculture*, *29*, 107-120.
- Staudt, M., El-aouni, B. J. H., Buatois, B., Lacroze, J. P., Poessel, J. L., Sauge, M. H., & Niinemets, U. (2010). Volatile organic compound emissions induced by the aphid *Myzus persicae* differ among resistant and susceptible peach cultivars and a wild relative. *Tree Physiology*, *30*(10), 1320-1334.
- Stephens, W., Othieno, C. O., & Carr, M. K. V. (1992). Climate and weather variability at the Tea Research Foundation of Kenya. *Agriculture and Forest Meteorology*, *61*, 219-235.
- Stranden, M., Liblikas, I., Konig, W. A., Borg-Karlson, A. K., & Mustaparta, H. (2003). (-)-Germacrene D receptor neurones in three species of heliothine moths: Structure reactivity relationship. *Journal of Comparative Physiology A*, *189*(7), 563-577.
- Sudoj, V. (1989). Effects of nitrogen fertilizers on yield and incidence of mites attacking tea in Kenya: Preliminary indications. *Tea*, *10*, 187-191.
- Sudoj, V. (1991). Effect of nitrogen rates and frequency of application on yield and incidence of red crevice mite *B. phoenicis*. *Tea Research Foundation of Kenya Annual Report*, pp 195-197. . Kericho, Kenya: Tea Board of Kenya.



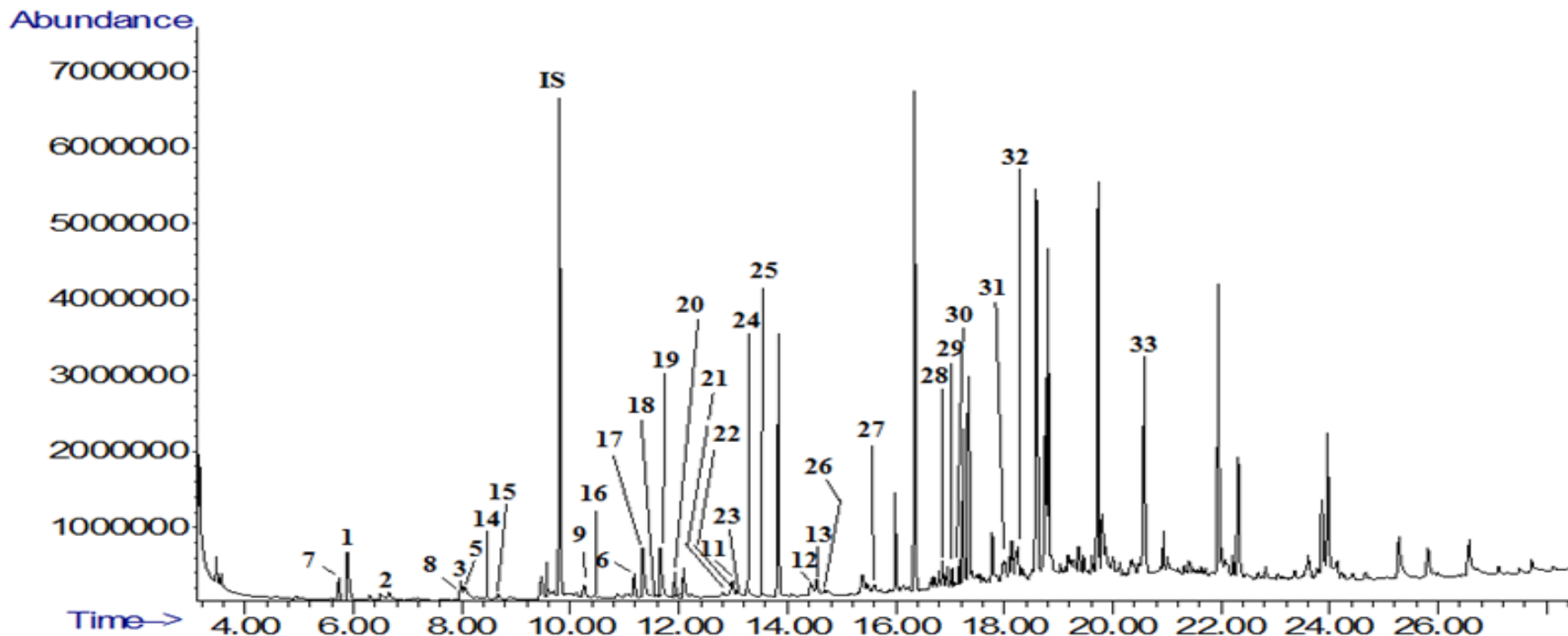
- Sudoj, V. (1993). Effect of rates of NPKS on incidence of red spider mite (*Oligonychus coffeae*) at Timbilil Estate., *Tea Research Foundation of Kenya Annual Report*. (pp. 169-172). Kericho, Kenya: Tea Board of Kenya.
- Sudoj, V. (1997). Tea pests with special reference to mites: Research achievements and future thrusts. *Tea*, 18, 156-165.
- Sudoj, V., Cheramgoi, E., Langat, J. K., & Wachira, F. N. (2011). Screening of Kenyan clones for susceptibility to mite at different ecological zones. *International Journal of Current Research*, 33, 328-332.
- Sudoj, V., Khaemba, B. M., & Wanjala, F. M. E. (1994). Screening of Kenyan tea clones for their resistance to *Brevipalpus phoenicis* Geijskes (Acari: *Tenuipalpidae*) attack. *Tea*, 15, 105-109.
- Sudoj, V., Khaemba, B. M., & Wanjala, F. M. E. (1996). Influence of soil applied nitrogen (NPKS 25: 5: 5:5) on *Brevipalpus phoenicis* Geijskes (Acari: *Tenuipalpidae*) mite incidence and damage symptoms on tea. *Annals of Applied Biology*, 128, 13–19.
- Sudoj, V., Khaemba, B. M., & Wanjala, F. M. E. (2001a). In search of factors responsible for resistance in tea clones to red spider mite *Oligonychus coffeae* Nietner (Acari: *Tetranychidae*) and possibility of their future use in screening programme. *International Journal of Pest Management*, 47, 207-210.
- Sudoj, V., Khaemba, B.M., Wanjala, F.M.E. (1996). Screening of Kenyan tea clones for their resistance to *Brevipalpus phoenicis* Geijkes (Acari: *Tenuipalpidae*) attack. *Journal of Plantation Crops*, 24, 291-295.

