PHYTOCHEMICAL ATTRACTANTS AND REPELLENTS FROM SELECTED KENYAN VARIETIES OF MANGO (*Mangifera indica* L.) AS CONTROL TOOLS FOR MANGO FRUIT FLY, *Bactrocera invadens* (DIPTERA: TEPHRITIDAE)

WETUNGU MARTIN WAMALWA

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DECLARATION

Declaration by the candidate

This thesis is my original work and has not been submitted for any academic award in any institution; and shall not be reproduced in part or full, or in any format without prior written permission from the author and/or University of Eldoret.

WETUNGU MARTIN WAMALWA
SC/D.PHIL/001/10

Signature: …… Date……

Declaration by the Supervisors

This thesis has been submitted with our approval as University supervisors:

PROF. PAUL KIPKOSGEI TARUS
Associate Professor of Organic Chemistry, Department of Chemistry and Biochemistry, University of Eldoret.

Signature: …… Date……

PROF. MAURICE VINCENT OMOLO
Associate Professor of Organic Chemistry, Department of Pure and Applied Chemistry, Masinde Muliro University of Sceince and Technology.

Signature: …… Date……

PROF. FRED K. SEGOR
Associate Professor, Department of Chemistry and Biochemistry,
University of Eldoret.

Signature: …… Date……

DEDICATION

I dedicate this thesis to my late parents Paul and Berita Wetungu both of whom were inspirational in my life

I also dedicate it to my wife Josephine Simiyu and all our children who have been my source of inspiration to work hard and finish this work.

ABSTRACT

Infestation of mangoes (*Mangifera indica* L.) by the mango fruit fly, *Bactocera invadens* (Drew, Tsuruta and White) is a big threat to mango production in Kenya. The pest infestation leads to poor quality fruits that cannot fetch good prices both locally and internationally. This study was carried conducted at Cheptebo in Kenya where observations in the mango orchard revealed variation in the infestation of the fruit fly among different mango varieties. The aim of this study was to identify the plant host chemical cues that possess the attractive or repellent effects to the fruit fly. The essential oils were extracted from six mango varieties: Ngowe, Apple, Keitt, Boribo, Tommy Atkins and Van Dyke by hydro distillation using a modified Clevenger-like apparatus. Volatiles from the six varieties together with Kent and Sabre were collected from the fruit juices on porapak Q adsorbent filters (mesh 40-80µm) using air entrainment kit. The oils and the volatiles were analyzed through gas chromatograph fitted with a flame ionization detector and coupled to a mass spectrometer. It was found that the chemical profile of all the mango varieties were qualitatively and quantitatively different. The oils were rich in monoterpenes and sesquiterpenes with minor quantities of their analogues. Trace amounts of nonterpenoid and oxygenated hydrocarbons were also identified. Among the identified compounds, δ-3-carene was most dominant in the leaf mango oils of Keitt, Tommy Atkins and Van Dyke. Five compounds namely; α-pinene, β-pinene, α-copaene, α-gurjunene and α-humulene were present in significant but varying amounts in all the oils of the six varieties. The aroma components of the eight mango volatiles were classified on the basis of their richness in α-pinene, myrcene and δ-3-carene as the dominant compounds. The compounds that were selected for bioassay were those that were major both in the oil and the volatile profiles. The fruit flies were subjected to crude essential oils, fruit juices and blends of chemical standards comprising esters (ethylbutanoate, ethyl hexanoate ethyloctanoate and methyl salicylate), nonoxygenated monoterpenes (myrcene, δ-3-carene and α-pinene) as lures in both Laboratory Dual choice olfactometric and field bioassays. The bioassay of the individual compounds was also done in the field. Methyl eugenol was used as positive control. There was no significant difference ($P>0.05$) in the mean number of flies (4.75±0.25) lured by the blend of myrcene, α-pinene, and ethyl butanoate and the positive control (5.25±0.25) of methyl eugenol in the laboratory test. In the field bioassay there was no significant difference ($P>0.05$) in the attractiveness of Keitt juice volatiles (mean catch of 4.571±1.445) and the positive control (mean catch of 5.857±1.724) of fruit flies. In terms of individual components, Boribo oil, fruit juices and ethyl butanoate exhibited attractive effects while Ngowe oil and methyl salicylate showed repellent effects. The individual pure compounds were not attractive to the fruit fly in the field bioassay.This study shows that mango contains volatile compounds that can be exploited as natural, environmentally friendly lures for the fruit fly and the compounds can only be effective when used as a blend.

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LIST OF ABBREVIATIONS, ACRONYMS, AND SYMBOLS

CUE	Cuelure
EAD	Electroantennographic Detector
EAG	Electroantennography
EI	Electron Impact
EU	European Union
FAO	Food and Agricultural Organization
FAOSTAT	Food and Agriculture Organization of the UN Statistical Database
FPEAK	Fresh Produce Exporters Association of Kenya
GC-EAD	Gas Chromatography-Electroantennographic Detection
GC-FID	Gas Chromatography-Flame Ionization Detector
GC-MS	Gas Chromatography-Mass Spectrometry
HCDA	Horticultural Crops Development Authority
HD	Hydrodistillation.
HS-SPME	Headspace Solid Phase Microextraction
ICIPE	International Centre for Insect Physiology and Ecology
IPM	Integrated Pest Management
ISPM	International Standards for Phytosanitary Measures
LSD	Least Significant Difference
ME	Methyl Eugenol
MT	Metric Tons
NIST/NBS	National Institute of Standards and Technology /National Bureau of Standards
ORNs	Olfactory Receptor Neurones
RI	Retention Indices
SE	Standard Error
SPME	Solid-Phase Microextraction
STDF	Standards and Trade Development Facility
TML	Trimedlure
WHO	World Health Organization

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cooperation and assistance right from the field survey, sample collection and setting up of the traps for field bioassays. His willingness to assist me in identification of the mango varieties, providing me with the samples and allowing me to carry out field bioassay on his farm played a key role in the overall success of this research.

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CHAPTER ONE

INTRODUCTION

1.1 Background information

Mango (*Mangifera indica* L) is one of the most popular tropical fruits widely grown in Kenya. It belongs to genus *Mangifera*, in the family of Anacardiaceae (Kostermans and Bompard, 2013). *Mangifera indica* is native to India and Southeast Asia (Singh *et al*., 2016) but it is also produced in almost every tropical country, and even some subtropical regions such as Florida, Egypt and Southern Latin America (Sandoval *et al*., 2007).

Asian continent is the largest global producer of mango. Seven years ago, this continent accounted for approximately 76.49 % of the global mango production while America and Africa accounted for approximately 12.62 % and 10.77%, respectively (FAOSTAT, 2017).

Mango is the second most important fruit crop in Kenya after bananas in terms of production volumes (USAID-KAVES, 2014). In 2012, the estimated annual mango production was 781,706 MT, with a value of KSh13.5 billion (HCDA, 2012). It is also of growing importance as an export crop, accounting for 15.6 percent of the total value of fruit exports and 3.6 percent of the total value of horticulture exports in 2012. Mango export earnings increased from KSh 623 million (US$7.2 million) in 2009 to KSh1 billion (US$11.8 million) in 2011.

Africa's mango production is considered to be below its potential as a result of the ever increasing production costs and the reduction of the quality and quantity of marketable produce due to fruit flies (Sebstad and Snodgrass, 2005). Fruit flies constitute one of the major threats to horticultural production, causing substantial produce losses in East, Central and West Africa (White and Elson-Harris, 1992; ICIPE, 2008; Van Melle *et al*., 2007).

Sub-Saharan Africa is home to about 915 fruit fly species from 148 genera, developing in either wild or cultivated hosts or in both (Ekesi, 2010). The commonest fruit fly

species in Kenya are *Ceratitis cosyra* and *Bactrocera invadens* (Ekesi *et al*., 2006). Their wide distribution, fast proliferation, significant populations, polyphytophagous nature (feeding on multiple host crops) and the difficulty to control them using insecticides cause momentous yield losses in fruit and vegetable crops (Baral *et al.*, 2006; Ndiaye *et al*., 2008).

Fruit flies threaten the production and marketability of fruits and vegetables by reducing their quantity and quality. This curtails the expansion of domestic and international trade for these crops, triggering huge economic losses that deprive producers of massive revenues. Fruit flies are easily transported across borders without being detected. This has made them acquire a worldwide quarantine insect pest status (STDF, 2010). Countries in the European Union (EU) importing fresh produce impose strict quarantine measures such that the detection of only one larva at the entry port of a destination country leads to interception, confiscation and destruction of the entire mango consignment and a possible outright ban for the exporting country.

These losses have been estimated to cause annual economic losses of more than USD 42 million in Africa and USD 1 billion worldwide (STDF, 2010). Most countries in Sub-Saharan Africa have been banned from exporting their mangoes to markets in the EU and the United States of America following detection of the larvae of fruit fly in mangoes (Lux *et al*., 2003; Ndiaye *et al*., 2008; Vayssieres *et al.*, 2009; STDF, 2010). In addition, the economic damage caused by fruit fly infestation in Africa worsened after South Africa banned imports of mangoes from Kenya (Rwomushana, 2008).

Female fruit flies puncture the periscarp and lay their eggs under the skin of mango fruit. Then, the eggs hatch into larvae which feed on the decaying flesh of the fruit. Infested fruits rot quickly causing considerable losses (Vayssieres *et al*., 2009). The control of this pest at the destructive larval stage is difficult because insecticides in form of dust or sprays cannot reach them. One of the ways to deal with them is to target adult flies before they start laying eggs by trapping them or using insecticides to control their populations. In the absence of natural enemies, fruit fly populations are a menace such that sometimes, damage is so sporadic and acute that all fruits in the orchard can be attacked simultaneously (Vayssieres *et al*., 2009).

The attraction of male *Bactrocera* flies to the parapheromone compound methyl eugenol is well known (Vargas *et al.,* 2000), and the compound is used for monitoring and male annihilation (Vargas *et al.*, 2010). Monitoring and more efficient management of *Bactrocera* flies will however require the development of a female-specific or at least a female-biased trapping system that complements the male lures in fruit fly management. Luring females can reduce damage directly caused by oviposition (Siderhurst and Jang, 2006).

Olfactory receptor systems in phytophagous insects enable the insects to perceive volatile plant compounds (in odours) that evaporate from the plant surface and then diffuse into the environment. The perceived odours constitute the chemical cue/message (Visser, 1986) and the insects can recognize individual molecular structures (Toby and Pickett, 2011). Through the chemical cue(s) the insects are able to: recognize their host plants for feeding and oviposition, avoid non-host plants/organisms, locate feeding/oviposition sites within the host, locate their sex partners/mates and copulate on host plants. Different responses can occur to a whole blend compared to individual components. Components of the host blend may not be recognized as host when they are perceived outside the context of that blend (Toby and Pickett, 2011).

Female fruit flies are attracted to blends of chemicals in odors emanating from leaves and fruits of trees. These chemicals may therefore be promising sources of attractants (Siderhurst and Jang, 2006). The blends to which fruit flies respond are complex and vary in composition during ripening (Alagarmalai *et al.,* 2009), making the isolation and identification of attractants tedious. Polyphagous fruit flies may however orient to different fruits by using odors that are shared by host fruits. In this study, the attractiveness of the leaf essential oils and fruit volatiles of the host mango varieties to *B. invadens* in an olfactometer and field bioassay was investigated to identify blends of the compounds that are behaviorally active as attractants or repellents of *B. invadens*.

1.2 Statement of the problem

Infestation of the mangoes by the mango fruit fly, *Bactrocera invadens,* is a big threat to mango production in Kenya in terms of loses not only in quality and quantity but also prices of the fruits (HCDA, 2008; Drew *et al*, 2005). The fruit flies can cause either

direct damage through oviposition in fruits and tissues of vegetative parts and feeding by the larvae or indirect damage through decomposition of plants tissues by invading microorganisms. (Koyama *et al*., 1984; Iwahashi *et al*., 1996). Mango exports are therefore declining despite the expansion in demand for the fresh fruits in Europe, Africa and Asia (HCDA, 2012). Consequently, domestic market is also not sufficiently supplied with the good quality and quantity of mangoes.

1.3 Rationale for the study

The loss caused by fruit fly alone has been estimated to be as high as 40%-50% of the production (Griesbach, 2003; Hasyim *et al*., 2007). Currently, mango growers mainly use synthetic pesticides for the control of insect pests of orchards. Management with these chemicals causes many problems such as pest resurgence, insect resistance to pesticide, secondary pest out-breaks and environmental pollution (Latif and Abdullah, 2005). These pesticides may also be toxic to non-target beneficial organisms such as the pollinator bees.

Consequently, there is an urgent need to search for more effective alternative control agents of the mango fruit fly that are species specific and environment friendly. This solution lies in nature, which has various semio-chemicals (repellents and attractants) that insects exploit in looking for suitable food, sex mates and good breeding sites. There is however, differential susceptibility among mango varieties to oriental fruit fly infestation (Jayanthi and Verghese, 2008). This fact has further been confirmed by physical assessment of various varieties of mangoes in a farm at Cheptebo in Kerio Valley. In this farm, Van dyke, Boribo and Ngowe varieties were least attacked by the fruit fly as compared to others like Apple, Keitt and Tommy Atkins.

1.4 Research question

Do mango aroma, essential oils and volatile chemicals have any role in the resistance or susceptibility of some mango varieties to the infestations by the *Bactocera invadens* mango fruit fly?

1.5 Hypotheses

1. The aroma, essential oils and volatile chemicals blend components of some mango varieties may be responsible for the refractive nature of these mangoes to the *B. invadens* fruit fly.
2. The susceptibility of some mango varieties to *B. invadens* fly may be due to some of the aroma, essential oils and volatile chemicals blend components from these mango varieties.

1.6 Objectives of the study

Main objective

To investigate and identify the chemical cues that are responsible for the susceptibility and refractiveness of some mango cultivars to the mango fruit fly, *B. invadens* and evaluate their potential as lures and repellents of the fruit fly for control of the insect pest.

Specific objectives

i) To identify the chemical constituents of the leaf essential oils and aroma volatiles of the ripe fruit pulp of mango cultivars used in the study.
ii) To determine the behavioural response of *Bactrocera invadens* mango fruit fly on essential oils and aroma volatiles of the mango cultivars used in the study.
iii) To identify the chemical blends of compounds responsible for the repellent and/or susceptibility (attractiveness) of some mango cultivars to attack by *B. invadens.*
iv) To identify the individual chemical components responsible for the repellent and/or susceptibility (attractiveness) of some mango cultivars to attack by *B. invadens.*

CHAPTER TWO

LITERATURE REVIEW

2.1 Origin of *Mangifera indica* L.

The genus *Mangifera* belongs to the order Sapindales in the family Anacardiaceae of mainly tropical species with 73 genera. The word "mango" originated as early as 16th century from the ancient Tamil word '*mangai*' (Singh *et al.,* 2016). Wild species of genus *Mangifera* are spread throughout South and South-East Asia. Paleocene mango leaf fossils which were discovered near Damalgiri, West Garo Hills, Meghalaya, point to the origin of the genus in peninsular of India before joining of the Indian and Asian continental plates (Singh *et al.,* 2016).

2.2 Mango Production in the world

World production of mango was estimated at 31 million tonnes in 2010, accounting for nearly 50% of the world tropical fruit production (Ambele *et al.,* 2012). Mangoes are produced in more than 90 countries worldwide with Asia as a continent accounting for approximately 77% of the world mango production (FAOSTAT, 2017). Fruit production provides essential components of diet/nourishment for the people of Asia, Central and South America and Africa and also acts as a source of income. For several tropical fruits, the production is mainly by small landholders and a large proportion is intended for the rapidly growing local urban market (Mwatawala *et al*., 2006). Mango is traded and consumed as fresh or in processed form. A number of mango products such as mango juice, mango pulp, mango flavour, mango kernel oil, mango pickles and powder have been well introduced and accepted in different market segments (Mwatawala *et al*., 2006).

2.3 Mango production in Kenya

Mango is cultivated in a variety of different agroecological zones across Kenya including not only sub-humid and semi-arid zones but also areas that are mainly unsuitable for other cash crops (Kehlenbeck *et al*., 2010). The main areas of mango production include the Eastern and Coast regions (responsible for 85 percent of national

mango production), followed by Central Region and other areas such as Nyanza, Rift Valley, North, and Western Region (HCDA, 2012). Kilifi County, accounts for the largest mango production in Kenya at 18% followed by Kwale (16%), Machakos (8%), Meru (8%), Makueni (8%), Embu (7%), Migori (5%), Bungoma (4%), Tana River (4%) and Lamu (4%) (HCDA, 2012). Additional growth comes from non-traditional production areas in North Eastern, Nyanza, Western and Rift Valley regions. Production is estimated to reach 878,000 MT in 2017 and 1,415,000 MT in 2022 (USAID-KAVES, 2014).

There are over 200,000 small-scale farmers that derive their livelihood from mango production. Many more benefit from the mango value chain in trading, transport, export and processing. Within the Eastern and Coast regions, more than 1.5 million new mango trees were established in the last five years, pointing to the growing importance of the fruit to small-scale farmers (USAID-KAVES, 2014). These farmers face challenges related to proper orchard management, access to quality planting material, pests and diseases, and market access. Mangoes are also driving the growing fruit processing industry for domestic and export markets. However, most of the mango processing firms operate at or near 40 percent capacity, due to a lack of raw material suitable for processing companies (USAID-KAVES, 2014).

2.4 Period of production

Mango is grown on a wide range of soils such as sandy soils at the Coast line as well as on loam, black cotton and also murram soils at other elevations. Mangoes mature at different times, depending on the variety, due to differences in climatic conditions. The tree itself is easy to grow and once well established, it is relatively tolerant to drought, occasional flooding and poor soil conditions (Griesbach, 2003). There are two supply seasons in the main production area of the Coast region. The first season runs from November to February and the second from June to August. In Central region, particularly Murang'a and Mwea, the peak of the harvest season is in February and March, the onset of which is 4-6 weeks later than at the Coast (Griesbach, 2003).

2.5 Mango Varieties

The mangoes grown in Kenya are generally classified as local and exotic or improved varieties. The exotic varieties, which are grown mainly for the export market, are usually grafted on local mangoes. The local varieties are Ngowe, Boribo, Batawi, Sabre and Dodo, while the improved varieties include Tommy Atkins, Kent, Van Dyke, Apple, Sabine, Sensation, Pafin, Maya, Kenston, Gesine and Haden. Both local and exotic varieties are grown in Eastern region of Kenya. Overall, the Counties with higher percentage of improved mango varieties are Kiambu (Thika), Embu, Tharaka Nithi (Mbeere), Meru (Meru Central, & Meru South), Makueni, Machakos and Kitui. Local varieties are predominant in Coastal region and these include Ngowe (70% production) with others like Boribo, Batawi and a few minor ones. The main exotic variety at the Coast is apple which is mainly grown in Lamu, Malindi and Kilifi (Griesbach, 2003).

The main varieties of mango grown in Kenya are Apple and Ngowe. Apple is mainly grown in Eastern region and contributes 50% of mango produced from this region. Ngowe variety is mainly grown at the Coast and comprises 49% of mango produced from this region. These two varieties account for 39% and 17% of national mango production respectively (HCDA, 2012). Apple is the variety of choice for export and fresh fruit domestic market because of its colour and aroma when ripe. The Ngowe variety is mainly used for processing due to its large size, high quality, high brix content resulting in high quality and quantity pulp (Griesbach, 2003).

Different mango varieties may have different amount and type of flavor compounds present depending on their origin. Quantitatively, the major volatile components may or may not be important contributors to the aroma of a fruit, while minor components may have high oduor characteristic/property. Thus many volatile components are not aromatic active at all (Mahattanatawee *et al.*, 2005).

2.5.1 Ngowe

Ngowe tree originated from Zanzibar and was planted in Lamu approximately 106 years ago (Griesbach, 2003), This cultivar, whis is also called Lamu mango, can now be found all along the coastline and has also adapted well to medium altitude locations (ITC, 2015). Ngowe is easily recognized as it is large, oblong and slender with a very

pronounced hook-like beak at the apex (Plate 2.1). The fruit develops from pale green to yellow to orange colour when ripe. It has deep yellow flesh which is almost free from fibre, melting, and carries no turpentine taste. The average length of the fruit is 14 cm with a width of 9.5 cm, and a weight range of 425-600 g. The seeds are polyembryonic which means progeny develops more or less true-to-type (Griesbach, 2003).

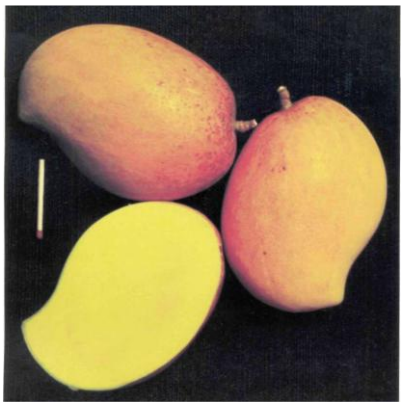


Plate 2. 1: Photo of Ngowe mango fruit (Source: Griesbach, 2003).

2.5.2 Apple

This cultivar originated from the Kenya coastline, most probably around the Malindi area. It is a chance seedling with unknown parentage (Griesbach, 2003). Apple has adapted to Coastal and lowland areas, and is very susceptible to rust in high altitude areas. Fruits are large, round and apple shaped, and has rich yellow-orange to red colour. They are fleshy, juicy, fibreless and with firm texture and matures early in the season between November and January. Apple weighs on average 379 g (ITC, 2015). The fruits are almost round in shape and have a yellow/orange to red colour when ripe (Plate 2.2). Average length measures 9.7 cm by 11 cm in width, and the weight is 280-580 g (mean: 397 g) (Griesbach, 2003).

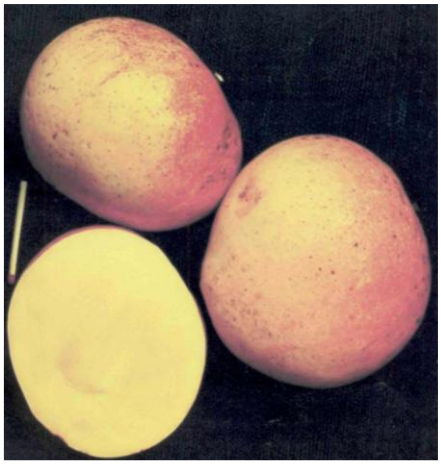


Plate 2. 2 : Photo of Apple mango fruit (Source: Griesbach, 2003).

2.5.3 Keitt

It is an open pollinated seedling which originated from Homestead (Florida) in 1946 (Griesbach, 2003). Keitt matures late and may be left on the trees long after the normal harvesting time. The fruit has an average length of 11.7 cm and a width of 9.2 cm. Its average weight is 456 g and has a greenish-yellow colour with pink or red blush and lavender bloom (Plate 2.3) with numerous white or yellow/red lenticels on the skin. The fruit is ovate in shape, plump with no beak and has a rounded base. It has deep yellow flesh which is fairly firm but tender, melting, juicy and with only a little fibre near a small (7.5% of fruit weight) monoembryonic seed. The fruit has a rich and sweet flavor with a pleasant aroma and excellent quality.The tree is medium-sized, moderately vigorous, producing long arching branches and has a scraggy open appearance (Griesbach, 2003).

Plate 2. 3 : Photo of Keitt mango fruit (Source: Griesbach, 2003).

2.5.4 Boribo

It originated from a chance seedling found at the Kenyan coast. The tree is extensively grown in the Malindi area. The fruit is larger than Ngowe and is oblong, only slightly curved with an obscure beak (Plate 2.4). The average fruit dimensions are: 11.5 cm long by 7.8 cm broad with a weight range of 430-640 g (mean: 511 g). The fruits are pale olive green with bloom and yellow apricot when ripe. The quality of the fruit is good to excellent with a flesh that is deep orange in colour. It has very little fibre, juicy and with strong typical mango flavour. Propagation by Boribo seed is possible (Griesbach, 2003).

Plate 2. 4: Photo of Boribo mango fruit (Source: Griesbach, 2003).

2.5.5 Tommy Atkins

This cultivar originated from a seed planted at Fort Lauderdale in Florida in the 1920s and released in 1948 (Griesbach, 2003). The fruit has a broadly rounded base (Plate 2.5) and measures an average length of 12.6 cm, 9.9 cm wide with an average weight of 522 g. The skin is smooth, tough and thick with yellow to deep yellow-coloured flesh which is firm and medium juicy with a moderate amount of fibre (ITC, 2015). It is mild and sweet with a strong pleasant aroma. The eating quality is fairly good; the seed is mono-embryonic and covered in a thick, woody stone which is 6.6% of the total fruit weight (Griesbach, 2003).

Plate 2. 5: Photo of Tommy Atkins mango fruit (Source: Griesbach, 2003).

2.5.6 Van Dyke

This cultivar belongs to a selected group of seedlings known for greater resistance to anthracnose and its origin is Homestead (Florida). Van Dyke has very good shelf life and shipping qualities (Griesbach, 2003). The fruit is ovate, small- to medium-sized (average weight 280 g) and very attractive showing a bright red colour with a heavy crimson blush and prominent beak (Plate 2.6) The average fruit dimensions are: 10.5 cm length by 7.9 cm width. The skin is thick, though easily separating and covered with numerous white/yellow lenticels (ITC, 2015). The tree yields poorly and suffers heavy fruit drop (Griesbach, 2003).