- Sudoj, V., Wanyoko, J. K., Owuor, P. O., & Lang'at, J. K. (2001b). Prospects for NPKS 25:5:5:5 fertilizer as a component of *Brevipalpus phoenicis* Geiskes (Acari: *Tenuipalpidae*) mite pest management. *Tea*, 22, 13-19.
- Sun, X. L., Wang, G. C., Cai, X. M., Jin, S., Gao, Y., & Chen, Z. M. (2010). The tea weevil, *Myllocerinus aurolineatus*, is attracted to volatiles induced by conspecifics. *Journal of Chemical Ecology*, 36, 388–395.
- Takeo, T. (1981). Variation in amounts of linalool and geraniol produced in tea shoots by mechanical injury. *Phytochemistry*, 20, 2149-2151.
- Takeo, T. (1983). Effect of clonal specificity of monoterpene alcohol composition of tea shoots on black tea aroma profile. *Japan Agricultural Research Quarterly*, 17, 120-124.
- Takeo, T. (1984). Effect of withering on volatile compounds formation during black tea manufacture. *Journal of the Science of Food and Agriculture*, 35, 84-87.
- Tholl, D., Sohrabi, R., Huh, J. H., & Lee, S. (2011). The biochemistry of homoterpenes--common constituents of floral and herbivore-induced plant volatile bouquets. *Phytochemistry*, 72, 1635-1646.
- Tura, D., Failla, O., Bassi, D., Attilio, C., & Serraiocco, A. (2013). Regional and cultivar comparison of Italian single cultivar olive oils according to flavor profiling. *European Journal of Lipid Science and Technology*, 115, 196–210.
- Unsicker, S. B., Kunert, G., & Gershenzon, J. (2009). Protective perfumes: the role of vegetative volatiles in plant defense against herbivores. *Current Opinion in Plant Biology*, 2, 479–485.
- Vancanneyt, T., Sanz, C., Farmaki, T., Paneque, M., Ortego, F., Castanera, P., & Sanchez-Serrano, J. J. (2001). Hydroperoxide lyase depletion in transgenic potato plants lead to an