Plate 2. 6: Photo of Van Dyke mango fruit (Source: Griesbach, 2003).

2.5.7 Sabre

The mango cultivar originated from South Africa (Griesbach, 2003). Sabre fruits are oblong, kidney-shaped and small to medium sized measuring on average 11.8 cm long and 6.9 cm broad and weigh an average of 233 g (range: 180-290 g), the apex being broadly rounded and curved into a prominent beak (Plate 2.7). The fruit has a smooth-surfaced tough leathery skin which is yellow-green, often with a reddish blue colour. It is easily removed from the flesh and matures at around January and February (ITC, 2015). The flesh has a deep orange colour with a melting texture and a medium amount of fibre. It has a fair eating quality, is sweet to insipid flavoured and normally has a turpentine aftertaste. The seed is large comprising up to 9.4% of total fruit weight (Griesbach, 2003).

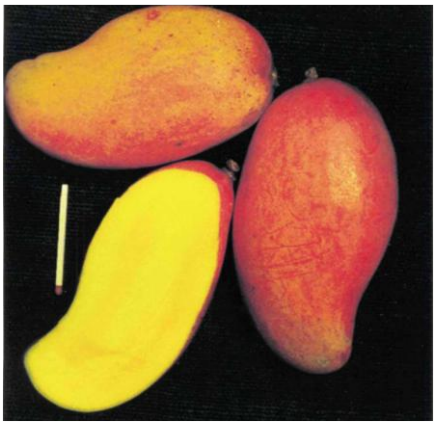


Plate 2. 7: Photo of Sabre mango fruit (Source: Griesbach, 2003).

2.5.8 Kent

This is an open pollinated seedling of the cultivar Brooks. It originated in Miami, Florida, and was released in 1944 (Griesbach, 2003). It is similar looking cultivar to Keitt but matures earlier (March) than Keitt. The fruit is large with average length of 12.4 cm with a width of 9.7 cm and an average weight of 545 g. It is greenish-yellow in colour with a red or crimson blush on the shoulder. The fruit has regular ovate shape with a rounded base and often with two slight beaks (Plate 2.8). The skin is thick, tough and with small yellow lenticels. The flesh is juicy, sweet melting, deep yellow, fibreless

and of a rich flavor (ITC, 2015). The seed, embedded in a thick, woody stone (8.5% of fruit weight) is mono-embryonic. It is susceptible to black spot disease. To enrich the skin colour, ethylene treatment is required (ITC, 2015).

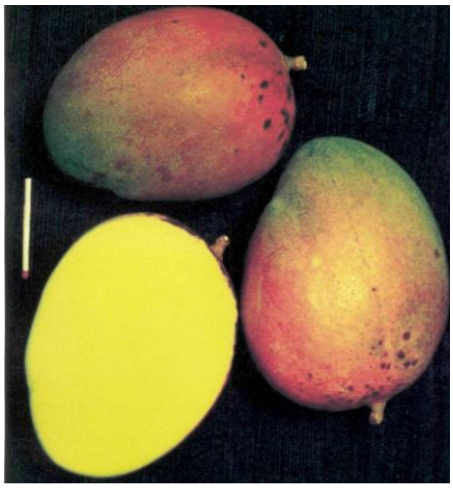


Plate 2. 8: Photo of Kent mango fruit (Source: Griesbach, 2003)

2.6 Mango pests and Diseases

An insect species is considered a pest if it can cause important damage to the crops or stored-products or livestock production. Insect pests feed on leaves or burrow in stems, fruits, roots and stored grains (Suliman *et al*., 2015). Those that feed on foliage are called phytophagous; whereas those that feed on grains, especially the stored-grains are called stored-grain pests. The phytophagous are further categorized into three groups on the basis of food (host) preference. Monophagous insects feed on plants within a single genus, oligophagous have hosts in different genera within the same plant family and polyphagous attack a large number of plants of different families (Hsiao, 1985).

Mango is attacked by a wide range of insect pests and diseases (Veerish, 1989). Such insect pests include the mango mealybug (*Drosicha mangiferae*), mango gall midges (*Erosomyia indica*, *Dasineura amaramanjarae*), mango shoot gall psylla (*Apsylla cistellata*, *Pauropsylla brevicornis*), fruit flies (*Bactrocera*, *Ceratitis* and *Anastrepha* spp.), fruit-sucking moths (*Eudocima*, *Achaea*), mango aphid (*Toxoptera odinae*) and the mango seed weevil (*Sternochetus mangiferae*). Some 25 fungus diseases affect mango with the most serious and widespread being anthracnose (*Glomerella cingulata*) which infects shoots, flowers and fruits. Powdery mildew (*Oidium mangifera*) is also

an important fungal disease and can cause substantial crop losses as it affects flowers and fruitlets as well as the leaves. Infection symptoms include leaf spots and various storage rots of the fruit. Fruit flies such as the Mango or Marula Fruit Fly (*C. cosyra*) and the African Invader Fly (*B. invadens*) have been described as the most devastating pests of mangoes in Africa (Ekesi *et al.*, 2009; Ambele *et al.*, 2012).

Majority of Kenyan mango farmers have reported problems with pests and diseases (ABD *et al*., 2009; ABD, 2011). Mango in Kenya is mainly affected by two insect pests (fruit fly and mango seed weevil) and two major fungal diseases (powdery mildew and anthracnose) (Griesbach, 2003; Varela *et al.*, 2006). These pests, diseases, their symptoms and control measures are summarized in Table 2.1

Table 2. 1: **Major mango pests and diseases, their symptoms and control methods in Kenya**

Pest/Disease	Symptoms	Control methods
Mango fruit fly (*Ceratitis* spp., *Bactrocera invadens)*	• Premature fruit ripening • Fruit dropping • Maggots in the destroyed fruit pulp	• Orchard hygiene (collection /destruction of fallen fruits). • Using poison–bait traps or spray (molasses mixed with an insecticide such as Malathione) • Spraying synthetic
Mango seed weevil (*Colletotrichum gloeosporioides*)	• Almost no outside signs • Seeds inside the husk eaten by the larvae • Fruit rotten inside	• Orchard hygiene (collection/destruction of fallen fruits and any waste) • Repeated brushing/spraying of the tree's trunk with contact insecticides during the flowering season to prevent the adult beetle from climbing up the tree from their hibernation sites • Biweekly spraying of fruits with organophosphate insecticides
Anthracnose (*Colletotrichum gloeosporioides*)	• Attacks all tree parts, particularly during high humidity. • Attacks harvested fruits • Small brown–black spots, enlarging • On fruits often tear–stain lines of black spots	• Selection of more tolerant varieties, (*e.g*., Tommy Atkins, Van Dyke • Orchard hygiene (pruning of dead, infested branches, removal of fallen leaves) • Biweekly spraying of the whole tree with fungicides during flowering and once per month during fruiting • Cold storage of harvested fruits
Powdery mildew (O*idium mangiferae*)	• Attacks all tree parts apart from older fruits, particularly during cold, cloudy weather. • Infected parts are coated with the white, powdery fungus • Defoliation, drop of flowers	• Selection of more tolerant varieties (*e.g*., Sensation, Van Dyke, Tommy Atkins, Sabine) • Biweekly spraying of the whole tree with fungicides during flowering until fruit set

Sources: Griesbach, 2003; Varela *et al*., 2006.

Kenya's export varieties (Kent, Apple, Tommy Atkins) and local ones (Boribo, Dodo, Ngowe) are infested although local ones show lower infestation compared to the export varieties (ICIPE, 2008).

Both fruit fly and seed weevil are quarantine pests (Ekesi and Billah, 2007) and the high infestation levels have negative consequences on the mango export. The infestation by the two pests is rampant in Kenya since mango farmers do not adequately control pests and diseases. A survey carried out in the coast region revealed that over 46% of mango farmers did nothing against mango pests and diseases (ABD *et al.*, 2009). Only 8% of the surveyed farmers applied pesticides and 33% use some alternative methods such as keeping orchards clean. The coast region is dominated by tall-growing local varieties which hinders efficient application of pesticides. The situation may differ in Eastern and Central Kenya, with improved varieties grafted on dwarfing root stocks.

2.7 Insect Behavior and Olfactory Signal Transduction

An Insect depends on chemosensory or chemoreceptor organs located on antennae, mouthparts, wings, legs and ovipositors to live. In general, the chemosensory are gustatory receptors involved in sense of taste and olfactory receptors are involved in sense of odor (Suliman *et al*., 2015). The odor molecules enter through the openings (pores) located on the cuticle where the dendrites of several (usually up to five) sensory neurons are exposed (Suliman *et al*., 2015).

In sensory neuron cells, there are receptor genes encoding proteins, which mediate odor signal transduction. These small soluble proteins called Odorant Binding Proteins (OBPs) are secreted in large quantities by support cells surrounding the Olfactory Sensory Neurons (OSNs) (Swarup *et al.*, 2011) They bind odorant messages allowing therefore, an insect to locate food source, aggregate, and mate (Wyatt, 2003 ; Sun *et al*., 2012).

2.8 Tephritid Fruit Flies

Tephritidae is one of the two fruit fly families that are key pests that most adversely affect the production and market value of fruits and vegetables around the world. The

second family is Lonchaeidae. The tephritids insert the ovipositor to drop their eggs into the living tissues of host plants, such as green fruit, fruit in process of maturation or ripe fruits (Uchôa, 2012).

2.8.1 General classification

The Tephritidae (true fruit flies), with about 4,000 species in 500 genera, is one of the most species-rich families within the order Diptera (Boykin *et al.*, 2014). Larvae of most species develop in fruits of wild and cultivated plants and the family is therefore commonly known as 'fruit flies' and are not to be confused with the Drosophilidae (vinegar flies) which share this common name (Skaife, 1979).

At present there is no generally accepted higher classification of the family Tephritidae. Korneyev (1999) revised the family of Tephritidae, subdividing it into 6 sub families: Dacinae, Trypetinae, Tephritinae, Phytalmiinae, Tachiniscinae, and Blepharoneurinae. Dacinae has a major tribe, Dacini consisting of mainly 2 genera, *Bactrocera* and *Dacus. Bactrocera* is a large genus consisting of 629 described species out of 880 in the tribe Dacini (Drew, 2004).

2.8.2 Biology and ecology of the fruit fly

Typically, fruit flies lay their eggs in semi-mature and ripe fruit. Mated female fruit flies puncture the fruits with their sharp egg-laying appendages (ovipositors), which are located at the tip of the abdomen, and oviposit their eggs in batches of 1 to 8 depending on the species, quality and density of the host (Aluja *et al*., 2001). The eggs are white, banana shaped and nearly 1 mm long. Infested fruit may show ‘sting’ marks on the skin and may be stung more than once by several females (Plate 2.9, right).

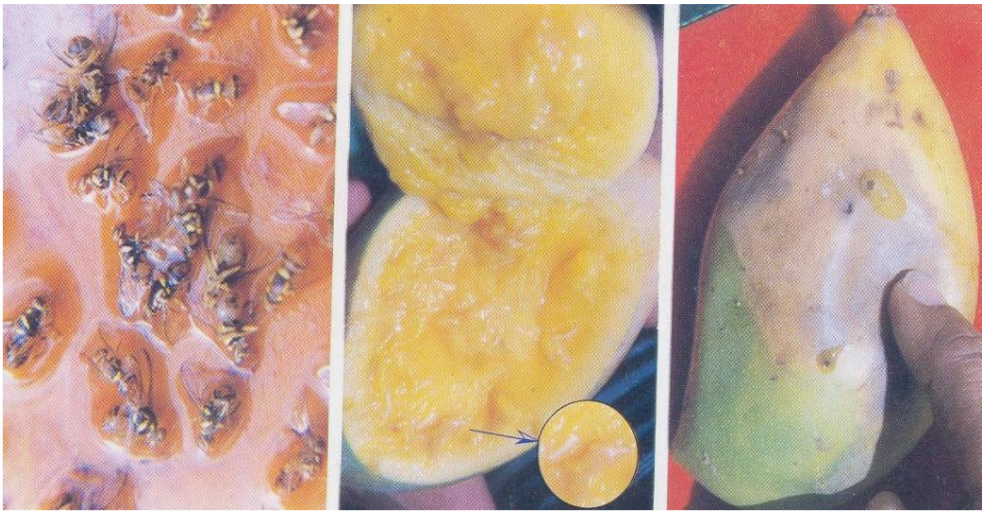


Plate 2. 9: Photo of traped flies (left), Maggots in pulp (centre) and infested fruit (right) (Source: Prakash, 2012)

The eggs develop into larvae within 3 to 12 days which feed on the fleshy part of the fruit. When fully grown, the maggots exit the fruit and bury themselves in the soil, where they pupate (Vargas *et al*., 1996). In a few species, pupation may occur inside the host. The flies become sexually mature approximately one week after emergence, and mating ensues. Depending on the climatic conditions and abundance of host fruits, fruit flies of the tropics may complete more than twelve generations in a year (Wih-Kwasi, 2008).

Female flies may associate with bacteria resident in their gut, which they regurgitate onto the fruit before ovipositing. Most of the damage sustained by the fruit fly is actually caused by the bacteria and the maggots simply lap up the juice.

The mouth hooks enable the larvae to easily tear the fruit flesh and develop through three instars before becoming about 9 mm long and pale yellow when fully grown. Several larvae can develop in a single fruit, leaving the fruit when fully developed and falling into the soil under the tree. They then burrow down about 5 cm to form a hard, brown, barrel-like pupal case from its own skin where it completes its development (Wih-Kwasi, 2008).

Many larvae leave the fruit when it is already on the ground. Most insects cannot pupate successfully in the presence of excess moisture and fruit flies have a pre-pupal stage when they can turn themselves over presumably to distance themselves from the host

fruit. The duration of pupal developmental is dependent on temperature with each stage taking from nine days to several weeks to complete (Fletcher, 1987).

Adult flies emerge from their pupal cases in the soil and burrow towards the surface where they fly away. Adults are able to mate within a week of emerging, living for many weeks with females continuing to lay eggs throughout their life cycle (Fletcher, 1987; Plant Health Australia, 2011). Adult fruit flies feed on carbohydrates from sources such as fruit and honey dew secretions from aphids and scale insects, as well as natural protein sources, including bird droppings and bacteria (CABI, 2007).

2.8.3 Effect of altitude on seasonal activity and distribution of fruit flies

Insects are not actually affected by altitude *per se* but by the climatic changes that occur along the altitudinal gradient. Altitudinal gradients can serve as spatial analogues for climate change. Ecological factors like host plants and predators, as well as physical parameters like temperature, rainfall and humidity encountered along an altitudinal transect can affect the density, diversity, and life history of insects requiring phenotypic flexibility and genotypic adaptability of many species (Geurts *et al.*, 2012).

Of all the climatic and environmental factors such as temperature, rainfall and host plants, it appears temperature is most important as it decreases by about 0.5-0.6 °C with every 100 m increase in altitude (Ekesi *et al.*, 2006; Kovanci and Kovanci, 2006). Temperature therefore has an important role in determining development rates and is responsible for the timing of the population processes and their synchronization with changes in the environment. The fruit flies are seasonal in abundance in temperate climates. Multivoltine species such as *Ceratitis capitata* for instance increase their numbers up to a peak in later summer and early autumn and then decline rapidly (Radonjic *et al.*, 2013). A species with a wide altitudinal range appears later in the season at higher altitudes, and at these locations it must be adapted to a shorter season. Emergence of *Rhagoletis cerasi* flies for example must coincide with the ripening period of sweet cherries in order to be able to lay eggs. Adult emergence dates may however vary from one location to another because locations may differ in climatic regimes even though they might be close together geographically (Randall, 1982).

Studies have shown that there is a significant inverse in relationship between numbers of flies per kilogramme of fruit and elevation at which the fruit was collected. High levels of infestation were recorded at low elevations and were seen as an indication that *B. invadens* may well be adapted to a hot climate and thus represent a real threat to mango crops grown in the warmer low elevation regions of Kenya (Ekesi *et al.*, 2006).

2.8.5 Indigenous fruit fly pest species in Sub-Saharan Africa

Subsaharan Africa is the home of several species of highly damaging fruit flies such as *Ceratitis cosyra (*Walker), *C. quinaria* (Bezii), *C. frasciventris* (Bezzi), *C. rosa* (Karsch), *C. anonae* (Graham) and *C. capitata* (Wiedemann). Other important native *Ceratis* species in the region include *C. rubivora* (Coquillet), *C. puntata* (Wiedemann), *C. discussa* (Munro), *C. ditissima* (Munro), C. *pedestris (*Bezz) that attack a variety of important fruits and vegetables (Lux *et al.*, 2003). Several *Ducus* species such as *D. bivittatus*, (Bogot), *D. iounsburyii* (Coquillet), *D. ciliates* (Loew), *D. puntatifrons* (Wiedemamman), *D. frontalis* (Becker) and *D. vetebratus* (Bezz) also inflict considerable losses on vegetable crops especially the cucurbits (De Meyer *et al*., 2017).

2.8.6 Exotic fruit fly pest species in Sub-Saharan Africa

Although Africa is known to be the place of several fruit fly establishments worldwide, the most notorious species is the Mediterranean fruit fly, *C. capila*. The continent has also become vulnerable to introduction of elien fruit fly species. *B. zonata* was introduced into Egypt in 1997 (De Meyer, 2017). In 2003, *B. invadens* was detected and described for the first time in Africa as a junior synonym *of B. dorsalis* (Drew *et al.*, 2005). In 2006 *Solanum* fruit fly *B. latifrons*, a primary pest of solanaceous crops, was detected in Tanzania (Mwatawala *et al.*, 2009). The melon fruit fly *B. cucurbitae* has also been in Africa for years with no clear date of introduction (White and Elson-Harris, 1992).

Among all the native and exotic fruit fly species, one species *B. invadens*, also called the African invader fly, has been linked to extensive economic losses to the horticultural crops in Africa since it was first detected in 2003 (Lux *et al*., 2003). The rapid spread and devastating impact of *B. invadens* in Sub-Saharan Africa has been a matter of serious concern to the horticulture industry (De Meyer, 2017).

2.8.7 Economic Impact of Tephritid Fruit Flies

Fruit flies cause direct damage by puncturing the fruit skin to lay eggs. During egg lying, bacteria from the intestinal flora of the fly are introduced into the fruit. These bacteria cause rotting of the tissues surrounding the egg. When the eggs hatch, the maggots feed on the fruit flesh, making galleries. These provide entry for pathogens and increase fruit decay and thereby making fruits unsuitable for human use (Ekesi and Muchugu, 2007). Direct damage caused by the fruit flies usually range from 20 to 80% (Ekesi *et al*., 2009). Larvae of the Mediterranean fruit fly (*C. capitata*) for instance, can cause extensive damage to fruit crops of up to 100% by tunneling into the fruit, making it unfit for human consumption. The larval tunnels also provide entry points for bacteria and fungi that cause the fruit to rot. These factors normally lead to reduced income and increased costs of control. Production losses and costs of field control are the direct impacts of fruit fly attack, while indirect losses result from the implementation of regulatory controls and loss of export markets (Boykin *et al*., 2014).

In Kenya, where about 90,000 tonnes of mangoes are produced annually, losses of between 20-80% due to fruit fly infestation have been reported (Lux *et al*., 1998). A similar situation prevails in the other mango-producing African countries such as Côte-d'Ivoire, Mali, Senegal and Burkina Faso, where the infestation has been reported to reach 70% in small-holder orchards. The production of mangoes is threatened by *C. cosyra* and *B. invadens* with the latter being described as the most devastating fruit fly pest in Africa (Allotey *et al*., 2010; Ambele *et al*., 2012). In Benin, fruit fly infestation led to losses of more than 60% on the main mango cultivars in the second half of the mango season resulting in uprooting of mango plantations in one area (Borgou) at one time (De Meyer *et al*., 2010). Mauritian and South African governments banned the importation of mangoes from Kenya as a result of the threat posed by invasive fruit flies (Ekesi *et al.*, 2009).

Tephritid fruit flies have a greater impact on international marketing and world trade in agricultural produce than other insects. Fruit flies as major quarantine pests of fruits and vegetables have triggered implementation of trans-boundary control programmes (IAEA, 2013). As a result of the discontinuous distribution of some species of fruit fly, and their enormous potential as fruit pests, several species are subject to quarantine legislation in different countries, involving restriction of importation of fruit likely to

contain the larvae. The main species involved are *C. capitata*, *B. dorsalis*, *B. tyroni* and *B. invadens* (CABI, 2007).

2.9 The Genus *Bactrocera*

Bactrocera, which was previously known as *Dacus* (Drew, 1989), is a Tephritid fruit fly genus in the subfamily Dacinae which has more than 500 species subdivided into 28 sub-genera (Drew, 2004). A number of species in this genus which are of major economic importance include the polyphagous *B. zonata*, *B. tryoni* (Plate 2.10), *B. cucurbitae* and *B. dorsalis/invadens* (Clarke *et al*., 2011) which utilize a variety of fruit species in numerous plant families. A few species such as *B. olea* and *B. cacuminata* have monophagous larvae utilizing closely related host species (Drew, 2004).

Plate 2. 10: Photo of *Bactrocera tyroni* fruit fly (Source: Yonow and Sutherst, 1998).

The taxonomy of members of this group, particularly of those in the *B. dorsalis* complex, is unsettled and constantly reassigned with the addition or omission of new siblings or species. For instance, the African invader fruit fly *B. invadens* was described as a new species more than a decade ago (Lux *et al*. 2003, Drew *et al*., 2005). Recent literatures however show that it is the same species as the oriental fruit fly *B. dorsalis* (Bo *et al*., 2014, Schutze *et al*., 2014). Some species such as *B. invadens/dorsalis*, *B. zonata* and *B. cucurbitae* have invaded Africa (De Meyer *et al*., 2010). Multiple independent species delimitation tests have shown that B. *invadens B. papaya and B. philippinensis* are the same biological species as B. *dorsalis* (Clarke, 2011). An important behavioural characteristic of the genus *Bactrocera* is that, males of many species are highly attracted to either of the parapheromones: methyl-eugenol (ME) (4-

allyl-1, 2-dimethoxybenzene) (**1**) or cue-lure (CUE) [4-(p-ace toxyphenyl)-2-butanone] (**2**) (Drew and Hooper, 1981).

2.9.1 *Bactrocera invadens*, African Invader Fly/Asian Fruit Fly

The eggs of *B. invadens* are small, white and slender (0.8 mm x 0.2 mm). Larvae are white maggots. Adults have a pair of black spots on the face, one under each antenna. Wing length is 4-7 mm with distinctive dark markings along the anterior edge. Two raised areas just below and behind the wing base are yellow. There is a lateral pair of broad yellow stripes on the side of the thorax (Plate 2.11). The abdomen has distinctive markings; a clear, black, mid-longitudinal line with broad lateral markings almost rectangular in shape. A distinctive characteristic is that the males are attracted to the chemical lure of methyl eugenol (**1**) (Ekesi and Muchugu, 2007; Allotey *et al.*, 2010).

Plate 2. 11: Photo of *Bactrocera invadens* (Source: Nboyine *et al*., 2012)

Following a preliminary survey of fruit flies of Bangladesh, Leblanc *et al* (2013) observed that the species in Bangladesh exhibited a broad range of scutum colour pattern variation which ranged from predominantly pale to dark. This was similar to the scutum colour pattern variation documented in *B. invadens* from Sri Lanka. *Bactrocera invadens* is multivoltine in nature, and has all year host availability (Ekesi and Muchugu, 2007; Allotey *et al*., 2010).

2.9.2 Host Range of *B. invadens*

Bactrocera invadens is a polyphagous pest of both cultivated and wild fruit species and has been recorded to infest over 50 plant species belonging to over 25 families (Allotey *et al*., 2010). Among the cultivated fruits are mango (*Mangifera indica*), guava (*Psidium guajava*), citrus (*Citrus limon, Citrus reticulata* and *Citrus sinensis*), avocado (*Persea americana*) and paw paw (*Carica papaya*). The most preferred hosts among the wild fruits are Marula (*Sclerocarya birrea*) and *Terminalia catappa* (Rwomushana *et al*., 2008). The invader fly, *B. invadens* also attacks vegetable crops such as solanaceous plants like tomato (*Lycopersicon esculentum*), pepper (*Cucumis annum*) and curcubits (*Curcubita pepo*) (Allotey *et al*., 2010; Manrakhan *et al*., 2011).

2. 10 Fruit Fly Monitoring

Fruit fly monitoring helps to identify fruit fly pests in an area, determine distribution of pest species, identify local hot spots with high populations of the pest, track changes in population levels, determine efficacy of control measures and facilitate early detection of new fruit fly pests in a particular area. Tools used in fruit fly monitoring consist of attractant-based traps and host fruit surveys (Manrakhan, 2000).