- increase in aphid performance. *Proceedings of the National Academy of Sciences of the United States of America*, 98(14), 8139-8144.
- Veromann, E., Toome, M., Kannaste, A., Kaasik, R., Copolovici, L., Flink, J., Gabriella Kovacs, G., Narits, L., Luik, A., & Niinemets, U. (2013). Effects of nitrogen fertilization on insect pests, their parasitoids, plant diseases and volatile organic compounds in *Brassica napus*. *Crop protection*, 43, 79-88.
- Visser, J., & Avé, D. (1978). General green leaf volatiles in the olfactory orientation of the Colorado beetle, *Leptinotarsa decemlineata*. *Entomologia Experimentalis et Applicata*, 24, 738-749.
- Wachira, F. N., Nge'tich, W. K., Omolo, J., & Mamati, G. E. (2002). Genotype X environment interactions for tea yields. *Euphytica*, 127, 289-296.
- Wang, L. Y., Wei, K. J., Jiang, Y. N. H., Cheng, J., & Zhou, W. (2011). Seasonal climate effects on flavanol and purine alkaloids in tea *Camellia sinensis* L. *European Food Research and Technology*, 233, 1049-1055.
- Wang, Y. T., Huang, S. W., Liu, R. L., & Jin, J. Y. (2007). Effects of nitrogen application on flavor compounds of cherry tomato fruits. *Journal of Plant Nutrition and Soil Science*, 170, 461-468.
- Wason, E. L., Agrawal, A. A., & Hunter, A. M. D. (2013). Genetically-based latitudinal cline in the emission of herbivore-induced plant volatile organic compounds. *Journal of Chemical Ecology*, 39, 1101-1111.
- Wee, S. L., El-Sayed, A. M., Gibb, A. R., Mitchell, V., & Suckling, D. M. (2008). Behavioural and electrophysiological responses of *Pantomorus cervinus* (Boheman) (Coleoptera: Curculionidae) to host plant volatiles. *Australian Journal of Entomology*, 47, 24-31.

- Wenda-Piesik, A. (2011). Volatile organic compound emissions by winter wheat plants (*Triticum aestivum* L.) under *Fusarium spp.* infestation and various abiotic conditions. *Poland Journal of Environmental studies*, 20, 1335-1342.
- Yang, N. W., Li, A. L., Wan, F. H., Liu, W. X., & Johnson, D. (2010). Effects of plant essential oils on immature and adult sweet potato whitefly, *Bemisia tabaci* biotype B. *Crop protection*, 29, 1200–1207.
- Yatagai, M., Makihara, H., & Oba, K. (2002). Volatile components of Japanese cedar cultivars as repellents related to resistance to *Cryptomeria* bark borer. *Journal of Wood Science*, 48, 51-55.
- Yuan, J. S., Himanen, S. J., Holopainen, J. K., Chen, F., & Stewart, C. N. (2009). Smelling global climate change: mitigation of function for plant volatile organic compounds. *Trends in Ecology and Evolution*, 24, 323-331.
- Zapata, N., & Smagghe, G. (2010). Repellency and toxicity of essential oils from the leaves and bark of *Laurelias empervirens* and *Drimys winteri* against *Tribolium castaneum*. *Industrial Crops and Products*, 32(3), 405-410.
- Zeiss, M. R., & Braber, K. (2001). *Tea Integrated Pest Management Ecological Guide*. Ha Noi, Viet Nam: So 6 Duong so 4, Khu A.
- Zhang, Y. R., Wang, R., Lin, F. Y., Lu, P. F., & Luo, Y. Q. (2015). Identification of Caragana plant volatiles, overlapping profiles, and olfactory attraction to *Chlorophorus caragana* in the laboratory. *Journal of Plant Interactions*, 10, 41–50.
- Zhu, J., & Park, K. C. (2005). Methyl salicylate, a soybean aphid induced plant volatile to the predator *Coccinella septempunctata*. *Journal of Chemical Ecology*, 31(8), 1733-1746.