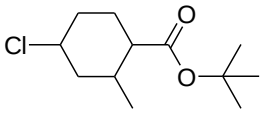
2. 11 Attractants and lures

The three main types of attractants used in fruit fly monitoring include male and female lures, and food baits. Fruit fly trapping systems used for fruit fly surveys consist of the attractants (pheromones, para pheromones or food attractants), killing agents (dry/wet) and a trap/trapping device. Lures are excellent tools for fruit fly pest management and are either male specific or female biased (Tan *et al*., 2014) as there is no female specific attractant for tephritid flies.

2.11.1 *Bactrocera* Male specific Parapheromones

Parapheromones are chemicals that are not naturally used in intraspecific communication but which do elicit responses similar to true pheromones. Parapheromones are available in both liquid form and polymeric plugs in the form of a controlled-release formulation. The most widely used traps, which contain para-pheromone attractants that are male specific, include trimedlure (TML) (**1**), methyl

eugenol (ME) (**2**) and cuelure (CUE) (**3**). Para-pheromones are volatile, and can be used with a variety of traps. TML, CUE and ME have controlled-release formulations which provide longer-lasting attractant for field use (ISPM, 2008).



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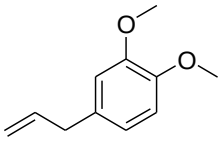

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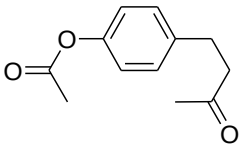

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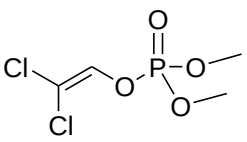
Fruit flies in the *Bactrocera dorsalis* complex are classified into three different groups; ME (**2**) responders, CUE responders and non-responders, based on the response to CUE (**3**) and ME (**2**) lures (Tan and Nishida, 2012). Nearly 200 species of *Bactrocera* are CUE (**3**) responders and 81 are ME responders, but most of these are not economically important (IAEA, 2013).

The parapheromone ME (**2**) captures many species of the genus *Bactrocera* (including *B. dorsalis*, *B. zonata*, *B. carambolae*, *B. invadens*, *B. philippinensis* and *B. musae*). The pheromone Spiroketal® captures *B. oleae*. The parapheromone trimedlure (TML) (**1**) captures species of the genus *Ceratitis* (*C. capitata* and *C. rosa*). The parapheromone cuelure (CUE) (**3**) captures other *Bactrocera* species, including *B. cucurbitae* and *B. tryoni* (ISPM, 2008). A sticky material can also be mixed with para-pheromones and applied to the surface of the panels. Dry forms of volatile toxicants such as DDVP (2, 2- Dichlorovinyl dimethyl phosphate) (**4),** Naled™ (1, 2-Dibromo-2, 2-dichloroethyl dimethyl phosphate) (**5**) and Malathion (Diethyl-2-dimethoxyphosphinothioylsulfanylbutanedioate) (**6**) are used as killing agents. They are usually applied in panels, delta-traps and in bucket traps, although some of these are repellent at higher doses (Manrakhan, 2000; IAEA, 2013).



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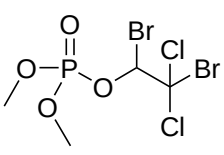

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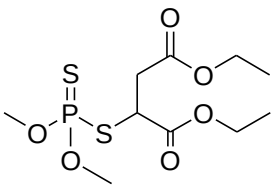

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Trimedlure **(1)** is a synthetic compound commonly used in control programs of *Ceratitis* species (including *C. capitata* and *C. rosa*) (ISPM, 2008). However, the underlying basis of male attraction remains unknown (Shelly and Villalobos, 1995). The pattern of male-female attraction of parapheromone trimedlure (**1**) has been found to possess an effect similar to the medfly male pheromone (Villeda *et al*., 1988). Methyl eugenol (**2**) occurs naturally in the essential oils of several plant species. The amount of methyl eugenol (**2**) in an essential oil extracted from a given type of plant depends on the variety, plant maturity at harvest, harvesting method, storage conditions and extraction method (Smith *et al*., 2002).

The parapheromone cuelure (CUE) **(3)** captures a large number of other *Bactrocera* species, including *B. cucurbitae* and *B. tryoni* (ISPM, 2008). Exposure of wild flies to cuelure (**3**) increases mating frequency compared to control males that have no prior exposure. Control and treated males have however similar mating success in tests performed 3 or 7 days after the treated males are exposed to the lure (Shelly and Villalobos, 1995).

2.11.2 Female-biased lures

Female-biased attractants are based on food or host odours (natural, synthetic, liquid or dry). Liquid protein attractants have been used to capture a number different fruit fly species, although they not as sensitive as the para-pheromone traps and also capture high percentages of non-target insects (ISPM, 2008). Ammonia and its derivatives have also been used to develop food-based synthetic attractants (ISPM, 2008).

New synthetic food attractant technologies are available for use, including the long-lasting three-component and two-component mixtures contained in the same batch, as well as the three components incorporated in a single cone-shaped plug. These attractant types have an advantage of being capable of detecting both male and female fruit flies at sexually immature stage earlier and at low population levels than liquid protein attractants (IAEA, 2013).

2.11.3 Food baits

Food baits, attract both male and female fruit flies, are species-specific and are known to have a lower efficiency compared to male lures but can also attract non-target insects, including beneficial ones. These baits are available in both liquid and synthetic forms with ammonia being the principal attractant (IAEA, 2013).

A variety of food baits available commercially include liquid protein hydrolysates, yeast products, ammonium salts and the three-component lure (consisting of putrescine, ammonium acetate and trimethylamine). Field longevity of liquid protein hydrolysates, yeast product and ammonium salts is usually between 1-2 weeks while the three-component lure and Questlure capsule can last between 4-6 weeks. Minimum distance interval between any two food-baited traps should range from 10-30 m (IAEA, 2013).

2.11.4 Host volatiles as potential female attractants

The success of Tephritidae fruit flies is dependent on among other factors, polyphagy, which was made possible primarily due to morphological adaptation in the ovipositor and behavioural adaptations in oviposition preference of the female (Fletcher, 1987). There exists a positive correlation between the choice of the ovipositing female and the performance of the larvae (Fletcher, 1987). The process of oviposition site choice therefore requires a sophisticated behavioural mechanism which involves the integration of various plant chemical cues and physical information such as colour, shape and texture of the fruit (Cardé and Willis, 2008). The oviposition process is probably regulated by short term memory (Liu *et al*., 2015).

Studies have shown that fruit flies strongly rely on odorants in host searching behavior (Jayanthi *et al*., 2012; Biasazin *et al*., 2014). Although fruits and leaves of host plants are the most extensively assessed organs for fruit fly volatile attractants, the search has been extended to include volatiles from non-host plants (Robacker *et al*., 2009). Electrophysiological studies have shown that fruit flies are sensitive to organic compounds such as monoterpenes, sesquiterpenes, pyrazine, carboxylic acids, alcohols, aldehydes, ketones and esters, most of which are typical volatiles of ripening fruits (Gikonyo *et al*., 2003; Jayanthi *et al*., 2012; Biasazin *et al*., 2014).

2.12 Fruit fly control strategies

Several techniques including male annihilation, sanitation, bait sprays and use of biological control agents have been used to suppress or eradicate fruit fly population from an infested area (Vargas *et al*., 2010). Other numerous preventive and post-harvest methods available include bagging or wrapping, early harvesting and post-harvest cold and heat treatments for fruit fly control (Ekesi and Billah, 2007). Some management techniques combine semiochemicals (male and female attractants) with killing agents in traps (Sivinski and Calkins, 1986).

2.12.1 Sanitation

All damaged and rotten fruits on the ground should be removed and burned, as these promote pest population build up (CABI, 2007). The fruit can be buried, cooked, and fed to poultry or swine. Cultivating the soil beneath the trees to expose larva and pupa to ants, poultry, lizards, and song birds is also a worthwhile idea (CABI, 2007).

2.12.2 Fruit picking

Picking all the fruit from a tree has been used primarily in eradication programs. All fruit from the tree is picked to remove any ovipositional sites that would be available for the continued development of the fruit fly population (Jacobi *et al*., 2001).

2.12.3 Wild host destruction

Elimination of non-economic or non-cultivated hosts that fruit fly populations need to survive is effective, especially in eradication programs (Smith, 2002). Fruit of these wild hosts provide a source for survival when the cultivated hosts are absent or not fruiting.

2.12. 4 Bagging of fruit

Bagging of fruit to prevent fruit fly oviposition has been used by many backyard growers and small farmers especially in Hawaii (Ekesi *et al.*, 2007). Small holes must be made in the paper bag in order for transpiration to take place. Plastic bags should not be used. The bag is removed 24-28 hours prior to harvest to allow natural colour of the

fruit to develop. Although labour-intensive, mechanical fruit protection is an effective method for high value fruit produced for export or fruits produced in backyard gardens for family use (Ekesi *et al.*, 2007).

2.12. 5 Biological control

Biological control is the use of fruit fly parasitoids, predators and pathogens to reduce the damage caused by the pest or related species of a pest (Elizinga, 2004; Ekesi *et al.*, 2007). The successes of classical biological control against fruit flies is attributed to the use of the egg parasitoid, *Fopius arisanus* against *B. dorsalis*, a close relative of *B. invadens*. This parasitoid is presently being released in Africa. There are many species of parasitoids and predators in fruits and vegetable agro-ecosystems which can contribute to the suppression of fruit flies. Native parasitoid species include *Tetrastichus giffaardi*, *Psyttalia cosyrae*, *P. concolor*, *Fopius caudatus*, *Dirhinus giffardi* and *Spalangia* spp. Predators include spiders, ants, carabid beetles and staphylinid beetles. The presence and foraging activity of the African weaver ant, *Oecophylla longinoda*, hinders the fruit flies from laying eggs (Vayssières *et al*., 2013).

A potent fungal pathogen isolate, *Metarhizium anisopliae*, has been found to be effective against both developmental stages of the major fruit fly species such as *B. invadens, B. cucurbitae, C. cosyra, C. fasciventris, C. rosa, C. capitata* and *C. anonae* (Ekesi *et al.*, 2007). Biological agents such as parasitoids and predators have not been effective mainly due to low fecundity of parasitoids as compared to fruit flies and poor searching ability of parasitoids to larval and pupal populations of fruit flies (Nadeem *et al.*, 2014).

2.12.6 Male Annihilation Technique

This is a control strategy that involves the deployment of traps consisting of male specific lures (parapheromones) combined with a killing agent. This technique is important since suppression or eradication of fruit fly without the involvement of Male Annihilation Technique (MAT) has been impossible (Cunningham, 1989). The traditional killing agents in MAT are generally organophosphorus compounds, such as Dichlorvos™ (DDVP) (**4**), Naled™ **(5**) and Malathion™ (**6)** (Vargas *et al.*, 2002). Recent studies have however focused on developing environmentally friendly

bioinsecticides such as spinosad (Vargas *et al*., 2014). The aim of MAT is to diminish the number of male fruit flies in a population to such low levels that mating and subsequent population build-up is reduced (suppression) or does not happen (eradication).

The use of methyl eugenol (**2**) traps is the most outstanding alternative among the various strategies. Methyl eugenol (**2**) has both olfactory as well as phagostimulatory action and attracts fruit flies from a distance of 800 m. When used together with an insecticide impregnated into a suitable substrate, methyl eugenol (**2**) forms the basis of male annihilation technique that has been successfully used for the eradication and control of several *Bactrocera* species (Ravikumar and Viraktamath, 2007).

2.12. 7 Sterile Insect Release Method

The sterile insect release method or sterile insect technique (SIT) is used to contain and exclude populations of fruit flies by releasing a large amount of sterile males to mate with any introduced wild female, resulting in the production of infertile eggs. The potential of SIT for controlling pests has been around since the 1960s and has some advantages including increased specificity and can be *targeted* to affected regions (Knipling, 1959). The SIT programs in the past have failed due to continual immigration into the areas being targeted.

The most common method used to make fruit flies sterile for SIT programs is to irradiate them when pupation is approximately 70% complete (Gilchrist and Crisafulli, 2006). The most effective irradiation dose rate for SIT programs should be at a level which makes an individual sterile without reducing its reproductive competitiveness. Irradiated males are not reproductively disadvantaged against normal males in terms of females re-mating (Harmer *et al*., 2006). The SIT has been used against *C. capitata* in Costa Rica, Italy, Mexico, Nicaragua, Peru, Spain, Tunisia and the USA (California and Hawaii) (CABI, 2007).

2.12.8 Baits

This method of fruit fly control involves the spot spraying of a combination of a dilute protein mixture, which serves as an attractant and an insecticide component that causes

death. This method targets both the male and female fruit flies. The effectiveness of this control method can be reduced if rain washes off bait spots making the pesticide to degrade. Malathion (**6**) is used as an insecticide in bait spraying as it has a short residual life and low mammalian toxicity (Gilchrist and Crisafulli, 2006). Bait spraying alone will not be enough to control high populations of fruit flies hence it should be used in combination with other control techniques and bait sprays are generally applied to foliage and not to the fruit (Dominiak, 2007).

The equipment used for applying the bait is simple, so the technique is appropriate for control of fruit flies at either village or commercial level (Allwood and Drew, 1997). The study on the effectiveness of GF-120 Fruit Fly Bait Concentrate®, a combination of protein bait and spinosad insecticide in suppressing *B. invadens* and other mango-infesting fruit flies revealed that the larval infestation by *B. invadens* and other native fruit fly species was significantly lower in plots treated with GF-120 than in untreated control plots (Vayssières *et al*., 2009).

2.12.9. Ground spraying

Ground spraying is applied under host trees, which are known to be infested with fruit flies and targets larvae and emerging adults in the soil (Dominiak, 2007). A spray of an appropriate insecticide such as chlorpyrifos is applied to the ground under infested trees. All compost heaps in the vicinity is also sprayed. No more than two ground sprays are usually necessary under one tree. In experiments conducted at ICIPE, it was found that application of a combination of Nulure/spinosad bait spray with soil inoculation of *Metarhizium anisopliae* reduced *Bactrocera invadens* population by 79% relative to control. Mean mango fruit infestation was 10% in the combined treatment of bait and fungus and was 73% in untreated control plots. Similarly, in field trials conducted during the 2006-2007 mango season, combined application of *M. anisopliae* and GF-120 spinosad bait spray achieved the highest reduction of *B. invadens* (92.1%) relative to the control of the last date of sampling (Papadopoulos, 2010).

2.12.10 Postharvest Regulatory Control

Many countries, such as Maryland USA, forbid the importation of susceptible fruit without strict post-harvest treatment having been applied by exporters (CABI, 2007).

Commodity treatments are needed in order to transport host fruits from areas infested with fruit flies through quarantine barriers into areas that are free of the pest. These include use of fumigants and lethal temperatures.

2.12.11 Integrated Pest Management (IPM)

Integrated Pest Management (IPM) approach is a combination of control methods and it is being promoted in today's agricultural practice. The use of single suppression techniques in many cases has been insufficient in reducing fruit flies from an area where they are well established. Most successful programs have therefore resorted to the use of integrated suppression techniques (Vargas *et al*., 2010). For instance, a nation-wide program was initiated to eradicate *Bactrocera dorsalis* from Taiwan in 1994. By the year 2002, a large amount of methyl eugenol (**2**) (40 metric ton) was used to supress 75% of the population, but further reductions with male annihilation alone was impossible. They subsequently incorporated bait sprays, sanitation, and fruit bagging which accomplished further suppression of *B. dorsalis* population (Vargas *et al*., 2010).

A successful eradication of *Bactrocera cucurbitae* was made possible by combining sterile insect technique (SIT) with other techniques that included mass trapping and bait sprays (Koyama *et al*., 2004). In South Africa, male annihilation combined with orchard sanitation and protein bait sprays with Malathion^{TM} (**6**) eradicated *Bactrocera dorsalis / invadens* from the northern most part of the Limpopo province (Manrakhan *et al*., 2011). For successful result, IPM system should also consider coexisting species of flies in the area of management because suppressing a major fruit fly pest to a significant level may through competitive exclusion cause other fruit fly species in the area to become pests.

The diversification of the approaches that form part of IPM is required for better environmental protection. Aromatic plants and their essential oils have been used as alternative strategies that have been found to cause fumigant and topical toxicity as well as antifeedant or repellent effects and also inhibit reproduction (Pemonge *et al*., 1997). Some essential oils and their components have been found to exhibit both repellent and larvicidal effects: *Ocimum* volatile oils including camphor, 1, 8-cineole, methyl eugenol, limonene, myrcene and thymol, strongly repelled mosquitoes and *O. basilicum* exerted a larvicidal activity (Chokechaijaroenporn *et al*., 1994).

2.13 Essential oil compounds reported in some mango varieties

The major compounds identified in the Brazillian mango variety Espada were terpinolene **(7**) and *δ*-3-carene **(8)** while the Rosa variety contained terpinolene **(7),** *α*-pinene **(9**) and *β*-pinene (**10**) (Ramos *et al.*, 2014)**.** Terpinolene **(7)** and with *δ*-3-carene **(8)** were also found as major components in the Australian Kensington and Irwin varieties (Loveys *et al.*, 1992).

Gebara *et al*. (2011) reported the major compounds found in the immature fruit of Brazillian *M. indica* var. coquinho as terpinolene **(7),** α-gurjunene (**11**), α-humulene **(12**), *E*-caryophyllene (**13**). The phenylpropanoid *p*-cymen-8-ol (**14**) was detected in significant amounts only in the volatile isolated by hydrodistillation (HD)**.** In immature leaves, terpinolene (**7**) α-humulene (**12**), *E*-caryophyllene (**13**) and cyperene **(15)** were the main compounds present. The oxygenated sesquiterpenes carotol **(16),** guaiol **(17**) and α-cadinol (**18**) were detected in the volatiles isolated only by HD. The major compounds extracted from the mature fruit were terpinolene (**7**), α-gurjunene (**11**), α-humulene **(12**) and *E*-caryophyllene (**13**). In mature leaves α-gurjunene (**11**), α-humulene (**12**), *E*-caryophyllene (**13**), cyperene (**15**) and α-cedrene (**19**) were the main substances detected. Hexadecanol was detected only in mature fruit while spathulenol (**20**) was detected in both mature fruit and leaf.

The essential oil isolated from the leaves and fruit peels of Nigerian *Mangifera indica* L. by hydrodistillation was rich in sesquiterpenes with the dominant compounds being δ-3- carene (**8**) α-gurjunene (**11**), E-caryophyllene (**13**) and β-selinene (**21**) , while fruit peel oil yielded mainly δ -3-carene (**8**) and α-pinene (**9**) (Ana *et al*., 2014). The structures of some essential oil compounds reported in mango varieties are shown in Figure 2.1.

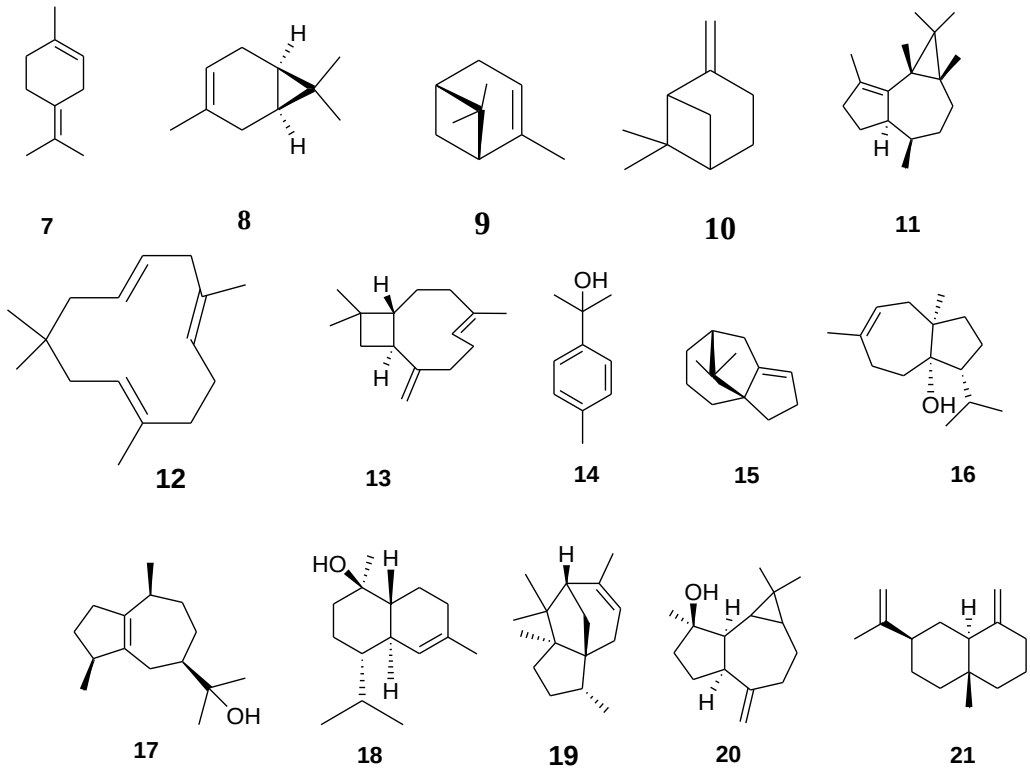


Figure 2. 1: Stuctures of essential oil compounds reported in some mango varieties

2.14 Volatile aroma compounds reported in some mango varieties

Chemical analysis of the aroma of several mango cultivars around the world have been reported (Macleod and De Troconis, 1982; Macleod *et al*., 1988; John *et l*., 1999; Pino *et al*., 2005). A wide range of compounds have been identified, including esters, lactones, mono- and sesquiterpenes. Monoterpene hydrocarbons such as α-pinene (**9**), β-pinene (**10**), *cis*-ocimene (**22**), myrcene (**23**) and limonene (**24**) seem to be particularly important contributors to the aroma of the fresh fruit, depending on the variety (Idsteom and Schreier, 1985; Pino *et al*., 2005).

The major classes of organic compounds identified in the flavor profile of Brazillian Tommy Atkins mango fruit were hydrocarbons, esters, terpenes, lactones and aromatic compounds. The principal compounds were δ-3-carene (**8**), myrcene (**23**), limonene (**24**), geranyl acetate (**25**), ϒ-octalactone (**26**), ϒ-nonalactone (**27**), citronellol (**28**), carvone (**29**), α-ionone (**30**), β-phellandrene (**31**) α-terpineol (**32)**, β-terpineol (**33**), toluene (**34**), benzaldehyde (**35**) and (*Z*)-3-hexen-o1 (**36**) (Narain and Galva˜o, 2002).

Canuto *et al*. (2009) investigated the effect of the maturation stages on the volatile chemical composition of the fruit of Tommy Atkins mango variety. The results showed that δ-3-carene (**8**) was the major component in all the stages, while terpinolene (**7**), *E*-caryophyllene (**13**) and α-pinene (**9**) succeded each other as the second most abundant constituent, during the ripening. The aroma of the ripe fruit was characterized by presence of short-chain ethyl esters (C_2-C_6), whereas the green mango contained the highest concentration of δ-3-carene (**8**).

An *et al*. (2015a) in their comparative analysis of volatile flavor compounds in Taiwan Apple mango and Philippines Carabao mango found out that δ-3-Carene (**8**) was the dominant flavor compound in these two mango cultivars. α-copaene (**37**), α-guaiene (**38),** germacrene D **(39),** α-bulnesene **(40),** and γ-gurjunene (**41**) were found only in Taiwan Apple mango, whereas terpinolene (**7**), myrcene (**23**), β-phellandrene (**31**), α-phellandrene (**42**) and cis-3-hexenyl butyrate (**43**) were identified in Philippine's Carabao mango. The δ-3-Carene (**8**) was also identified as the dominant compound in volatile flavor compounds in Jeju Apple mango by using different extraction methods, whereas terpinolene (**7**), α-pinene (**9**), limonene (**24**), α-phellandrene (**42**), γ-terpinene (**44**), trans-β-ocimene (**45**), and furaneol (**46**) were the next important compounds (An *et al*., 2015b).

The volatiles of nine Colombian mango varieties analysed by means of simultaneous distillation–extraction, GC and GC–MS showed that δ-3-carene (**8**) was dominant in Haden, Irwin, Manila and Tommy Atkins varieties. α-Pinene (**9**) was dominant in Hilacha and Vallenato. α-Phellandrene was dominant in Van Dyke and terpinolene in Yulima variety. Analysis of Volatiles of Mexican mango var. Ataulfo by Solid Phase Microextraction (SPME) and Capillary GC/MS Spectroscopy identified a complex mixture of monoterpenes and sesquiterpenes with terpinolene **(7),** δ 3-carene (**8**), α-pinene (**9**)) β-selinene (**21**), myrcene (**23**) and (-)-limonene (**24**) as dominant compounds (Quijano *et al*., 2007).

The volatile components of 20 Cuban mango cultivars investigated by means of simultaneous distillation-extraction, GC, and GC-MS showed that monoterpene hydrocarbons were the major volatiles of all cultivars. The dominant monoterpenes were δ-3-carene **(8**) (present in Haden, Manga amarilla, Macho, Manga Blanca, San Diego, Manzano, Smith, Florida, Keitt, and Kent), limonene (**24**) (present in Delicioso,

Super Haden, Ordon˜ez, Filipino, and La Paz). Both δ-3-carene and limonene were present in Delicia variety. Terpinolene **(7**) was dominant in Obispo, Corazo and Huevo de toro, while α-phellandrene (**42**) was dominant in Minin variety (Pino *et al*., 2005).

Malo *et al*. (2012) in their work found out that the compounds α-pinene (**9**), β-selinene (**21**), myrcene (**23**) and *trans*- β -ocimene (**45**) were the most abundant in Indian Amate mangoes, whereas δ-3-carene (**8),** terpinolene (**7**), α-pinene (**9**) and β-selinene (**21**), were the predominant compounds of Ataulfo cultivars. In the Coche mango, the predominant compounds were terpinolene Compounds 22 to (**7**), δ-3-carene **(8**), β-selinene **(21**) and limonene (**24**). Figure 2.2 shows the structures of some volatile aroma compounds reported in mango varieties.

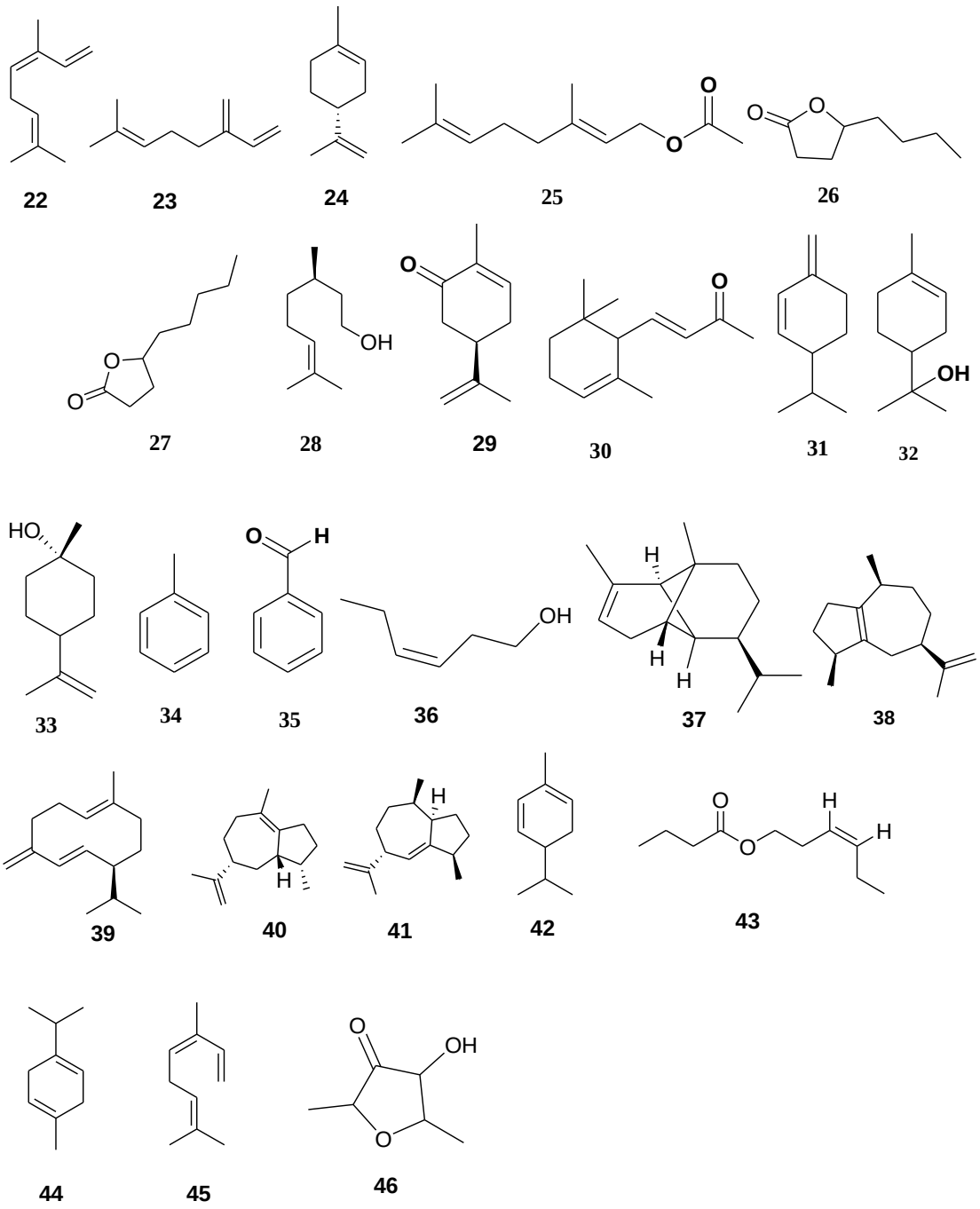


Figure 2. 2: Stuctures of volatile aroma compounds reported in some mango varieties

2.15 Fruit fly bioactive compounds reported in mango varieties.

Junwei *et al*. (2003) in their work found out that odour from overripe mango attracted vinegar flies, *Drosophila melanogaster*. Combined gas chromatography-electroantennographic detection (GC-EAD) analysis SPME and Tenax extracts of

overipe mango odors showed that several volatile compounds, including ethanol, acetic acid, amyl acetate (**47**), and 2-phenylethanol and phenylethyl acetate **(48**) elicited significant EAD responses from antennae of female flies in cage bioassays. However, in field trials, they were not as attractive as suggested by the bioassay.

Traps baited with a blend of α-pinene (**9**), myrcene (**23)** and trans- β-ocimene (**45**) have been found to capture more West Indian fruit fly, *Anastrepha obliqua* (Macquart) (Diptera: Tephritidae) females and males than control trap (Malo *et al.,* 2012). The flies were more attracted to the volatile extracts of Amate mango than to the three-component blend were formulated in a ratio of 1:1:1. There was however no significant difference between the numbers of flies caught by traps baited with Amate mango extracts and that caught by traps baited with the three-blend component. Traps baited with myrcene (**23**) caught fewer flies than traps baited with Amate mango extracts (Malo *et al.,* 2012).

Headspace samples of volatiles collected from two cultivars of mango, 'Alphonso' and 'Chausa' from South East Asia and Hawaii showed a strong positive behavioral response when female *B. dorsalis* were exposed to these volatiles in olfactometer bioassays (Jayanthi *et al*., 2012).

The EAG active compounds, from 'Alphonso', were identified, using GC-MS, as heptane, γ-octalactone **(26),** myrcene (**23**), (*E)*-ocimene (**45**), *(Z)*-ocimene (**49**), allo-ocimene (**50**) and *(Z)*-myroxide (**51**) with the two ocimene isomers being the dominant compounds. The EAG-active compounds from 'Chausa' were 3-hydroxy-2-butanone **(52**), 3-methyl-1-butanol (**53),** ethyl butanoate (**54**), ethyl methacrylate (**55**), ethyl crotonate (**56),** ethyl tiglate (**57),** 1-octen-3-ol (**58**)**,** ethyl hexanoate (**59**), 3- carene **(8**), p-cymene **(60**), ethyl sorbate (**61**), α-terpinolene **(7**), phenyl ethyl alcohol (**62**), ethyl octanoate (**63)**, and benzothiazole (**64**) (Jayanthi *et al*., 2012). Individual compounds were significantly attractive when a standard dose was tested in the olfactometer. The synthetic blends that had the same concentration and ratio of compounds as in the natural headspace samples were highly attractive (Jayanthi *et al*., 2012). Studies have shown that three terpenes, α- pinene (**9)** and β-pinene (**10**) and camphene **(65**) could be useful as biomarkers for susceptibility of mango to mango gall fly infestation (Augustyn *et al*., 2010). The structures of some fruit fly bioactive compounds reported in mango varieties are shown in Figure 2.3.

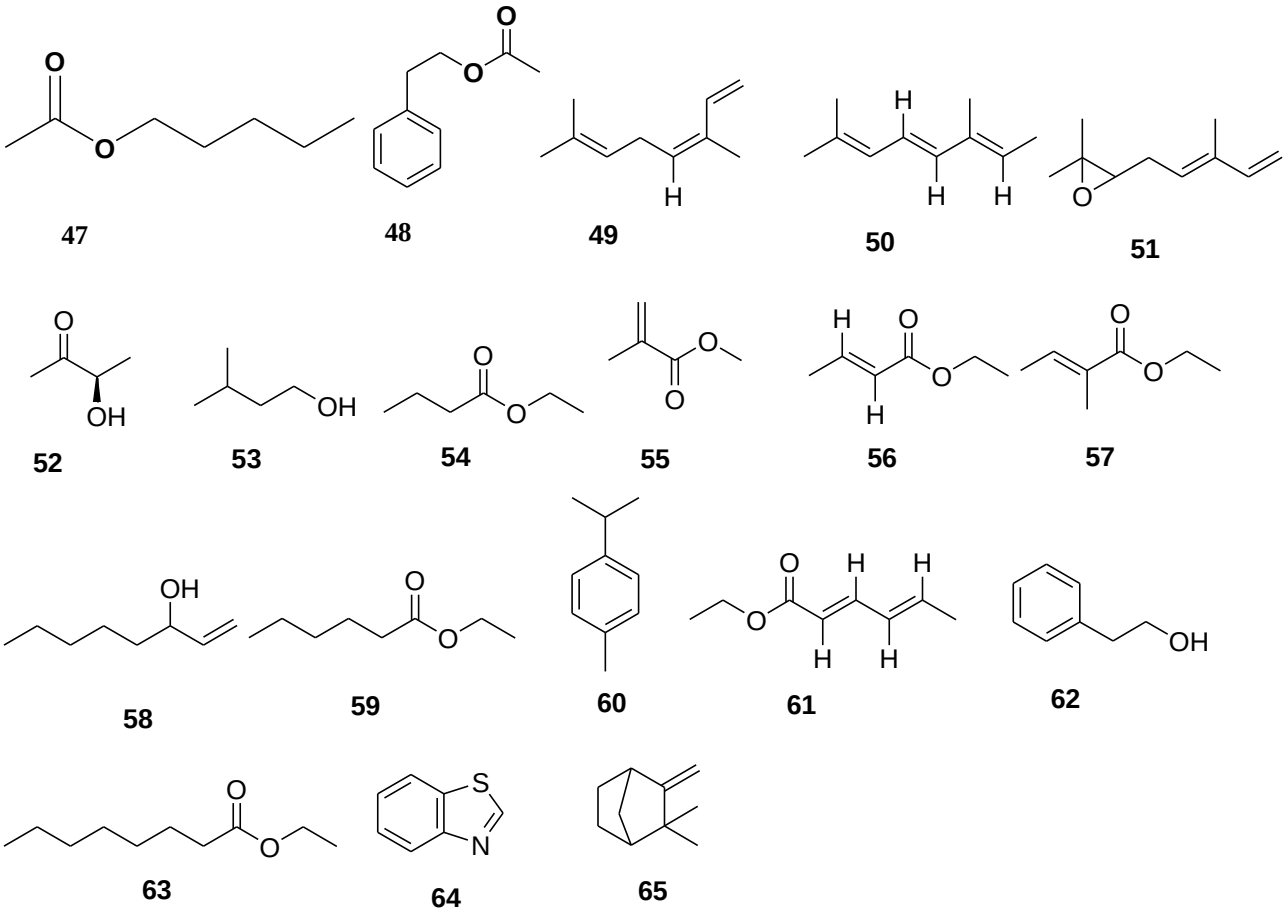


Figure 2.3: **Structures of some fruit fly bioactive compounds reported in mango varieties**

2.16 Biosynthetic pathways of plant monoterpenes

Monoterpenoids, like all terpenoids, are derived from the isopentenyl diphosphate (IPP) and its allylic isomer, dimethylallyl diphosphate (DMAPP) (Burke *et al*., 1999), using two separate pathways: plastidial Methyl-erythritol-4-phosphate (MEP) and cytosolic acetate-mevalonate (MVA) pathways to form the IPP (Ganjewala *et al*., 2009).

2.16.1 The Mevalonate (MVA) pathway

The plant MVA pathway is comparable to that existing in animal and yeast cells. The reaction starts by condensation of two units of Acetyl coenzyme A (Ac-CoA) into acetoacetyl-CoA through a Claisen type reaction catalyzed by acetoacetyl (AcAc)-CoA thiolase (AACT) (Figure 2.4). The enzyme, 3-hydroxy-3-methylglutaryl coenzyme A synthase (HMGS), catalyzes the thermodynamically favorable aldol condensation of

AcAc-CoA with Ac-CoA to form HMG-CoA. Mevalonate formation is accomplished by HMG-CoA reductase (HMGR). It catalyzes the reversible four-electron, two-step and NADPH dependent reduction of HMG-CoA into MVA (Bochar *et al*., 1999).

The remaining steps leading to IPP consist of two phosphorylation reactions that convert MVA to mevalonate 5-diphosphate (MVADP), catalyzed by mevalonate kinase (MK) and phosphomevalonate kinase (PMK). This is followed by an ATP-dependent decarboxy-lation of MVADP into IPP that is catalyzed by diphospho-mevalonate decarboxylase (PPMD. These organisms were proposed to follow a modified MVA route in archeabacteria that involves isopentenyl monophosphate phosphorylation into IPP (Grochowski *et al*., 2006). In the general scheme, isopentenyl-diphosphate isomerase (IDI) catalyzes the formation of DMAPP, the chemically active isoprene unit (Hemmerlin *et al*., 2012).

2.16.2 The Methyl-erythritol-4-phosphate (MEP) pathway

The MEP pathway comprises seven enzymatic steps that lead to the formation of the IPP and DMAPP from pyruvate and D-glyceraldehyde 3-phosphate (Figure 2.4). The enzymes of the MEP pathway are encoded by nuclear genes and targeted to plastids (Rodríguez Concepción and Boronat, 2002; Bouvier *et al*., 2005). The first step in this pathway is the condensation of pyruvate and glyceraldehyde 3-phosphate to form 1deoxy-D-xylulose-5-phosphate (DXP) by DXP synthase (DXS). DXP is then converted into MEP by DXP reductoisomerase (DXR), also called MEP synthase. MEP is then converted to 1-hydroxy-2-methyl-2-(E)-butenyl-4-diphosphate (HMBPP) by the consecutive enzymatic action of 2C-methyl-D-erythritol 4-phosphate cytidyl transferase (MCT), 4-diphosphocytidyl-2Cmethyl- D-erythritol kinase (CMK), 2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase (MDS) and 1-hydroxy-2-methyl-2-(E)-butenyl-4-diphosphate synthase (HDS).

The last step is the branching of HMBPP to IPP and the DMAPP is then catalyzed by the simultaneous enzymatic action of a single enzyme, 1-hydroxy-2-methyl-2-(E)-butenyl-4-diphosphate reductase (HDR) (Rohdich *et al*., 2003). Although both IPP and DMAPP are producesd by HDR in the MEP pathway (Tritsch *et al*., 2010), the plastid that localized IDI, also takes part in the substrate optimization phase by catalyzing IPP isomerization. The enzyme IDI is involved in the interconversion of IPP and DMAP.

IDI is required to produce DMAPP in the cytosol and mitochondria in plant cells, but not in plastids, where the MEP pathway produces both IPP and DMAPP (Adam *et al*., 2002).

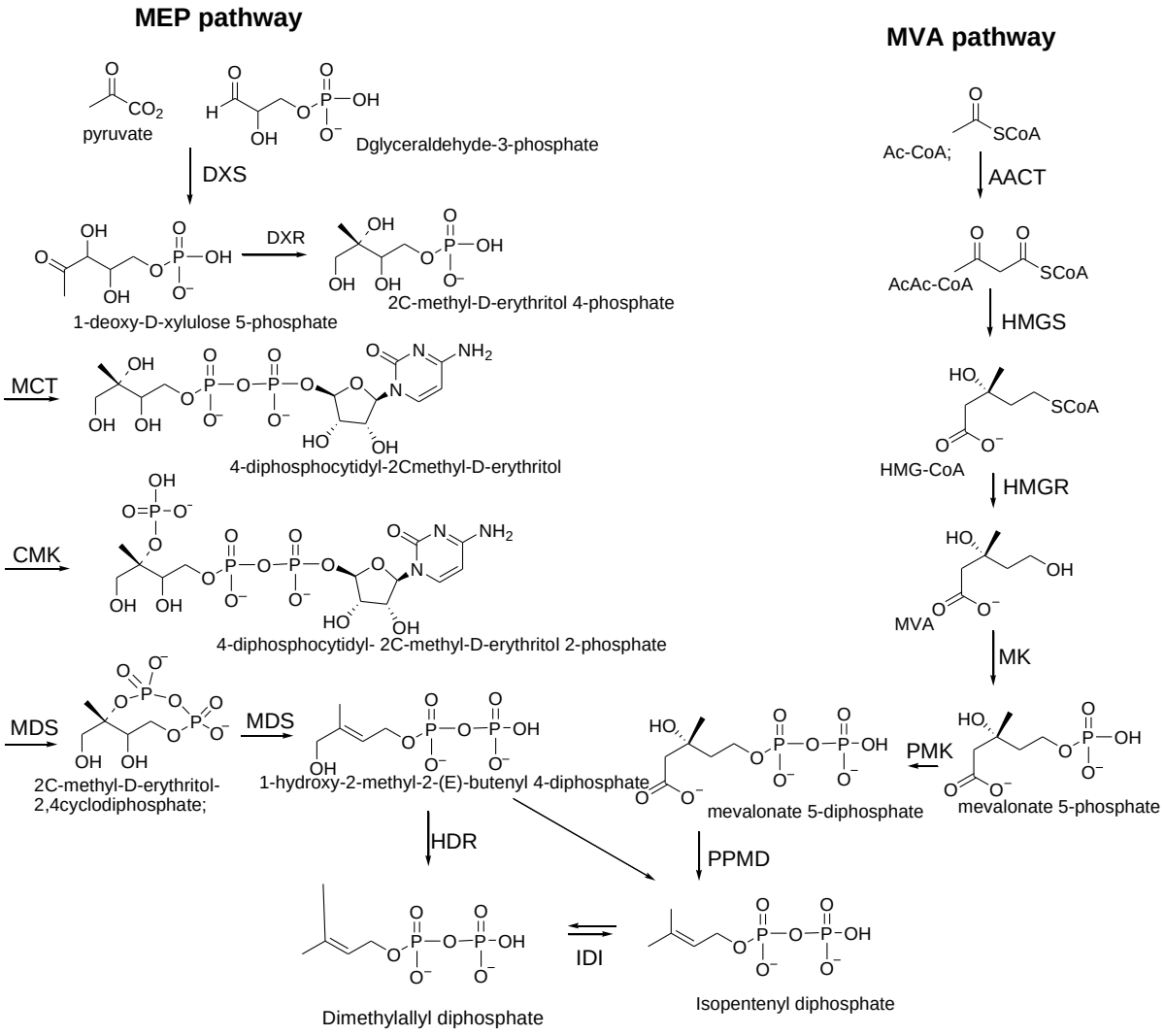
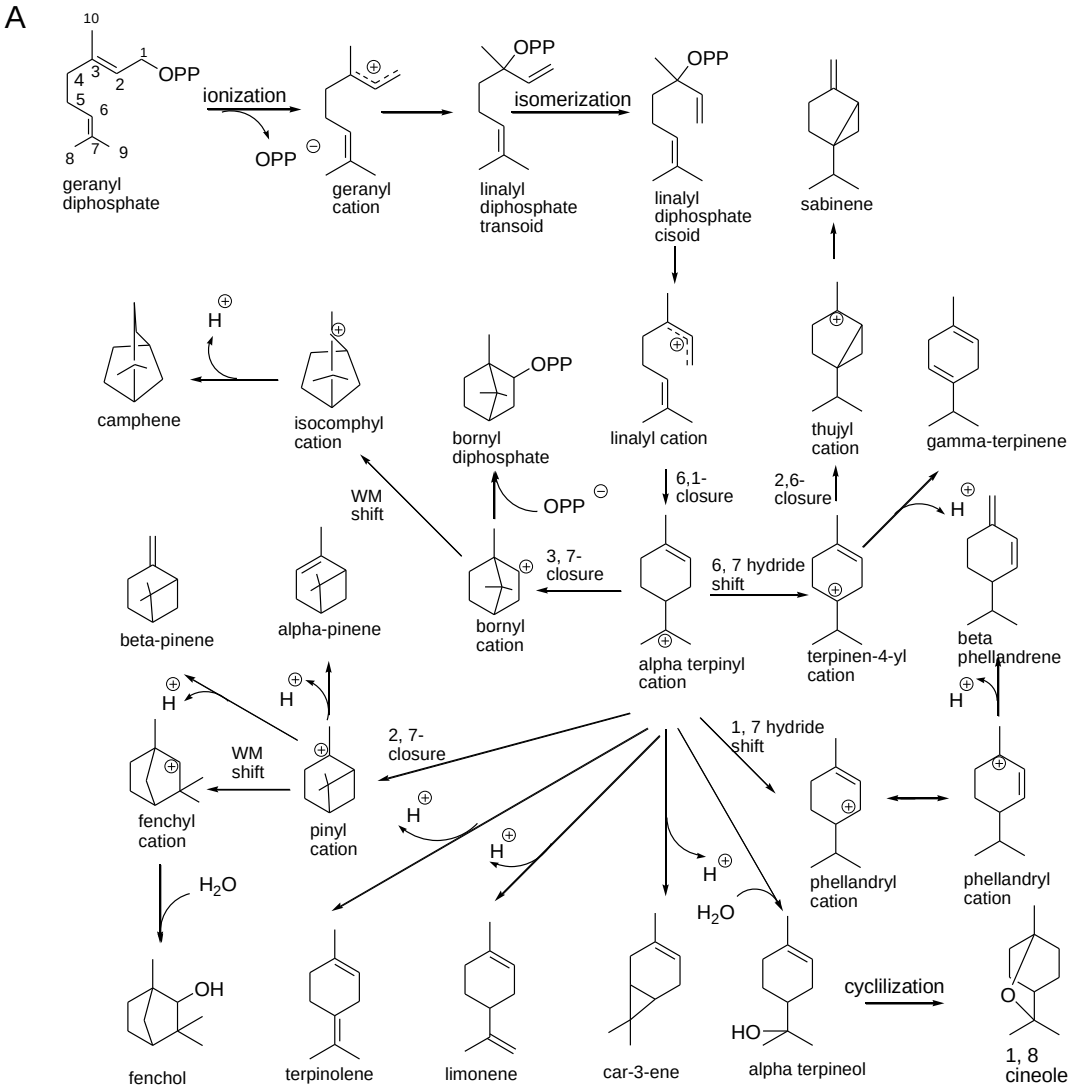


Figure 2.4: Isopentenyl diphosphate synthesis via the MEP or the via the MVA pathway (Hemerlin *et al*., 2012)

Ionization of the geranyl diphosphate substrate is the first step in the reaction mechanisms of all monoterpene synthases (Figure 2.5). The resulting carbocation can undergo a range of cyclizations and hydride shifts and rearrangements before reaching termination by deprotonation or water absorption. The mechanisms of cyclic (A) and acyclic (B) monoterpene synthases are shown separately. The formation of cyclic monoterpenes requires the initial isomerization of the geranyl cation to a linalyl intermediate, capable of cyclization (Ramak *et al*., 2014).



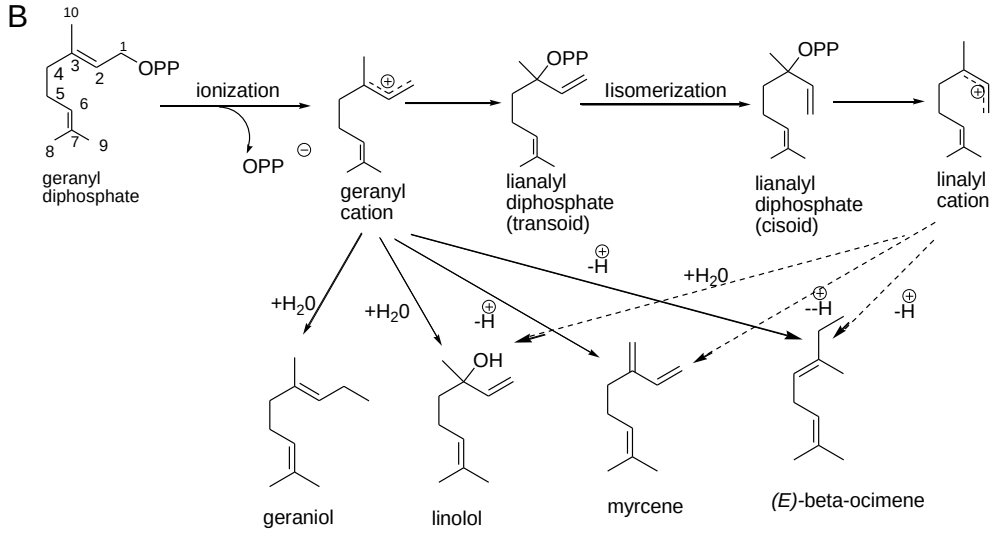


Figure 2. 5: Conversion of geranyl disphosphate to monoterpenes: cyclic (A) and acyclic (B) (Degenhardt *et al*. 2009)

2.17 Behaviourally fruit fly active phytochemicals

Bioassay of the essential oils extracted from four different plant materials *viz.*, *hinoki*, *Eucalyptus*, cinnamon and *Litsea* on the females of Oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) showed that significantly more number of female adults of *B. dorsalis* alighted on filter paper treated with *hinoki* oil, (R)-(+)-limonene, and *Eucalyptus* oil at 400 ppm. However, cinnamon was found to inhibit or repel alighting of the female adults at a dosage of 200, 400 and 800 ppm. Similar repellent activity was observed when filter paper was treated with essential oils extracted from *Litsea* leaves **(**Diongue *et al*., 2013).