**APPENDIX I : TYPICAL GAS CHROMATORGAM FOR OVOCS FROM *Camellia sinensis***



3.4004 = 1-penten-3-ol; 5.419 = Phenyl ethyl alcohol; 7 = *Z*-2-pentenol; 1 = Hexanal; 2 = Nonanal; 8 = *E*-2-hexenal; 3 = Ethyl benzene; 5 = *Z*-3-hexenal; 6 =  $\alpha$ -phellandrene; 9.188 = Heptanal; IS = Cumene (Internal Standard); 9 = Myrcene; 17 = *Z*-3-hexenyl acetate; 18 = Benzaldehyde; 19 = *E*- $\beta$ -ocimene; 20 = *Z*- $\beta$ -ocimene; 12.494 = Acetophenone; 21 = Linalool; 23 = 4,8-dimethyl-1,3(*E*),7-nontriene; 13.994 = Naphthalene; 14.285 = Terpinen-4-ol; 12 =  $\alpha$ -methyl styrene; 13 = *Z*-anethole; 26 = Methyl salicylate; 15.921 = *Z*-jasmone; 30 = *E*- $\beta$ -caryophyllene; 18.533 = *E*- $\beta$ -farnesene ; 17.52 =  $\alpha$ -cedrene; 31 =  $\beta$ -cedrene; 32 = Humulene; 18.944 = Germacrene D; 19.432 = Nerolidol; 19.638 = Longicamphenylone; 19.733 = Cedrol; 33= Benzophenone.

NB. Some peaks were not assigned numbers on the chromatogram. Their retention times indicate the position.

**APPENDIX II:** Compounds identified, their retention time (RT) and IUPAC names

SN	COMPOUND	RT	IUPAC
1	1-penten-3-ol ( <b>16</b> )	3.400	1-penten-3-ol
2	Phenyl ethyl alcohol ( <b>35</b> )	5.419	2-Phenylethanol
3	Z-2-pentenol ( <b>17</b> )	5.752	Z-2-penten-1-ol
4	Hexanal ( <b>18</b> )	5.887	Hexanal
5	Nonanal ( <b>19</b> )	6.626	Nonanal
6	E-2-hexenal ( <b>7</b> )	7.992	E-Hex-2-enal
7	Ethyl benzene ( <b>37</b> )	8.010	Ethylbenzene
8	Z-3-hexenal ( <b>6</b> )	8.050	Z-hex-3-enal
9	Z-3-hexenol ( <b>9</b> )	8.059	Z-hex-3-en-1-ol
10	P-xylene ( <b>41</b> )	8.305	1,4-dimethylbenzene
11	Indole ( <b>33</b> )	8.460	2, 3-Benzopyrrole
12	O-xylene ( <b>42</b> )	8.888	1,2-dimethylbenzene
13	Heptanal ( <b>18</b> )	9.188	Heptanal
14	Cumene ( <b>112</b> )	9.813	(1-methylethyl)-benzene
15	$\alpha$ -pinene ( <b>81</b> )	9.829	(1S,5S)-2,6,6-trimethylbicyclo[3.1.1]hept-2-ene
16	Myrcene ( <b>72</b> )	10.282	7-methyl-3-methylene-1,6-octadiene
17	$\alpha$ -phellandrene ( <b>78</b> )	11.284	2-methyl-5-(1-methylethyl)-1,3-cyclohexadiene
18	Z-3-hexenyl acetate ( <b>12</b> )	11.329	3-Z-hex-3-en-1-yl acetate
19	Benzaldehyde ( <b>30</b> )	11.374	Phenylmethanal
20	Phenyl acetaldehyde ( <b>34</b> )	11.461	2-phenylacetaldehyde
21	Geraniol ( <b>65</b> )	11.486	3,7-dimethyl-2,6-octadien-1-ol
22	Sabinene ( <b>85</b> )	11.525	4-methylene-1-(methylethyl)bicyclo[3.1.0]hexane
23	O-cymene ( <b>88</b> )	11.645	1-methyl-4-(propan-2-yl)benzene
24	P-cymene ( <b>87</b> )	11.675	1-methyl-4-(1-methylethyl)benzene
25	$\beta$ -phellandrene ( <b>79</b> )	11.730	3-methylene-6-(1-methylethyl)cyclohexene
26	Limonene ( <b>76</b> )	11.744	1-methyl-4-(1-methylethenyl)-cyclohexene
27	Phenol ( <b>49</b> )	11.846	Phenol
28	Z- $\beta$ -ocimene ( <b>74</b> )	11.910	$\beta$ : <i>cis</i> -3,7-dimethyl-1,3,7-octatriene
29	Benzyl alcohol ( <b>36</b> )	11.934	Phenylmethanol
30	Indene ( <b>43</b> )	12.068	Bicyclo[4.3.0]nona-1,3,5,7-tetraene
31	E- $\beta$ -ocimene ( <b>73</b> )	12.098	$\beta$ : <i>trans</i> -3,7-dimethyl-1,3,6-octatriene
32	Acetophenone ( <b>46</b> )	12.494	1-phenylethanone.
33	Linalool oxide ( <i>cis</i> ) furanoid ( <b>68</b> )	12.800	2-[(2R,5S)-5-ethenyl-5-methyloxolan-2-yl]propan-2-ol
34	Linalool oxide ( <i>trans</i> ) furanoid ( <b>67</b> )	12.821	2-[(2S,5S)-5-ethenyl-5-methyloxolan-2-yl]propan-2-ol
35	Linalool ( <b>66</b> )	12.986	3,7-dimethylocta-1,6-dien-3-ol
36	4,8-dimethyl-1,3(E),7-nonatriene (DMNT) ( <b>89</b> )	13.101	(3E)-4,8-dimethyl-1,3,7-nonatriene
37	P-mentha-1,3,8-triene ( <b>86</b> )	13.278	1-methyl-4-(prop-1-en-2-yl)cyclohexa-1,3-diene
38	Naphthalene ( <b>44</b> )	13.994	Bicyclo[4.4.0]deca-1,3,5,7,9-pentene
39	Diphenyl ether ( <b>48</b> )	14.209	Diphenyl ether
40	Terpinen-4-ol ( <b>83</b> )	14.285	4-isopropyl-1-methyl-1-cyclohexen-4-ol
41	$\alpha$ -methyl styrene ( <b>47</b> )	14.493	1-methyl-1-phenylethylene
42	Azulene ( <b>45</b> )	14.622	Prop-1-en-2-ylbenzene

APPENDIX: II cont...

SN	COMPOUND	RT	IUPAC
43	Z-anethole (38)	14.689	1-methoxy-4-[(Z)-prop-1-enyl]benzene
44	Methyl salicylate (32)	14.711	Methyl 2-hydroxybenzoate
45	Benzothiazole (39)	15.136	1,3-benzothiazole
46	Z-jasmone (15)	15.921	3-methyl-2-[(2Z)-pent-2-en-1-yl]cyclopent-2-en-1-one
47	$\alpha$ -copaene (106)	17.184	(1R,2S,6S,7S,8S)-8-isopropyl-1,3-dimethyltricyclo[4.4.0.0]dec-3-ene
48	E- $\beta$ -caryophyllene (94)	17.191	4,11,11-trimethyl-8-methylethylene-bicyclo[7.2.0]undec-4-ene
49	Longifolene (101)	17.623	(1R,2S,7S,9S)-3,3,7-trimethyl-8-methylenetricyclo[5.4.0.0]undecane
50	Italicene (105)	17.735	2,11-cycloacor-3-ene
51	$\alpha$ -cedrene (107)	17.712	(1S,2R,5S,7R)-2,6,6,8-tetramethyltricyclo[5.3.1.0]undec-8-ene
52	E- $\beta$ -fernesene (96)	17.780	3,7,11-trimethyl-1,3,6,10-dodecatetraene
53	E- $\gamma$ -muurolene (102)	17.800	1S,4as,8aR)-1-isopropyl-7-methyl-4-methylene-1,2,3,4,4a,5,6,8a-octahydronaphthalene
54	$\beta$ -cedrene (108)	17.822	(1S,2R,5S,7R)-2,6,6-trimethyl-8-ethylidenetricyclo[5.3.1.0(1,5)]undecane
55	Humulene (100)	18.227	2,6,6,9-tetramethyl-1,4-8-cycloundecatriene
56	Germacrene D (95)	18.944	(s,1Z,6Z)-8-isopropyl-1-Methyl-5 methylenecyclodeca-1,6-diene
57	Calamenene (103)	19.056	(1S,4R)-1,6-dimethyl-4-propan-2-yl-1,2,3,4-tetrahydronaphthalene
58	Nerolidol (99)	19.432	(3S,6Z)-3,7,11-trimethyldodeca-1,6,10-trien-3-ol
59	Longicamphenylone (104)	19.638	3,3,7-trimethyltricyclo[5.4.0.0]undecan-8-one
60	Cedrol (109)	19.733	(1S,2R,5S,7R,8R)-2,6,6,8-tetramethyltricyclo[5.3.1.0]undecan-8-ol
61	Benzophenone (40)	20.486	Diphenylmethanone