Siderhurst and Jang (2006) in their work found out that the ethanol extracts of almond fruit and *Terminalia catappa* L showed female biased attraction of oriental fruit fly, *B. dorsalis* (Hendel) in indoor bioassays. The outdoor olfactometer tests with the ethanol extract showed strong attraction over the control, but in contrast to the laboratory bioassays, there were no differences between the numbers of males and females caught. Activity of the male fruit fly was attributed to the presence of methyl eugenol (**7**), identified in the *T. catappa* extracts by GC-MS analysis.

It has been found that some mango varieties such as Alfa and Espada Stahl are resistant to fruit fly (Rossetto *et al.*, 2006). However, no scientific reason has been given in support of non-preference for oviposition. Studies focusing on the use of host-plant resistance to control this insect, revealed ovipositional non-preference of *B. dorsalis* to selected polyembryonic varieties of mango through choice/ no-choice bioassays (Jayanthi and Verghese, 2008).

Studies of the preference of fruit fly (*B. dorsalis* complex) on 5 mango varieties (Gedong, Arumanis, Cengkirand Bapang and TO) showed that there was a correlation between the attractiveness and fruits character such as colors, odor, sugar contents and the thickness of skin (Susanto and Syamsudin, 2017)**.** Extract of *Elsholtzia pubescens*, which contained camphor as a major component, attracted male *Bactrocera tau* fruit flies in passion orchards in West Sumatra, Indonesia (Hasyim *et al*., 2007).

2.18 Reaserch gaps

There is no report on comparative chemical analysis of the leaf essential oil and fruit volatiles of Tommy Atkins, Apple, Keitt, Van dyke, Boribo, Ngowe, Sabre and Kent varieties in Kenya. No particular host cues have been attributed to the attractiveness or repellent effect on the mango fruit fly. The research sought to establish if the susceptibility to attack of some mango varieties by fruit fly can be attributed to specific chemicals or blend of chemicals and whether the resistant varieties lack the said chemicals/ blend component(s) or contain other chemicals or blend of chemicals, which repel the fly.

Previous studies have mainly focused on either the chemical composition of mango volatiles or bioassays involving other mango varieties from different countries. Moreover, there is no report of any attempt to exploit the potential of host plant attractants and repellents in controlling the flies in a “push-pull” control strategy where by the repellents will push the flies away from the plants as the attractants pull them into a trap or killing device baited with such volatiles.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Plant material

This study was based on eight varieties of mango, namely: Ngowe, Apple, Keitt, Boribo Tommy Atkins Van dyke, Sabre and Kent varieties of mango collected from a mango farm at Cheptebo, Kerio Valley in Kenya which lies on latitude 00º 31.312 N and longitude 035º 34.504 E at an altitude of 1205 m above sea level.

3.1.1 Collection and treatment of Leaves

The leaves of each variety, approximately 5 months old were picked. After collection, the samples were packed in bag nets and transported to the laboratory for hydrodistillation in fresh status.

3.1.2 Collection and treatment of mango Fruits

Fresh ripe mango fruits were obtained from mango study varieties. Fruits at ripe stage were transported to the laboratory in small cardboard boxes. The fruits did not have any application of inhibitor or accelerator for the control of maturation. Fruits that had no apparent skin damage were selected for volatile collection.

3.2 Hydrodistillation of Essential oils

The method for hydrodistillation was adopted from Liu *et al* (2010) with some modification. Briefly, fresh leaves (750 g of each variety) were subjected to hydrodistillation in a 5 L round bottomed flask fitted with Clevenger-like apparatus for 4 hours and the distillate collected over water (Plate 3.1). The procedure was repeated two more times to allow the collection of reasonable amounts of oils. The wet essential oil extracts were dried using anhydrous Na_2SO_4 and stored in amber vials in a refrigerator at -4°C for further use.

Plate 3. 1: Photo of Clevenger apparatus used in hydrodistillation of essential oils (Source: Author, 2016)

3.3 Volatiles isolation

The fruit was washed with distilled water, then the skin and kernel got separated manually by using a stainless steel knife and 200 g of macerated pulp were placed into a glass bottle container (with diameter 10 cm and height 20 cm) with metallic cap that had a small hole (5 mm i diameter) at the center. The capture of volatile components was undertaken on different soxhlet cleaned porapak Q adsorbent filters using an air entrainment kit powered by car battery (12 volts D. C) (Rayaisse *et al*., 2010) as shown in plate 3.2. After collection, the tubes were heat-sealed in glass ampoules and stored at -4°C, for further analysis.

Plate 3. 2: Photo showing set up system for extraction and collection of volatile constituents (Source: Author, 2016)

3.4 Chemical analysis for essential oils and volatiles

3.4.1 Preparation of samples for analysis

The trapped volatiles of mango juice were eluted with 100 µL pure GC-grade DCM, which was then followed by collection of samples of 5µL for GC-MS analysis from which 1µL for each sample was injected into the GC-MS equipment.

3.4.2 GC-MS Instrumentation

Qualitative and quantitative analyses of the volatiles were performed using GC-FID and GC-MS. The GC-FID analyses were carried out on a GC HP-5890 II equipment linked to a split-splitless injector, attached to HP-5 column (25 m x 0.32 mm, 0.52 mm film thickness) and fitted to FID. Carrier gas flow rate (He) was 1 mL/min, split ratio 1:30, injector temperature was 250°C, detector temperature 270°C, while column temperature was linearly programmed from 40–240°C (at rate of 5°C/min). The same analytical conditions were employed for GC/MS analysis, where HP G 1800C Series II GCD system equipped with HP-5MS column (30 m x 0.25 mm, 0.25 mm film thickness) was used. Transfer line was heated at 270°C. Mass spectra were acquired in EI mode (70 eV), in *m/z* range 40–400 a.m.u, scan time 1.5 s with the filament solvent delay time set at 3 min.

3.4.3 Identification of compounds

Identification of oil components was achieved on the basis of their retention indices (RI), which was determined with reference to a homologous series of normal alkanes using the following generalized van den dool and Kratz equation (H´erent *et al*., 2007).

$$I_X = 100\left(\frac{t_x - t_n}{t_{n+1} - t_n} + n\right)$$

Where I_x = Retention Index (RI) of a component

t_x = retention time of component

n = carbon number of preceding n-alkane

n+1 = carbon number of subsequent n-alkane

The identification of unknown compounds was achieved by comparing the retention index (RI) of unknown volatile compound with those recorded in RI databases, and matching the experimental mass spectrum with those stored in MS libraries (Jennings, 1980; Kondjoyan & Berdague 1996; Adams, 2007). Since several compounds may share an identical RI (Goodner, 2008) and the structurally related compounds may produce similar fragments in mass spectra, the two methods were combined to improve the accuracy of identification (Bianchi *et al*., 2007). For the purpose of quantitative analysis area percentage data obtained by GC-FID were used as the base and tabulated.

3.5 Behavioral Assays

The crude mango leaf essential oils, mango juice and compounds identified from the essential oil and the juice aroma were tested for the activity on fruit fly both at the laboratory and field situations. The identified compounds from mango fruit, *M. indica,* were tested for attractiveness or repellency with both male and female flies in two separate bioassays: Dual-choice laboratory olfactometer tests and outdoor bioassay tests using sensus trap (Jang *et al*., 1997). Subtraction behavioural assays were employed in evaluating the role/contribution of each component to the activity of the blends.

3.6 Preparation of the solutions for bioassay

Neat essentials oils were used in the bioassay. The amounts of the chemical standard solutions used were calculated with reference to the internal standard whose concentration was known and constituted to mimic the natural composition of the compounds in the mango varieties.

3.6.1 Essential oils from the leaves of the mangoes

The neat essential oils from the leaves of Ngowe, Apple, Keitt, Boribo, Tommy Atkins; and Van Dyke mango varieties were prepared by placing 5µL and 50 µL of each oil in a 1 mL sample vials for laboratory and field experiments, respectively. The vials had caps through which a capillary tube of 1 mm internal diameter was fitted.

3.6.2 Blends of chemical standards

The amounts of chemical standards of some of the major compounds that were identified in the leaf essential oils and fruit juices of the mango varieties were calculated with reference to the internal standard whose concentration was known as shown below. The calculations were based on the chemical composition of leaf essential oil or ripe fruit volatiles which contained the highest percentage of the respective compounds as shown in Tables 4.1- 4.14 in sections 4.1 and 4.2, and whose concentration was determined as shown below.

Internal standard $1000\ \mu L\ (1mL) \rightarrow 1mg$

Amount injected $= 1\mu L \rightarrow 0.001\ mg$

For α-pinene (Density =0.858 g/cm^3)

It was highest in Apple ripe fruit volatiles. From the chemical composition of Apple ripe fruit volatiles, α-pinene was represented by 34.9 % area.

If $7.8\% \rightarrow 0.001mg$ for the internal standard, then

$34.9\% = \frac{0.001\times34.9}{7.8} = 0.045\ mg/\mu L = 0.0045g/mL$ of α-pinene

$$\text{volume} = \frac{Mass}{Density} = \frac{0.0045g}{0.858\ g/cm^3} = 0.0052\ mL\ \ = 5.2\ \mu L/mL \text{ of } \alpha\text{– pinene}$$

For β-Pinene (Density =0.872 g/ cm^3)

For β-Pinene was highest (21.9%) in Boribo leaf essential oil

If 3.3% → $0.001mg$ for internal standard, then

$21.9\% = \frac{0.001\times21.9}{3.3} = 0.007\ mg/\mu L = 0.007g/mL$ of β-pinene

$$\text{volume} = \frac{Mass}{Density} = \frac{0.007g}{0.872\ g/cm^3} = 0.008\ mL\ \ = 8\ \mu L/mL \text{ of } \beta\text{– pinene}$$

For α-Phellandrene (density =0.846 g/cm^3)

α-Phellandrene was highest in Apple mango leaf essential oil was represented by 3.7% area.

If 0.09% → $0.001mg$ for internal standard, then

$3.7\% = \frac{0.001\times3.7}{0.89} = 0.0042\frac{mg}{\mu L} = 0.0042g/mL$ of α-phillandrene

$$\text{volume} = \frac{Mass}{Density} = \frac{0.0042\ g}{0.846\ g/cm^3} = 0.005\ mL\ \ = 5\ \mu L/mL \text{ of } \alpha\text{– phillandrene}$$

For myrcene (Density=0.794 g/cm^3)

Myrcene was highest in Boribo ripe fruit volatiles and was represented by 57.0% area.

If 7.8% → $0.001mg$ for internal standard, then

$$57\% = \frac{0.001 \times 57}{7.8} = 0.0073\frac{mg}{\mu L} = 0.0073\frac{g}{mL} \text{ of myrcene}$$

$$\text{volume} = \frac{Mass}{Density} = \frac{0.0073\ g}{0.794\ g/cm^3} = 0.0092\ mL\ \ = 9.2\ \mu L/mL \text{ of myrcene}$$

For δ-3-Carene (Density=0.867 g/cm^3)

It was highest in Kent ripe mango fruit volatiles and was represented by 35.0% area.

If 26.7% → $0.001mg$ for internal standard, then

$$35\% = \frac{0.001\times35}{26.7} = 0.0013\frac{mg}{\mu L} = 0.0013\ g/mL \text{ of δ-3-Carene}$$

$$\text{volume} = \frac{Mass}{Density} = \frac{0.0013\ g}{0.867\ g/cm^3} = 0.1.5\ mL\ \ = 5\frac{\mu L}{mL} \text{ of δ– 3– Carene}$$

For Camphene (Density=0.842 g/cm^3)

Camphene was higest in the Boribo mango leaf essential and was represented by 1.7% erea.

If 3.3 % → $0.001mg$ for internal standard, then

$$1.3\% = \frac{0.001\times1.7}{3.3} = 0.00051\frac{mg}{\mu L} = 0.00017\frac{g}{mL} \text{ ofcamphene}$$

$$\text{volume} = \frac{Mass}{Density} = \frac{0.00051\ g}{0.842\ g/cm^3} = 0.0006\ mL\ =\ 0.6\frac{\mu L}{mL}\ \text{ of camphene}$$

For Ethylbutanoate (Density=0.879 g/cm^3)

The compound was highest in Ngowe mango ripe fruit volatiles and was represented by 15.1% area.

If 13.1 % → $0.001mg$ for internal standard, then

$$15.1\% = \frac{0.001\times15.1}{13.1} = 0.0012\frac{mg}{\mu L} = 0.0012\ g/mL$$

$$\text{volume} = \frac{Mass}{Density} = \frac{0.0012\ g}{0.879\ g/cm^3} = 0.0013\ mL$$

$$=\ 1.3\ \frac{\mu\ L}{mL}\ of\ ethylbutanoate$$

For Methyl salicylate (Density=1.17 g/cm^3)

The chemical standard was highest in Kent ripe fruit volatiles and was represented by 9.3% area.

If 7.8% → $0.001mg$for internal standard, then

$$9.3\ \% = \frac{0.001 \times 9.3}{26.7} = 0.00035\frac{mg}{\mu L} = 0.00035\frac{g}{mL}\ of\ methyl\ salicylate$$

$$\text{volume} = \frac{Mass}{Density} = \frac{0.00035\ g}{1.17\ g/cm^3} = 0.0003\ mL\ \ = 0.30\ \frac{\mu L}{mL} of\ methyl\ saicylate$$

For Ethyl hexanoate (Volatiles): Density=0.869 g/cm^3

Ethyl hexanoate was highest (4.1%) in Keitt ripe mango fruit volatiles.

If 20.9% → $0.001mg$ for internal standard, then

$4.1\% = \frac{0.001\times4.1}{20.9} = 0.0002\frac{mg}{\mu L} = 0.0002\ g/mL$ of ethyl hexanoate

$$\text{volume} = \frac{Mass}{Density} = \frac{0.0002\ g}{0.869\ g/cm^3} = 0.00023mL$$

$$= 0.23\ \frac{\mu L}{mL}\ \ of ethyl\ hexanoate$$

For Ethyl Octanoate (Volatiles): Density=0.847 g/cm^3

It was highest (14%) in Keitt ripe mango fruit volatiles.

If 20.9% → $0.001mg$ for internal standard, then $14\% = \frac{0.001\times14.0}{20.9} = 0.0007\frac{mg}{\mu L} =$ $0.0007\frac{g}{mL}\ of\ ethyl\ octanoate$

$$\text{volume} = \frac{Mass}{Density} = \frac{0.0007\ g}{0.847\ g/cm^3} = 0.0082\ \ = 0.82\ \frac{\mu L}{mL}\ of\ ethyl\ octanoate$$

3.7 Laboratory bioassays

Behavioral observations were made in a glass dual choice Y-tube olfactometer in still-air arena using the crude mango leaf essential oils and chemical standards as described

by Canale *et al* (2015). The Y-tube olfactometer was fabricated from Perspex with the following dimensions: runway length of 105 cm diameter of 23 cm connected to two arms each of 22 cm in length and 13 cm diameter (Plate 3.3).

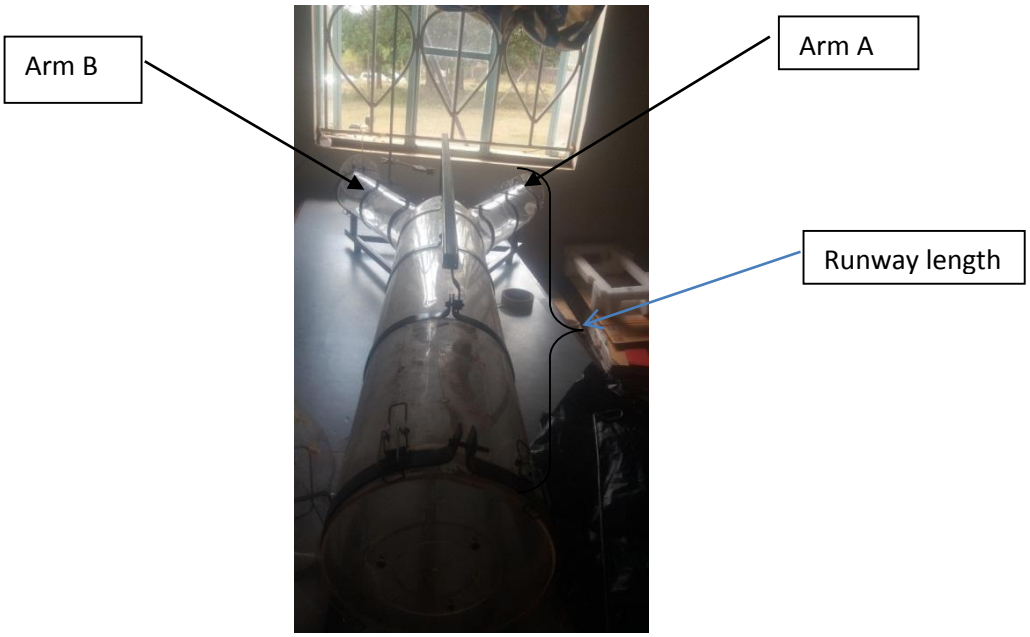


Plate 3. 3: Photo of Y-tube Olfactometer (Source: Author, 2016)

The tube was placed on a table in a room with the arms facing an open window that allowed in fresh air current. In each experiment a sample was placed in one arm while the positive control (methyl eugenol) in the second arm. Tests were done by releasing 10 *Bactrocera invadens* flies that were allowed enough time to choose between the side enriched with the sample and the opposite side which had methyl eugenol (positive control) (Canale *et al*., 2015). The number of fruit *Bactrocera invadens* flies in each arm were counted and recorded. Each test was replicated four times. At the end of each test, the olfactometer was cleaned with distilled water then the sample and control were alternated in the next test to account for positional biasness. The percentages of the flies attracted to the test preparation and to control treatment were calculated from the means of the flies per treatment and dividing the mean value by 10, the total number of flies released in each experiment.

3.8 Field bioassays

In all the experiments completely randomized Latin square block experimental design was used to evaluate the behaviour of the fruit flies towards the test samples. The test samples were placed into 1 ml sample vials (internal diameter =20 mm) with tops having septum through which a capillary tube of 1 mm diameter and 2 cm length was inserted to provide pathway for the volatiles diffusion into the environment. The vials were suspended inside the modified Nzi-like traps (Byamungu, *et al*., 2016) at the same height as the holes in the traps to optimize the rate of diffusion (Plate 3.4).

Plate 3. 4: Photo of fruit fly trap in Field Bioassay set up (Source: Author, 2016)

The traps baited with test samples were placed in the mango farm using completely randomized Latin Square block experimental Design model for 10 hours per day (Williams *et al*., 2006). Surveillance was done at 15 minutes' intervals and the number of *Bactrocera invadens* flies captured by the traps were collected then recorded.

3.8.1 Bioassay of the mango leaf essential oils

The crude essential oil extracts from Ngowe, Apple, Keitt, Boribo, Tommy Atkins and Van Dyke, which were prepared as earlier described under section 3.6.1, were

bioassayed in the field using the modified Nzi-trap (Byamungu, *et al*., 2016) in a 7×7 completely randomized Latin square block experimental design (Appendix II) that included F (methyl eugenol) as the seventh treatment besides the six mango essential oils. In this experiment which took 7 days each treatment was exposed to all the seven sites on different days.

3.8.2 Bioassay of the mango juice extracts

The juice from each of the six mango varieties was put in 1ml vial which got closed using a Teflon tape and the 1mm diameter capillary tube inserted to allow the mango fruit to diffuse into the environment. These six treatments together with F (methyl eugenol control) were tested on *B. invadens* fruit fly in a 7×7 completely randomized Latin square block experimental design (Appendix V).

3.8.3 Bioassay of the chemical standards

The purpose of bioassaying the chemical standards was to identify the specific compound or blend of compounds that would exhibit biological activity when tested on *B. invadens* in the field. The compounds were prepared as described in section 3.6.1.2 and the blends mixed in a ratio that mimicked their natural composition in the mango as shown in Tables 4.1 to 4.8. The bioassay of the compounds was done using the following blends or individual compounds:

- Blend of esters (ethyl butanoate, methyl salicylate, ethyl hexanoate and ethyl octanoate) using subractional assay in a 5×5 completely randomized Latin square block experimental design (Appendix IX).
- Monoterpenes and one ester (myrcene, δ-3-carene, α-penene and ethyl butanoate) using subractional assay in a 5×5 completely randomized Latin square block experimental design (Appendix XII).
- Individual compounds (camphene, α-phillandrene, ethyl butanaoate, α-pinene, myrcene, β-pinene, δ-3-carene and methyl euginol (positive control)) in 8×8 completely randomized Latin square block experimental design (Appendix XV).

3.9 Data Analysis

Results of the Dual-choice olfactometric bioassay and the field bioassay were compared using *t*-tests (Proc TTEST) (SAS, 2012). Multiple-choice test results were analyzed using ANOVA, and means compared using t-tests (LSD) (SAS, 2012). All analyses of significance were made at the $p < 0.05$ level of significance or lower.

CHAPTER FOUR

RESULTS

4.1 Essential oil chemistry of the Mango leaf oils

Chemical analysis of the essential oils from the mango cultivars by GC-MS lead to the identification of various compounds, which are given in sections 4.1.1 to 4.1.6 in the essential oils of 6 mango oil varieties. The same compounds identified in different mango oil varieties have the same peak labels.

4.1.1 Essential oil composition of Ngowe mango leaf oil

The analysis of the chemical constituents of the leaf oil of Ngowe mango revealed a total of twenty one (21) compounds representing 69.3% of the total leaf oil, which included generally oxygenated and non-oxygenated monoterpenes (38.4%), sesquiterpenes (34.4%) and non terpenoid hydrocarbons (12.7%) (Figure 4.1; Table 4.1). The major monoterpenoids identified include β-pinene (**10),** peak 5a (7.7%); α-pinene (**9**), peak 3a (5.8%) and β-phellandrene (**31**), peak 11a (4.9%). The major oxygenated monoterpene included cryptone (**66**), peak 20a (2.8%) while sesquiterpenes identified were α-gurjunene (**11**), peak 29a (4.2%); α-humulene (**12**), peak 31a (3.4%); β-selinine (**21**), peak 38a (2.0%); valencene (**67**), peak 39a (4.3%) and the oxygenated sesquiterpene was spathulenol (**20**), peak 47a (14.8%) (Table 4.1).

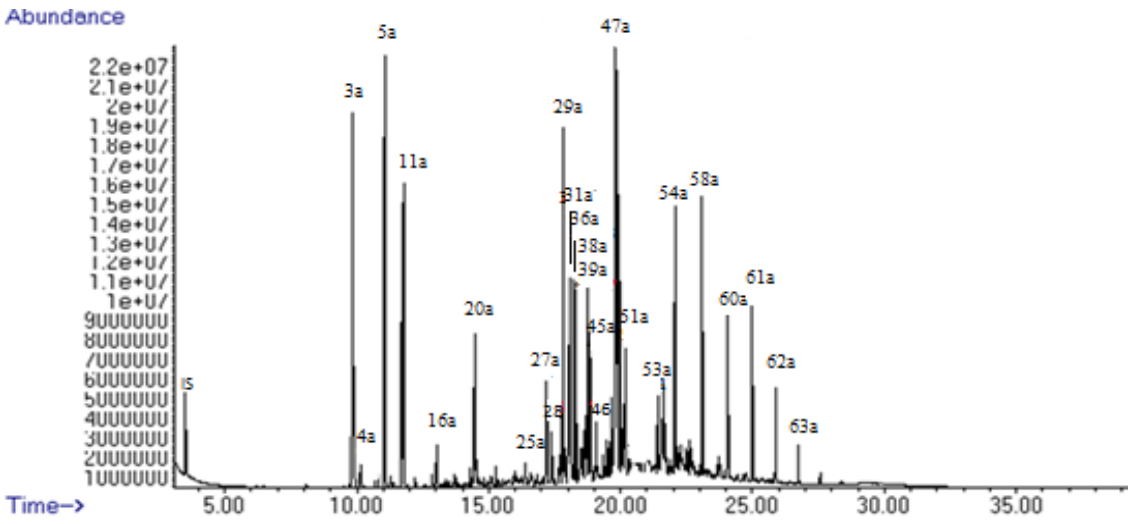


Figure 4.1: Representative total ion chromatogram of Ngowe leaf essential oil

N/B: Peak numbers correlate to compounds listed in Table 4.1.

Table 4.1: Ngowe leaf essential oil chemical composition

S/No	Peak	RI	Compound/Class	% Composition
	IS	688	1–heptene	0.5
1	3a	936	α-Pinene	5.8
2	4a	948	Camphene	0.3
3	5a	976	β-Pinene	7.7
4	11a	1028	β-Phellandrene	4.9
5	16a	1100	3-(4-methyl-3-pentenyl)-Furan	0.8
6	20a	1184	Cryptone	2.8
7	25a	1314	4-Hydroxy-Cryptone	0.7
8	27a	1374	α-Copaene	1.8
9	28a	1389	β-Elemene	1.4
10	29a	1409	α-Gurjunene	4.2
11	31a	1452	α-Humulene	3.4
12	36a	1478	ϒ- Muurolene	0.9
13	38a	1489	β-Selinene	2
14	39a	1494	Valencene	4.3
13	45a	1525	Unidentified E	2
16	46a	1528	cis- Calamenene	1.6
17	47a	1577	Spathulenol	14.8
18	51a	1650	Unidentified F	2.2
19	53a	1672	Unidentified H	2.4
20	54a	1700	Heptadecane	3.8
21	58a	1898	Nonadecane	3.9
22	60a	2000	Unidentified K	3.1
23	61a	2100	Heneicosane	2.3
24	62a	2200	Docosane	1.4
25	63a	2400	Tetracosane	0.5
			Non oxygenated Monoterpenes	18.7
			Non oxygenated Sesquiterpenes	19.6
			Oxygenated monoterpenes	3.5
			Oxygenated sesquiterpenes	14.8
			Non-terpenoid hydrocarbons	11.9
			Non-terpenoid oxygenated hydrocarbons	0.8
Total monoterpene, sesquiterpene and non terpenoid constituents				69.3

Key: IS = Internal Standard

4.1.2 Essential oil composition of Apple mango leaf oil

A total of twenty four (24) compounds were identified in the Apple mango leaf essential oil representing 76.1% of the total leaf oil (Figure 4.2; Table 4.2). The identified compounds comprised oxygenated and non-oxygenated monoterpenes (40.1%), oxygenated and non oxygenated sesqueterpenes (34.5%) and non terpenoid oxygenated hydrocarbons (1.5%) (Table 4.2). The major monoterpenes identified were α-pinene (**9**), peak 3a (10.3%); β-pinene (**10**), peak 5a (6.8%); α-phellandrene (**42**), peak 7a (3.7%) and β- phellandrene (**31**), peak 11a (12.1%). The major sesqueterpenes indentified were α-gurjunene (**11**), peak 29a (9.7%); α-humulene (**12),** peak 31a (2.6%); allo-aromadendrene (**68**), peak 33a (3.1%); ϒ-gurjunene (**41**), peak 35a (2.4%) and δ-cadinene (**69**), peak 42a (8.4%) (Table 4.2).

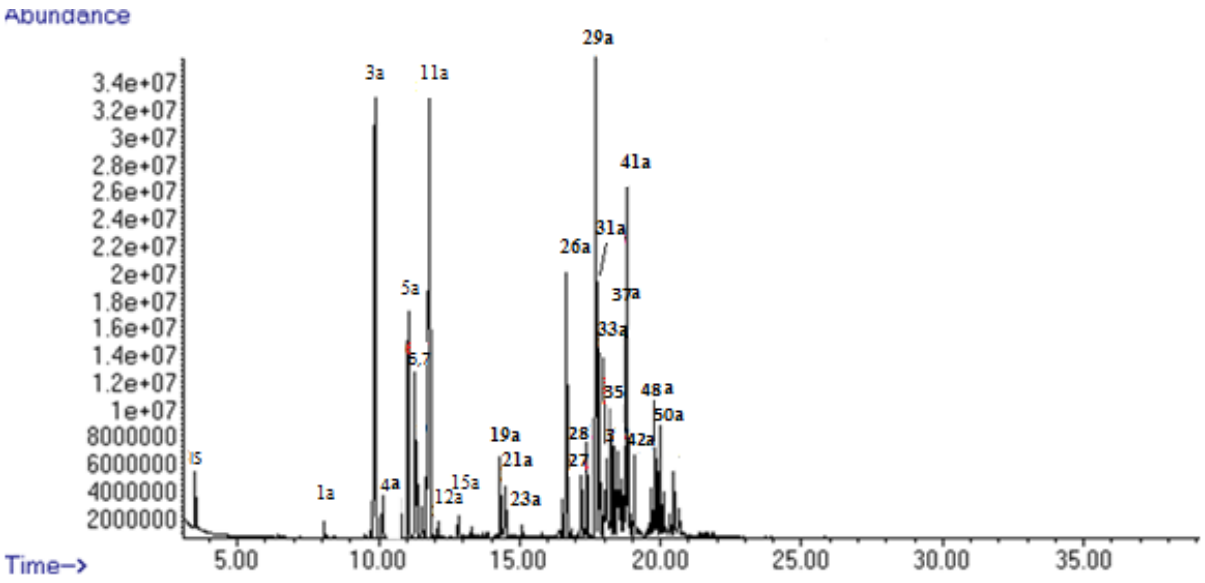


Figure 4.2: Representative total ion chromatogram of Apple leaf essential oil

N/B: Peak numbers correlate to compounds listed in Table 4.2.

Table 4.2: Apple leaf essential oil chemical composition

S/No	Peak	RI	Compound/Class	% composition
	IS	688	1-heptene	0.09
1	1a	852	*3Z*- Hexenol	0.4
2	3a	936	α-Pinene	10.3
3	4a	948	Camphene	0.8
4	5a	976	β-Pinene	6.8
5	6a	1000	δ-2-Carene	0.6
6	7a	1005	α-Phellandrene	3.7
7	11a	1028	β-Phellandrene	12.1
8	12a	1044	E-β- Ocimene	0.5
9	15a	1090	p-Cymenene	0.7
10	19a	1176	Terpinen-4-ol	1.1
11	21a	1188	α-Terpineol	1.3
12	23a	1300	3Z-Hexenyl valerate	1.1
13	26a	1326	Unidentified	3.1
14	27a	1374	α-Copaene	1.5
15	28a	1389	β-Elemene	1.5
16	29a	1409	α-Gurjunene	9.7
17	31a	1452	α-Humulene	2.6
18	33a	1458	*Allo*-aromadendrene	3.1
20	35a	1475	ϒ-Gurjunene	2.4
21	37a	1484	Germacrene D	1.8
22	41a	1500	Bicyclogermacrene	1.6
23	42a	1520	δ-Cadinene	8.4
24	48a	1602	Ledol	1.9
25	50a	1642	4-Allyloxyimino-2-carene	2.2
			Non oxygenated monoterpenes	35.5
			Non oxygenated sesquiterpenes	32.6
			Oxygenated monoterpenes	4.6
			Oxygenated sesquiterpenes	1.9
			Non-terpenoid hydrocarbons	0
			Non-terpenoid oxygenated hydrocarbons	1.5
Total monoterpene, sesquiterpene and non terpenoid constituents				76.1

Key: IS = Internal Standard

4.1.3 Essential oil composition of Keitt mango leaf oil

A total of seventeen (17) compounds were identified in the leaf essential oil of Keitt mango, representing 76.2% of the total leaf oil (Figure 4.3; Table 4.3). The compounds comprised of oxygenated and non-oxygenated monoterpenes (37.0%) and sesquiterpenes (49.2%) (Table 4.3). The major monoterpenes included α-pinene (**9**), peak 3a (2.4%) and δ-3-carene (**8**), peak 8a (19.4%). The major sesqueterpene identified included α-copaene (**37**), peak 29a (4.9%); β-elemene (**70**), peak 28a (4.1%); α-gurjunene (**11**), peak 29a (17.4%); α-humulene (**12**), peak 31 (2.7%); ϒ-muurolene

(**71**), peak 36a (3.0%); germacrene D (**39**), peak 37a (5.3%); viridoflorene (**72**), peak 40a (7.7%) and the sesquiterpene alcohol spathulenol (**20**), peak 47a (3.5%) (Table 4.3).

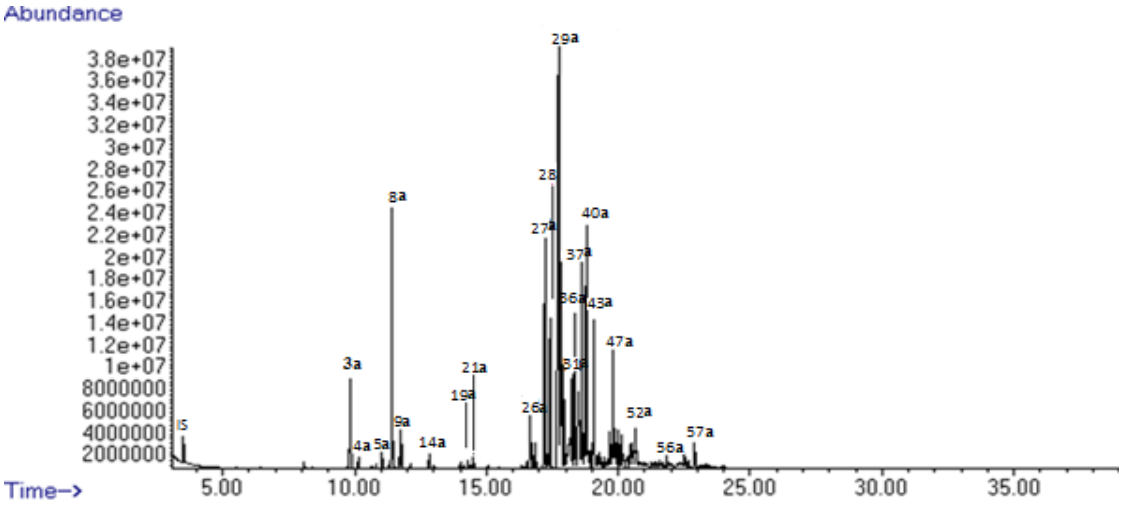


Figure 4.3: **Representative total ion chromatogram of Keitt essential oil**

N/B: Peak numbers correlate to compounds listed in Table 4.3.

Table 3: Keitt leaf essential oil chemical composition

S/No	Peak	RI	Compound/Class	% composition
	IS	688	1-heptene	0.6
1	3a	936	α-Pinene	2.4
2	4a	948	Camphene	0.5
3	5a	976	β-Pinene	1.9
4	8a	1008	δ-3- Carene	19.4
5	9a	1024	Limonene	1.0
6	14a	1087	Terpinolene	0.6
7	19a	1176	Terpinen-4-ol	0.6
8	21a	1188	α-Terpineol	0.6
9	26a	1326	Unidentified C	1.0
10	27a	1374	α-Copaene	4.9
11	28a	1389	β-Elemene	4.1
12	29a	1409	α-Gurjunene	17.4
13	31a	1452	α-Humulene	2.7
14	36a	1478	ϒ- Muurolene	3.0
15	37a	1484	Germacrene D	5.3
16	40a	1496	Viridiflorene	7.7
17	43a	1521	Unidentified D	3.1
18	47a	1577	Spathulenol	3.5
19	52a	1663	Unidentified G	1.2
20	56a	1774	epi-Cyclocolorenone	0.6
21	57a	1880	Unidentified I	0.9
			Monoterpenes	25.8
			Sesquiterpenes	45.1
			Oxygenated sesquiterpenes	4.1
			oxygenated monoterpenes	1.2
Total monoterpene, sesquiterpene and non terpenoid constituents				76.2

Key: IS = Internal Standard

4.1.4 Essential oil composition of Boribo mango leaf oil

A total of twenty two (22) compounds were identified from Boribo leaf oil representing 73.3% of the total leaf oil (Figure 4.4; Table 4.4). The identified compounds included both oxygenated and non-oxygenated monoterpenes (38.7%) as well as sesquiterpenes (32.8%) (Table 4.4). The major monoterpenes included α-pinene (**9**), peak 3a (10.9%); camphene (**65**), peak 4a (1.7%); β-pinene (**10**), peak 5a (21.9%) and limonene (**24**), peak 9a (1.8%). The major sesqueterpenes included β-elemene (**70**), peak 28a (1.6%); α-gurjunene (**11**), peak 29a (8.7%); α-humulene (**12**), peak 31a (2.5%); α-patchoulene (**73**), peak 32a (1.9%); allo-aromadendrene (**68**), peak 33a (2.7%); viridiflorene (**72**), peak 40a (2.9%) and the alcohol 13-hydroxy-valencene (**74**), peak 55a (4.6%) (Table 4.4).

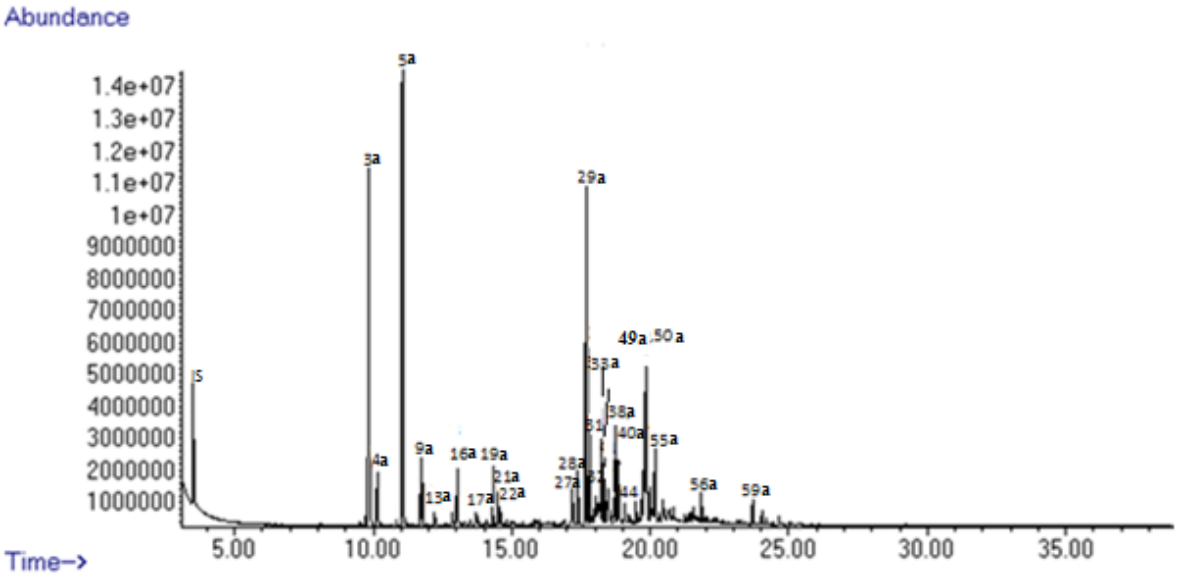


Figure 4.4: **Representative total ion chromatogram of Boribo leaf essential oil**

N/B: Peak numbers correlate to compounds listed in Table 4.4.

Table 4 4: Boribo leaf essential oil chemical composition

S/No	Peak	RI	Compound/Class	% composition
	IS	688	1-heptene	3.3
1	3a	936	α-Pinene	10.9
2	4a	948	Camphene	1.7
3	5a	976	β-pinene	21.9
4	9a	1024	Limonene	1.8
5	13a	106	Unidentified	0.5
6	16a	1100	3-(4-Methyl-3-pentenyl)- Furan	1.8
7	17a	1136	*trans*-Pinocarveol	0.4
8	19a	1176	Terpinen-4-ol	0.5
9	21a	1188	α-Terpineol	1.0
10	22a	1195	Myrtenal	0.5
11	27a	1374	α-Copaene	1.2
12	28a	1389	β-Elemene	1.6
13	29a	1409	α-Gurjunene	8.7
14	31a	1452	α-Humulene	2.5
15	32a	1454	α-Patchoulene	1.9
16	33a	1458	*allo*-Aromadendrene	2.7
17	34a	1460	Unidentified	0.8
18	38a	1489	β-Selinene	1.0
19	40a	1496	Viridiflorene	2.9
20	44a	1523	*trans*- Calamenene	1.0
21	49a	1608	Humulene epoxide II	3.3
22	50a	1642	4-Allyloxyimino-2-carene	4.6
23	55a	1768	13-Hydroxy-Valencene	4.6
24	56a	1774	*epi*-Cyclocolorenone	1.4
25	59a	1925	Unidentified J	0.9
			Monoterpenes	36.3
			Sesquiterpenes	23.5
			oxygenated monoterpenes	2.4
			Oxygenated sesquiterpenes	9.3
			Non-terpenoid hydrocarbons	0.0
			Non-terpenoid oxygenated hydrocans	1.8
Total monoterpene, sesquiterpene and non terpenoid constituents				73.3

Key: IS = Internal Standard

4.1.5 Essential oil composition of Tommy Atkins mango leaf oil

A total of seventeen (17) compounds were identified in the Tommy Atkins leaf essential oil which represented 83.6% of the total leaf oil (Figue 4.5; Table 4.5). The identified compounds comprised monoterpenes (oxygenated and non-oxygenated) (61.9%) and non-oxygenated sesqueterpenes (21.7%) (Table 4.5). The monoterpenes included α-pinene (**9**), peak 3a (24.5%); β-pinene (**10**), peak 5a (2.9%); δ-3-carene (**8**), peak 8a (29.2%) and β-phellandrene (**31**), peak 11a (2.7%). The major sesqueterpenes included α-gurjunene (**11**), peak 29a (10.3%); *E*-caryophellene (**13**), peak 30a (2.2%); β- selinine (**21**), peak 38a (3.0%) and viridiflorene (**72**), peak 40a (2, 2%) (Table 4.5).

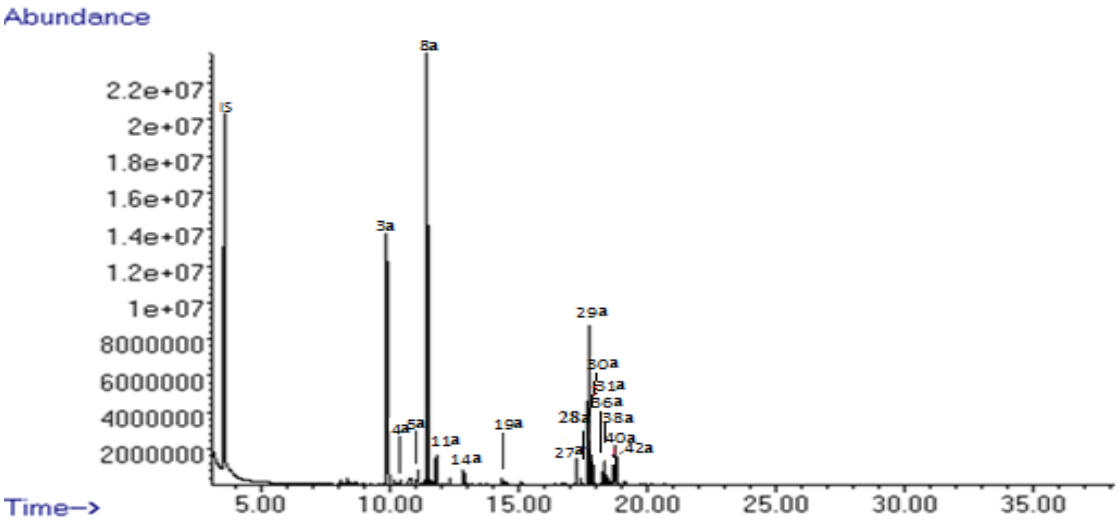


Figure 4.5: **Representative total ion chromatogram of Tommy Atkins leaf essential oil**

N/B: Peak numbers correlate to compounds listed in Table 4.5.

Table 4. 5: Tommy Atkins leaf essential oil chemical composition

S/No	Peak	RI	Compound/Class	% composition
	IS	688	1-heptene	9.4
1	3a	936	α-Pinene	24.5
2	4a	948	Camphene	0.5
3	5a	976	β-pinene	2.9
4	8a	1008	δ-3- Carene	29.2
5	11a	1028	β-Phellandrene	2.7
6	14a	1087	Terpinolene	1.4
7	19a	1176	Terpinen-4-ol	0.4
8	21a	1188	α–Terpineol	0.3
9	27a	1374	α–Copaene	1.4
10	28a	1389	β-Elemene	0.4
11	29a	1409	α–Gurjunene	10.3
12	30a	1417	E-Caryophyllene	2.2
13	31a	1452	α– Humulene	1.3
14	36a	1478	ϒ- Muurolene	0.6
15	38a	1489	β-Selinene	3.0
16	40a	1496	Viridiflorene	2.2
17	42a	1520	δ–Cadinene	0.3
			Monoterpenes	61.2
			Sesquiterpenes	21.7
			Oxygenated monoterpenes	0.7
			Oxygenated sesquiterpenes	0.0
			Non-terpenoid hydrocarbons	0.0
			Non-terpenoid oxygenated hydrocans	0.0
Total monoterpene, sesquiterpene and non terpenoid constituents				83.6

Key: IS = Internal Standard

4.1.6 Essential oil composition of Van Dyke mango leaf oil

A total of sixteen (16) compounds were identified in the Van Dyke leaf essential oil constituting 89.2% of the total leaf oil (Figue 4.6, Table 4.6). Two compounds at peak 18a and 24a could not be identified. The identified compounds comprised monoterpenes (44.4%), sesqueterpenes (43.9%) and non-terpenoid hydrocarbons (0.9%) (Table 4.6). The major monoterpenes included α-pinene (**9**), peak 3a (18.0%); β-pinene (**10**), peak 5a (4.3%); δ-3-carene (**8**), peak 8a (17.9%) and sylvestrene (**75**), peak 10a (2.5%); while major sesquiterpenes were α-copaene (**37**), peak 27a (1.5%); α-gurjunene (**11**), peak 29a (16.7%); *E*-caryophellene (**13**), peak 30a (3.7%); α-humulene (**12**), peak 31a (3.9%); β-selinene (**21**), peak 38a (9.9%) and viridiflorene (**72**), peak 40 (6.0%) (Table 4.6). The compounds from the Van Dyke essential oil are shown by the total ion chromatogram (Figure 4.6).

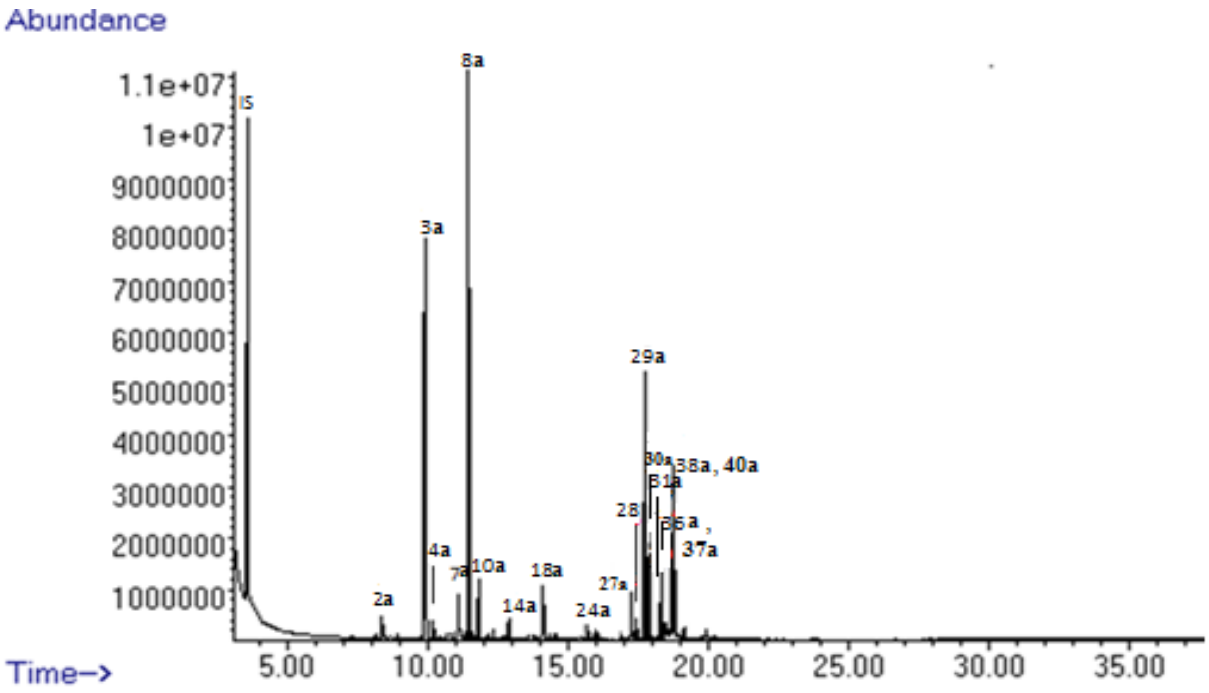


Figure 4.6: **Representative total ion chromatogram of Van Dyke essential oil**

N/B: Peak numbers correlate to compounds listed in Table 4.6.

Table 4.6: Van Dyke leaf essential oil Chemical composition

S/No	Peak	RI	Compound/Class	% composition
	IS	688	1-heptene	10.0
1	2a	860	1,3-dimethyl-benzene,	0.9
2	3a	936	α–Pinine	18
3	4a	948	Camphene	0.8
4	5a	976	β-pinene	4.3
5	8a	1008	δ-3- Carene	17.9
6	10a	1025	Sylvestrene	2.5
7	14a	1087	Terpinolene	0.9
8	18a	1150	Unidentified B	3.8
9	24a	1308	Unidentified C	0.8
10	27a	1374	α–Copaene	1.5
11	28a	1389	β-Elemene	0.9
12	29a	1409	α– Gurjunene	16.7
13	30a	1417	E-Caryophyllene	3.7
14	31a	1452	α– Humulene	3.9
15	36a	1478	ϒ- Muurolene	0.7
16	37a	1484	Germacrene D	0.6
17	38a	1489	β-Selinene	9.9
18	40a	1496	Viridiflorene	6
			Monoterpenes	44.4
			Sesquiterpenes	43.9
			oxygenated monoterpenes	0
			Oxygenated sesquiterpenes	0
			Non-terpenoid hydrocarbons	0.9
			Non-terpenoid oxygenated hydrocans	0
Total monoterpene, sesquiterpene and non terpenoid constituents				89.2

Key: IS = Internal Standard

4.2 Fruit pulp volatile chemistry of the ripe mango fruits

The GC-MS analysis of volatiles of eight mango varieties namely Ngowe, Apple, Keitt, Boribo, Tommy Atkins, Van Dyke, Sabre and Kent revealed a total of 58 different compounds. The identified compounds were different qualitatively and quantitatively and are presented in this section.

4.2.1 Volatile aroma chemistry of ripe Ngowe mango fruit pulp

The analysis of the chemical constituents of the fruit pulp of Apple mango revealed a total of twenty three (23) compounds representing 77.4% of the total fruit volatile (Figure 4.7; Table 4.7), which included oxygenated and non-oxygenated monoterpenes (38.0%), sesquiterpenes (2.4%), non terpenoid hydrocarbons (37.0%) (Table 4.7). The major monoterpenes were α-pinene (**9**), peak 11b (10.9%) and myrcene (**23**), peak 16b

(22.8%). The non terpenoid hydrocarbons included toluene (**34**), peak 3b (5.5%) and ethyl butanoate (**54**), peak 5b (15.1%) (Table 4.7).

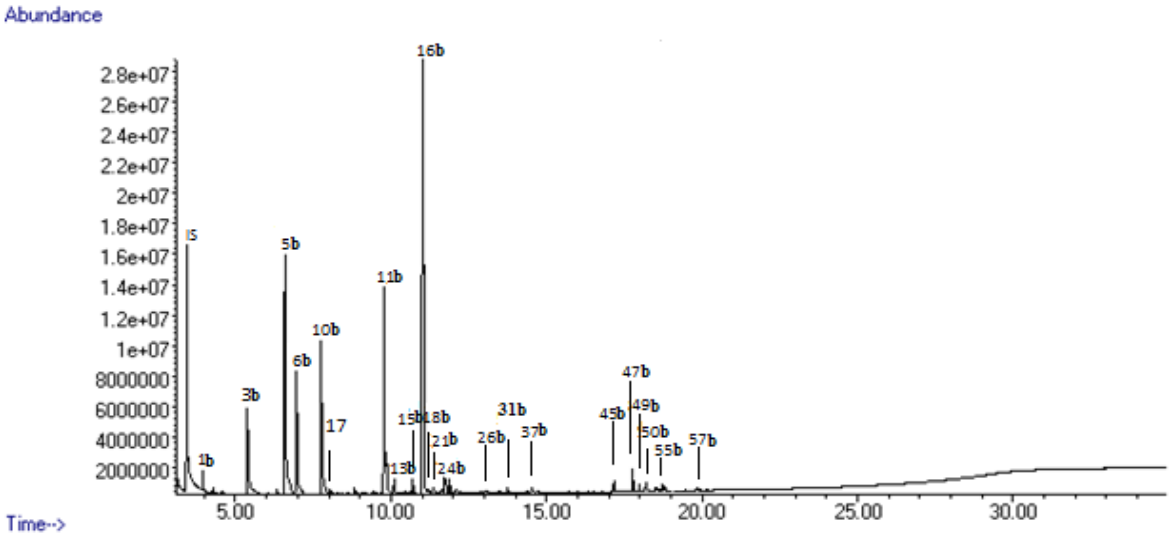


Figure 4.7: **Representative total ion chromatogram of Ngowe ripe fruit volatiles**

N/B: Peak numbers correlate to compounds listed in Table 4.7.

Table 4.7: Ngowe ripe fruit volatiles chemical composition

S/No	Peak	RI	Compound/Class	% composition
	IS	696	isopropylcyclobutane	13.1
1	1b	713	3-Hydroxy-2-butanone	1.0
2	3b	782	Toluene	5.5
3	4b	785	2,3-Butanediol	0.3
4	5b	800	Ethyl butanoate	15.1
5	6b	835	(E)-2-Butenoic acid, ethyl ester	5.9
6	10b	926	(Z)-2-Butenoic acid, ethyl ester	8.7
7	11b	932	α-Pinene	10.9
8	13b	946	Camphene	0.8
9	15b	974	β-Pinene	1.0
10	16b	988	Myrcene	22.8
11	18b	997	Ethyl hexanoate	0.2
12	21b	1008	δ-3-Carene	0.4
13	22b	1020	*o*-Cymene	0.2
14	24b	1025	β- Phellandrene	1.4
15	26b	1044	*(E)*-β-Ocimene	0.2
16	31b	1141	Camphor	0.3
17	37b	1196	Ethyl octanoate	0.3
18	44b	1374	α-Copaene	0.4
19	46b	1417	*(E-)* Caryophyllene	0.8
20	48b	1437	α-Guaiene	0.3
21	50b	1452	α-Humulene	0.4
22	55b	1528	*Cis*-Calamenene	0.3
23	57b	1582	Caryophyllene oxide	0.2
			Monoterpene hydrocarbons	37.7
			Oxygenated monoterpenes	0.3
			Sequiterpene hydrocarbons	2.2
			Oxygenated sesquiterpenes	0.2
			Non-terpenoid hydrocarbons	5.5
			Oxygenated non terpenoid compounds	31.5
Total monoterpene, sesquiterpene and non terpenoid constituents				77.4

Key: IS = Internal Standard

4.2.2 Volatile aroma chemistry of ripe Apple mango fruit pulp

The analysis of the chemical constituents of the fruit pulp of Apple mango revealed a total of twenty two (22) compounds representing 78.4% of the total fruit volatiles (Figure 4.8; Table 4.8). The identified compounds included oxygenated and non-oxygenated monoterpenes (65.9%), sesquiterpenes (1.7%) and non terpenoid hydrocarbons (10.8%). The major monoterpenes included α-pinene (**9**), peak 11b (34.9%); myrcene (**23**), peak 16b (24.4%) and β- phellandrene (**31**), peak 24b (4.0%) (Table 4.8). The total composition of all sesquiterpenes identified was less than 1%. The only major non terpenoid hydrocarbon was 3-hydroxy-2-butanone (**52**), peak 1b) (1.9%) (Table 4.8).

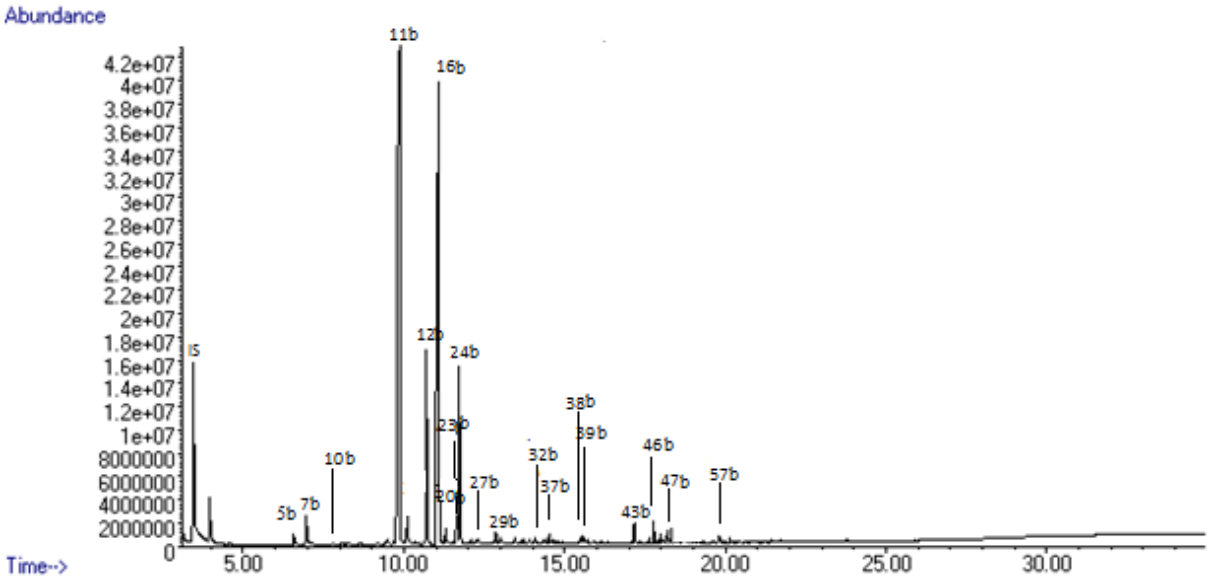


Figure 4.8: **Representative total ion chromatogram of Apple ripe mango fruit volatiles**

N/B: Peak numbers correlate to compounds listed in Table 4.8.

Table 4.8: Apple ripe fruit volatiles chemical composition

S/No	Peak	RI	Compound/Class	% composition
	IS	696	isopropylcyclobutane	7.8
1	1b	713	3-Hydroxy-2-butanone	1.9
2	5b	800	Ethyl butanoate	0.4
3	6b	835	(E)-2-Butenoic acid, ethyl ester	1.1
4	10b	926	(Z)-2-Butenoic acid, ethyl ester	0.1
5	11b	932	α-Pinene	34.9
6	12b	933	1,7,7-Trimethyl-Tricyclo[2.2.1.0(2,6)]heptane	5.3
7	13b	946	Camphene	1.3
8	16b	988	Myrcene	24.4
9	20b	1002	α-Phellandrene	0.5
10	23b	1022	1-Methyl-3-(1-methylethyl)-benzene	0.9
11	24b	1025	β- Phellandrene	4.0
12	27b	1062	ϒ-Terpinene	0.3
13	29b	1090	6,7-Epoxymyrcene	0.5
14	32b	1164	4-Ethyl-benzaldehyde,	0.3
15	37b	1196	Ethyl octanoate	0.3
16	38b	1260	ϒ -Octalactone,	0.2
17	39b	1272	*p*-Ethyl acetophenone	0.3
18	43b	1345	α-Cubebene	0.6
20	46b	1417	*(E-)* Caryophyllene	0.5
21	47b	1423	α-Caryophyllene	0.3
22	57b	1582	Caryophyllene oxide	0.3
			Monoterpene hydrocarbons	65.4
			Oxygenated monoterpenes	0.5
			Sequiterpene hydrocarbons	1.4
			Oxygenated sesquiterpenes	0.3
			Non-terpenoid hydrocarbons	6.2
			Oxygenated non terpenoid compounds	4.6
Total monoterpene, sesquiterpene and non terpenoid constituents				78.4

Key: IS= Internal Standard

4.2.3 Volatile aroma chemistry of ripe Keitt mango fruit pulp

The analysis of the chemical constituents of the fruit pulp of Keitt mango revealed a total of 13 compounds representing 69.9% of the total fruit volatiles (Figure 4.9; Table 4.9). The identified compounds included generally non oxygenated monoterpenes (38.6%), sesquiterpenes (1.7%) and non terpenoid hydrocarbons (26.6%) (Table 4.9). The major monoterpenes identified include β-pinene (**10),** peak 15b (10.2%); δ-3-carene (**8**), peak 24b (25.8%) and β-phellandrene (**31**), peak 24b (2.1%). There was no

major oxygenated monoterpene as all of them had less than 1% composition (Table 4.9).

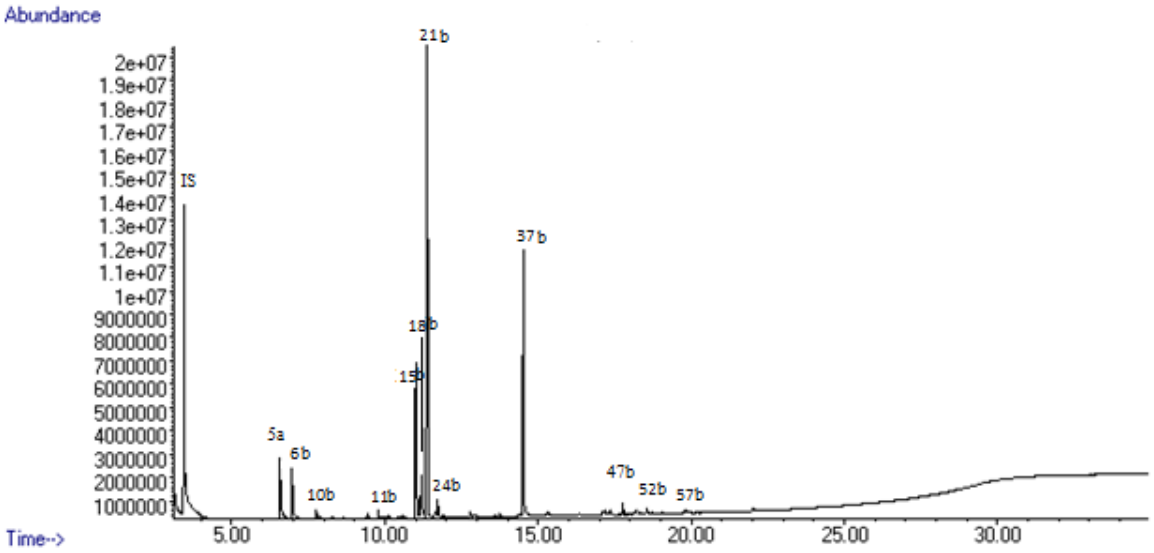


Figure 4. 9: **Representative total ion chromatogram of Keitt ripe fruit volatiles**

N/B: Peak numbers correlate to compounds listed in Table 4.9

Table 4.9: Keitt ripe fruit volatiles chemical composition

S/No	Peak	RI	Compound/Class	% composition
	IS	696	isopropylcyclobutane	20.9
1	5b	800	Ethyl butanoate	4.4
2	6b	835	(E)-2-Butenoic acid, ethyl ester	3.4
3	10b	926	(Z)-2-Butenoic acid, ethyl ester	0.7
4	11b	932	α-Pinene	0.5
5	15b	974	β-Pinene	10.2
6	18b	997	Ethyl hexanoate	4.1
7	21b	1008	δ-3-Carene	25.8
8	24b	1025	β- Phellandrene	2.1
9	37b	1196	Ethyl octanoate	14
10	44b	1374	α-Copaene	0.4
11	46b	1417	*(E-)* Caryophyllene	0.6
12	52b	1484	Germacrene D	0.4
13	57b	1582	Caryophyllene oxide	0.7
			Monoterpene hydrocarbons	38.6
			Oxygenated monoterpenes	0
			Sequiterpene hydrocarbons	1
			Oxygenated sesquiterpenes	0.7
			Non-terpenoid hydrocarbons	0
			Oxygenated non terpenoid compounds	26.6
Total monoterpene, sesquiterpene and non terpenoid constituents				69.9

Key: IS= Internal Standard

4.2.4 Volatile aroma chemistry of ripe Boribo mango fruit pulp

The analysis of the chemical constituents of the fruit pulp of Boribo mango revealed a total of twenty six (26) compounds representing 77.9% of the total fruit volatiles (Figure 4.10; Table 4.10), which included generally oxygenated and non-oxygenated monoterpenes (60.5%), sesquiterpenes (13.4%) and non terpenoid hydrocarbons (4.0%) (Table 4.10). The major monoterpenoids identified include myrcene (**23**), peak 16b (57.0%) The sesquiterpenes identified were *E*-caryophyllene (**13)** peak 46b (1.6%); ϒ-gurjunene (**11**), peak 51b (1.6%) and germacrene D (**39**), peak 52b (3.1%). The major oxygenated non terpenoid hydrocarbons were *(E)*-2-butenoic acid, ethyl ester (**76)**, peak 6b (2.4%) (Table 4.10).

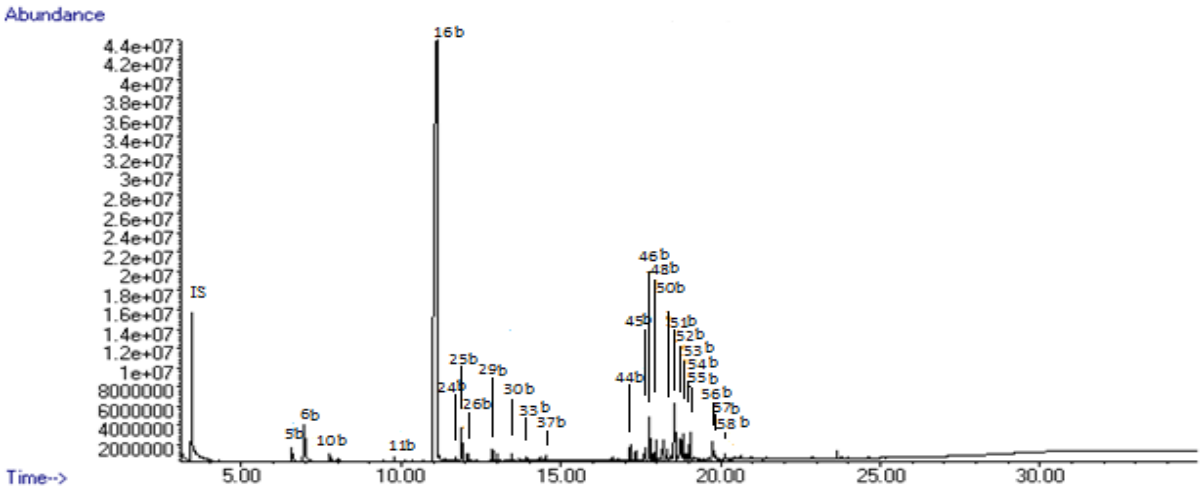


Figure 4.10: **Representative total ion chromatogram of Boribo ripe fruit volatiles**

N/B: Peak numbers correlate to compounds listed in Table 4.10

Table 4.10: Boribo ripe fruit volatiles chemical composition

S/No	Peak	RI	Compound/Class	% composition
	IS	696	isopropylcyclobutane	7.8
1	5b	800	Ethyl butanoate	0.8
2	6b	835	*(E)*-2-Butenoic acid, ethyl ester	2.4
3	10b	926	*(Z)*-2-Butenoic acid, ethyl ester	0.5
4	11b	932	α-Pinene	0.3
5	15b	974	β-Pinene	0.1
6	16b	988	Myrcene	57.0
7	24b	1025	β- Phellandrene	0.3
8	25b	1032	(Z)-β-Ocimene	0.8
9	26b	1044	*(E)*-β-Ocimene	0.5
10	29b	1090	6,7-Epoxymyrcene	0.8
11	30b	1130	*allo*-Ocimene	0.4
12	33b	1166	*p*-Mentha-1,5-dien-8-ol	0.3
13	37b	1196	Ethyl octanoate	0.3
14	44b	1374	α-Copaene	0.8
15	45b	1409	α-Gurjunene	0.6
16	46b	1417	*(E-)* Caryophyllene	1.6
17	48b	1437	α-Guaiene	0.9
18	50b	1452	α-Humulene	0.9
19	51b	1475	ϒ-Gurjunene	1.6
20	52b	1484	Germacrene D	3.1
21	53b	1509	α-Bulnesene	1.1
22	54b	1513	ϒ-Cadinene	0.3
23	55b	1528	Cis-Calamenene	1.2
24	56b	1577	Spathulenol	0.8
25	57b	1582	Caryophyllene oxide	0.3
26	58b	1688	Germacra-4(15),5,10(14)-trien-1-α-ol	0.4
			Monoterpene Hydrocarbons	59.4
			Oxygenated monoterpenes	1.1
			Sequiterpene Hydrocarbons	11.9
			Oxygenated sesquiterpenes	1.5
			Non-terpenoid hydrocarbons	0.0
			Oxygenatedon terpenoid compounds	4.0
Total monoterpene, sesquiterpene and non terpenoid constituents				**77.9**

Key: IS= Internal Standard

4.2.5 Volatile aroma chemistry of ripe Tommy Atkins mango fruit pulp

The analysis of the chemical constituents of the fruit pulp of Tommy Atkins mango revealed a total of thirty (30) compounds representing 73% of the total fruit volatiles (Figure 4.11; Table 4.11). The compounds generally included oxygenaned and non-

oxygenated monoterpenes (41.7%), sesquiterpenes (7.6%) and non terpenoid hydrocarbons (23.7%) (Table 4.11). The major monoterpenes identified include α-pinene (**9**), peak 11b (7.4%); δ-3-carene (**8**), peak 21b (27.1%) and β-phellandrene (**31**), peak 24b (2.1%). No major sesquiterpene was identified. The major oxygenated non terpenoid hydrocarbons identified were 3-hydroxy-2-butanone (**54**), peak 1b (4.8%); 2, 3-butanediol (**77**), peak 5b (6.7%); *(*E)-2-butenoic acid, ethyl ester (**76**), peak 6b (2.2%); *(Z)*-2-butenoic acid, ethyl ester (**78**), peak 10b (1.6%) and methyl salicylate (**79**), peak 36b (2.9%) (Table 4.11).

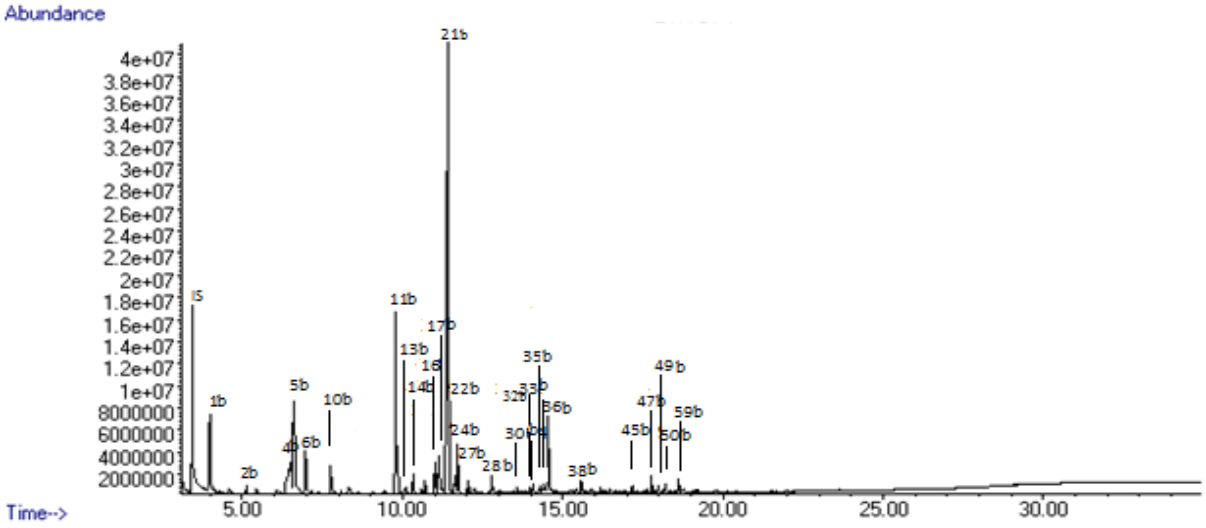


Figure 4.11: **Representative total ion chromatogram of Tommy Atkins ripe fruit volatiles**

N/B: Peak numbers correlate to compounds listed in Table 4.11

Table 4.11: Tommy Atkins ripe fruit chemical composition

S/No	Peak	RI	Compound/Class	% Composition
	IS	696	isopropylcyclobutane	8.4
1	1b	713	3-Hydroxy-2-butanone	4.8
2	2b	745	2,4,5-trimethyl-1,3-Dioxolane	0.3
3	4b	785	2,3-Butanediol	3.7
4	5b	800	Ethyl butanoate	6.7
5	6b	835	(E)-2-Butenoic acid, ethyl ester	2.2
6	7b	871	Ethylbenzene	0.2
7	10b	926	(Z)-2-Butenoic acid, ethyl ester	1.6
8	11b	932	α-Pinene	7.4
9	13b	946	Camphene	0.5
10	14b	958	Isobutyl butanoate	0.7
11	15b	974	β-Pinene	0.7
12	16b	988	Myrcene	1.1
13	17b	993	Butyl butanoate	1.9
14	20b	1002	α-Phellandrene	0.2
15	21b	1008	δ-3-Carene	27.1
16	22b	1020	*o*-Cymene	0.7
17	24b	1025	β- Phellandrene	2.1
18	27b	1062	ϒ-Terpinene	0.4
19	28b	1086	Terpinolene	1
20	30b	1130	allo-Ocimene	0.2
21	32b	1164	4-Ethyl-Benzaldehyde,	0.4
22	33b	1166	*p*-Mentha-1,5-dien-8-ol	0.3
23	35b	1179	*p*-Cymen-8-ol	0.3
24	36b	1190	Methyl salicylate	2.9
25	39b	1272	*p*-Ethyl acetophenone	1
26	44b	1374	α-Copaene	0.4
27	46b	1417	*(E-)* Caryophyllene	0.5
28	49b	1444	*p*-Acetylacetophenone	0.4
29	50b	1452	α-Humulene	0.3
30	59b	2344	Butyl dodecyl Succinicate	0.5
			Monoterpene hydrocarbons	41.4
			Oxygenated monoterpenes	0.3
			Sequiterpene hydrocarbons	1.2
			Oxygenated sesquiterpenes	6.4
			Non-terpenoid hydrocarbons	0.2
			Oxygenated non terpenoid compounds	23.5
Total monoterpene, sesquiterpene and non terpenoid constituents				**73**

Key: IS= Internal Standard

4.2.6 Volatile aroma chemistry of ripe Van Dyke mango fruit pulp

The analysis of the chemical constituents of the fruit pulp of Van Dyke mango revealed a total of seventeen (17) compounds representing 49.2% of the total fruit volatiles (Figure 4.12; Table 4.12). The identified compounds were generally oxygenated and non-oxygenated monoterpenes (34.2%), sesquiterpenes (2.1%) and non terpenoid hydrocarbons (12.9%) (Table 4.12). The major monoterpenes identified include α-pinene (**9**), peak 11b (3.9%); myrcene (**23**), peak 16b (1.4%), δ-3-carene (**8**), peak 21b (25.6%) and β- phellandrene (**31**), peak 24b (1.8%). The major oxygenated non terpenoid hydrocarbons identified were ethyl butanoate (**54**), peak 5b (3.4%) and *(E)*-2-butenoic acid, ethyl ester (**76**), peak 6b (4.9%) (Table 4.12).

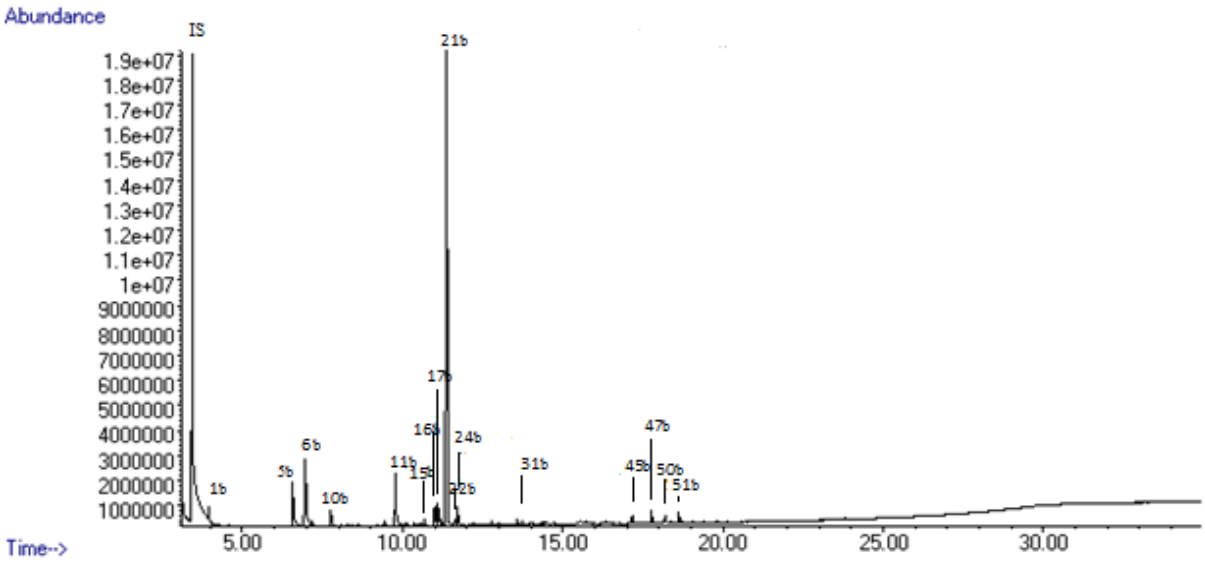


Figure 4.12: **Representative total ion chromatogram of Van Dyke ripe fruit volatiles**

N/B: Peak numbers correlate to compounds listed in Table 4.12.

Table 4.12: Van Dyke ripe fruit volatile chemical composition

S/No	Peak	RI	Compound/Class	% composition
	IS	696	isopropylcyclobutane	34.3
1	1b	713	3-Hydroxy-2-butanone	1.2
2	5b	800	Ethyl butanoate	3.4
3	6b	835	(E)-2-Butenoic acid, ethyl ester	4.9
4	10b	926	(Z)-2-Butenoic acid, ethyl ester	1.4
5	11b	932	α-Pinene	3.9
6	15b	974	β-Pinene	0.6
7	16b	988	Myrcene	1.4
8	17b	993	Butyl butanoate	2.0
9	21b	1008	δ-3-Carene	25.6
10	22b	1020	*o*-Cymene	0.5
11	24b	1025	β- Phellandrene	1.8
12	28b	1086	Terpinolene	0.6
13	31b	1141	Camphor	0.3
14	44b	1374	α-Copaene	0.5
15	46b	1417	*(E-)* Caryophyllene	0.6
16	50b	1452	α-Humulene	0.4
17	51b	1475	ϒ-Gurjunene	0.6
			Monoterpene hydrocarbons	33.9
			Oxygenated monoterpenes	0.3
			Sequiterpene hydrocarbons	2.1
			Oxygenated sesquiterpenes	0
			Non-terpenoid Hydrocarbons	0
			Oxygenated non terpenoid compounds	12.9
Total monoterpene, sesquiterpene and non terpenoid constituents				49.2

Key: IS= Internal Standard

4.2.7 Volatile aroma chemistry of ripe Sabre mango fruit pulp

The analysis of the chemical constituents of the fruit pulp of Sabre mango revealed twenty seven (27) compounds representing 65.2% of the total fruit volatiles (Figure 4.13; Table 4.13). The identified compounds were generally oxygenated and non-oxygenated monoterpenes (44.7%), sesquiterpenes (3.1%) and non terpenoid hydrocarbons (16.0%) (Table 4.13). The major monoterpoid identified include α-pinene (**9**), peak 11b (7.8%); δ-3-carene (**8**), peak 21b (30.3%) and β-phellandrene (**31**), peak 24b (1.8%). The major oxygenated non terpenoid hydrocarbons identified were methyl salicylate (**79**), peak 36b (3.8%) and *p*-Ethyl acetophenone (**80**), peak 39b (6.6%) (Table 4.13).

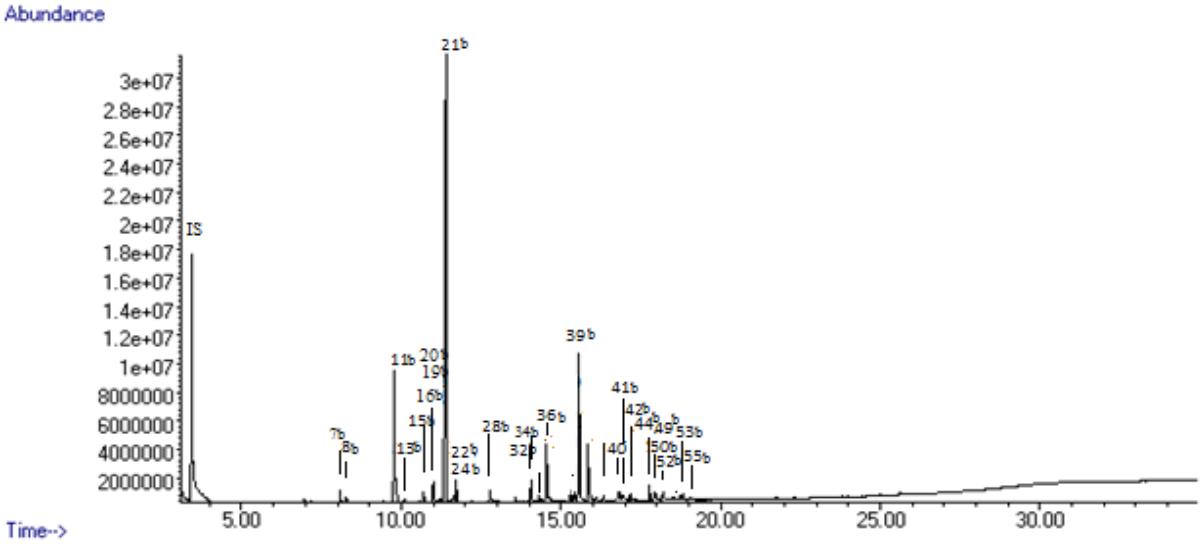


Figure 4.13: **Representative total ion chromatogram of Sabre volatiles**

N/B: Peak numbers correlate to compounds listed in Table 4.13.

Table 4.13: Sabre ripe fruit volatiles chemical composition

S/No	Peak	RI	Compound/Class	% composition
	IS	696	isopropylcyclobutane	18.4
1	7b	871	Ethylbenzene	0.9
2	8b	874	p-Xylene	0.7
3	11b	932	α-Pinene	7.8
4	13b	946	Camphene	0.3
5	15b	974	β-Pinene	1.1
6	16b	988	Myrcene	1.4
7	19b	999	Unidentified	0.3
8	20b	1002	α-Phellandrene	0.3
9	21b	1008	δ-3-Carene	30.3
10	22b	1020	*o*-Cymene	0.5
11	24b	1025	β- Phellandrene	1.8
12	28b	1086	Terpinolene	1.2
13	32b	1164	4-ethyl-Benzaldehyde,	0.8
14	34b	1166	Coahuilensol methyl ether	1.4
15	36b	1190	Methyl salicylate	3.8
16	39b	1272	*p*-Ethyl acetophenone	6.6
17	40b	1295	Methyl,2,4-dimethyl-Benzanoate	0.8
18	41b	1316	1-(2-hydroxy-5-methylphenyl)-Ethanone	1.3
19	42b	1341	4'-Ethylpropiophenone	0.2
20	44b	1374	α-Copaene	0.5
21	46b	1417	*(E-)* Caryophyllene	0.7
22	49b	1444	*p*-Acetylacetophenone	1.1
23	50b	1452	α-Humulene	0.7
24	51b	1475	ϒ-Gurjunene	0.6
25	52b	1484	Germacrene D	0.5
26	53b	1509	α-Bulnesene	0.4
27	55b	1528	*Cis*-Calamenene	0.4
			Monoterpene hydrocarbons	44.7
			Oxygenated monoterpenes	0
			Sequiterpene hydrocarbons	3.1
			Oxygenated sesquiterpenes	0
			Non-terpenoid hydrocarbons	1.4
			Oxygenated Non terpenoid compounds	16
Total monoterpene, sesquiterpene and non terpenoid constituents				65.2

Key: IS= Internal Standard

4.2.8 Volatile aroma chemistry of ripe Kent mango fruit pulp

The analysis of the chemical constituents of the fruit pulp of Kent mango revealed a total of twenty (20) compounds representing 57.3% of the total fruit volatiles (Figure 4.14; Table 4.14). The identified compounds were generally oxygenated and non oxygenated monoterpenes (41.4%), sesquiterpenes (2.4%) and non terpenoid hydrocarbons (13.5%) The major monoterpnoids identified was δ-3-carene (**8**), peak 21b (35.0%) and β- phellandrene (**31**), peak 24b (1.9%) (Table 4.14). Major oxygenated non terpenoid hydrocarbons identified was methyl salicylate (**79**), peak 36b (9.3%) (Table 4.14).

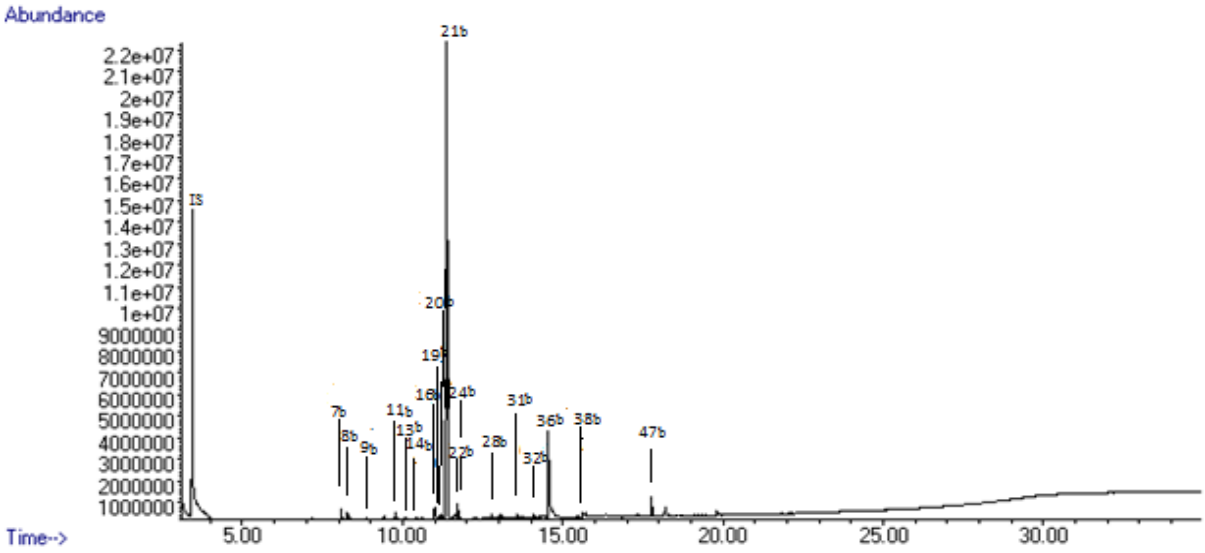


Figure 4.14: **Representative total ion chromatogram of Kent ripe fruit volatiles**

N/B: Peak numbers correlate to compounds listed in Table 4.14.

Table 4.14: Kent ripe fruit volatile chemical composition

S/No	Peak	RI	Compound/Class	% composition
	IS	696	isopropylcyclobutane	26.7
1	7b	871	Ethylbenzene	1.1
2	8b	874	p-Xylene	1.3
3	9b	888	1,3-Dimethyl-benzene	0.3
4	11b	932	α-Pinene	0.8
5	13b	946	Camphene	0.4
6	14b	958	Isobutyl butanoate	0.3
7	16b	988	Myrcene	1.4
8	19b	999	Unidentified	0.5
9	20b	1002	α-Phellandrene	0.3
10	21b	1008	δ-3-Carene	35
11	22b	1020	*o*-Cymene	0.5
12	24b	1025	β- Phellandrene	1.9
13	28b	1086	Terpinolene	0.7
14	31b	1141	Camphor	0.4
15	32b	1164	4-ethyl-benzaldehyde,	0.5
16	36b	1190	Methyl salicylate	9.3
17	39b	1272	*p*-Ethyl acetophenone	0.7
18	46b	1417	*(E-)* Caryophyllene	1.3
19	50b	1452	α-Humulene	0.7
20	52b	1484	Germacrene D	0.5
21	53b	1509	α-Bulnesene	0.4
			Monoterpene Hydrocarbons	41
			Oxygenated monoterpenes	0.4
			Sequiterpene Hydrocarbons	2.4
			Oxygenated sesquiterpenes	0
			Non-terpenoid Hydrocarbons	2.7
			Oxygenate Non terpenoid compounds	10.8
Total monoterpene, sesquiterpene and non terpenoid constituents				**57.3**

Key: IS= Internal Standard

The major compounds found in the leaf essential oils and ripe fruit volatiles are shown in Figure 4.15.

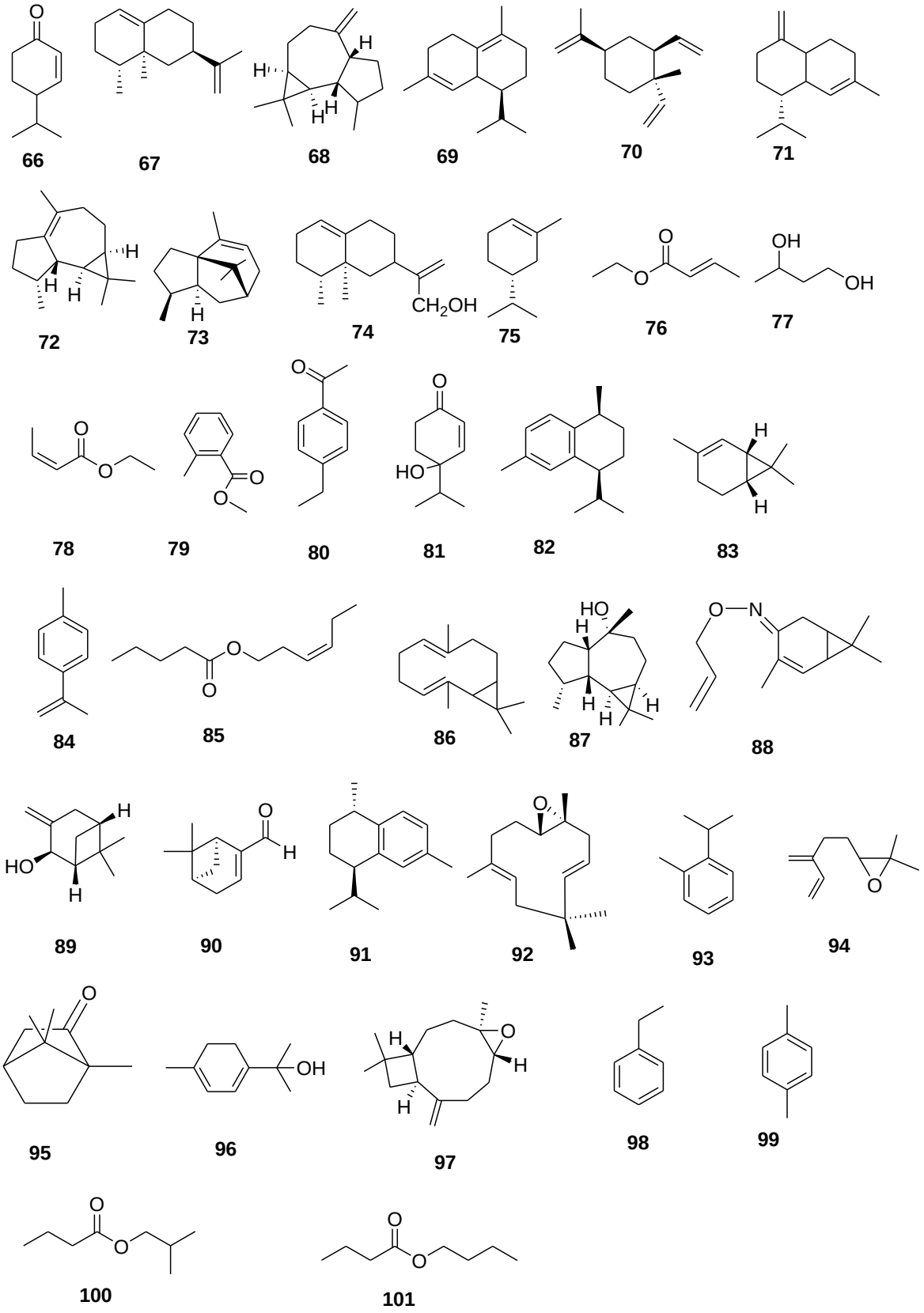


Figure 4.15: Structures of major Compounds found in the leaf essential oils and ripe fruit pulp of the different mango varieties

4.3 Bioassays

This section presents the results of the bioassays of the leaf essential oil extracts, mango juice and the chemical standards in the laboratory and field.

4.3.1 Bioassay of mango leaf essential oils under laboratory conditions

Results of the bioassay of essential oils showed that *B. invadens* fruit fly responded differently to odor that emanated from the different oils with methyl eugenol as positive control (Figure 4.16). The values with the same letter represent no significant difference in the mean number of *B. invadens* attracted by the different essential oil treatments when compared to the positive control (methyl eugenol). There was no significant difference ($p < 0.05$) in the number of flies caught by Boribo leaf essential oil (4.5 ± 0.288) and the positive control (5.5 ± 0.288). Keitt oil caught the least number of flies (3.0 ± 0.408).

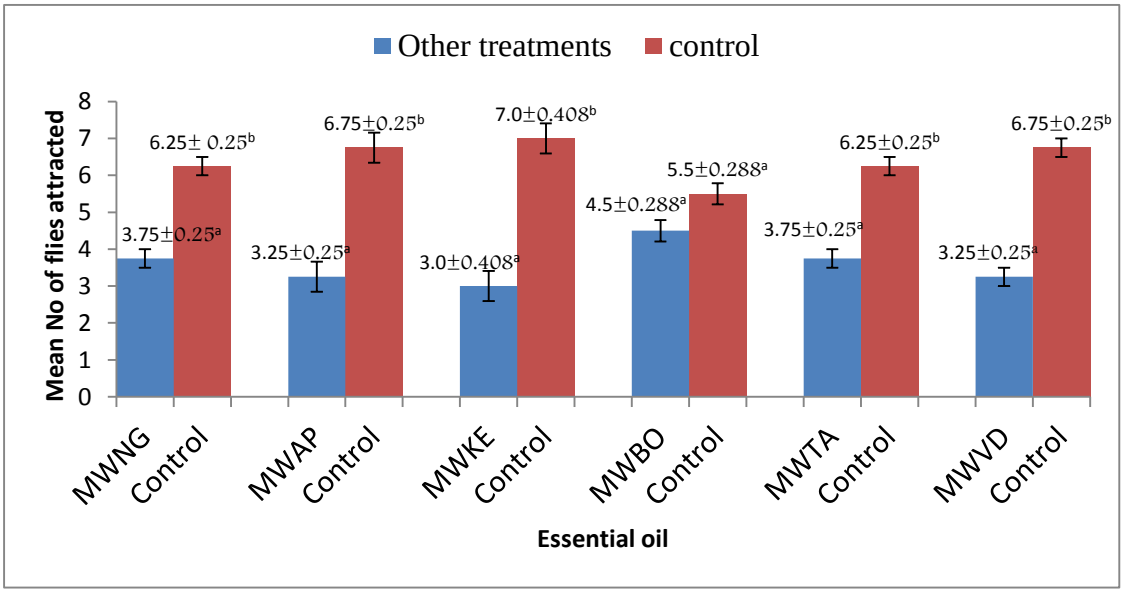


Figure 4.16: Number of *B. invadens* attracted by different mango leaf essential oils in comparison with methyl eugenol as control under laboratory conditions

Key: MWNG=Ngowe oil, MWAP= Apple oil, MWKE= Keitt oil, MWBO= boribo oil, MWTA= Tommy Atkins oil, MWVD= Van Dyke oil, Control=methyl eugenol

4.3.2 Bioassay of mango leaf essential oils in the field

Raw data showing the actual number of flies attracted by the different mango leaf essential oils under a 7× 7 completely randomized Latin square block experimental design (Appendix II) is shown in Appendix III. The analysed results for bioassay of the mango leaf essential oils in the field after ANOVA and LSD test (F=2.02, DF= 6, $p < 0.05$) are presented in Table 4.15 and Appendix IV. The number of flies attracted by positive control (5.143±1.47) was almost five times that attracted by Boribo as the best performing essential oil, while Ngowe did not attract any fly (Table 4. 16).

Table 4.15: Number of *B. invadens* attracted by mango leaf essential oils and methyl eugenol in the field

Treatment	mean No. of flies caught ±SE
Control (methyl eugenol)	5.143 ±1.471 [a]
Boribo essential oil (MWBO)	1.429±0.481[b]
Keit essential oil (MWKE)	0.4287± 0.297[b]
Apple essential oil (MWAP)	0.286±0.285[b]
Van Dyke essential oil (MWVD)	0.286± 0.184[b]
Tommy Atkins essential oil (MWTA)	0.143±0.142[b]
Ngowe essential oil (MWNG)	0.0000±0[b]

Number of replicates and observations made were 7 and 49, respectively. Means followed by the same letter are not significantly different at $P < 0.05$.

4.3.3 Bioassay of ripe mango fruit pulp under natural conditions

The raw data showing the number of flies caught by the different baits of mango juices in a 7×7 completely randomized Latin square block experimental design (Appendix V) is shown in Appendix VI. The analysed results for bioassay of the mango juices in field following ANOVA and LSD test (F=2.02, DF= 6, $P > 0.05$) are shown in Table 4.16 and appendix VII. The mean lure effect of Keitt mango juice (4.571±1.445) is as good as that of the positive control (5.857±1.724) and therefore not significantly different ($P<0.05$).

Table 4.16: Number of *B. invadens* caught by traps baited with different ripe mango fruit juices and methyl eugenol in field

Treatment	Mean No. of flies caught ±SE
Methyl eugenol (Control)	5.857±1.724[a]
Ripe Keitt mango juice (Kejuice)	4.571±1.445[ab]
Ripe Boribo mango juice (Bojuice)	2.857± 0.705[b]
Ripe Ngowe mango juice (NGJuice)	2.571±0.528[b]
Ripe Apple mango juice (APJuice)	2.429 ±0.649[b]
Ripe Tommy Atkins mango juice (TAjuice)	2.000 ±0.816[b]
Ripe Van Dyke mango juice (VDJuice)	2.000 ±0.723[b]

Number of replicates and observations made were 7 and 49 respectively. Mean followed by the same letter are not significantly different at P>0.05.

4.3.4 Bioassay of chemical standards in Laboratory conditions

The performance of ethylbuteme (4.75±0.478) and MYPIEB (4.75±0.25) in luring the fruit fly is the same and not significantly different (P<0.05) from that of methyl eugenol as positive control (5.25±0.478). Results of the attractiveness of the rest of the blends of chemicals and individual compound(s) towards *B. invadens* under laboratory conditions are shown in Figure 4.17. The mean values followed by the same letters represent treatments that have no significant difference in the attractiveness of *B. invadens.*

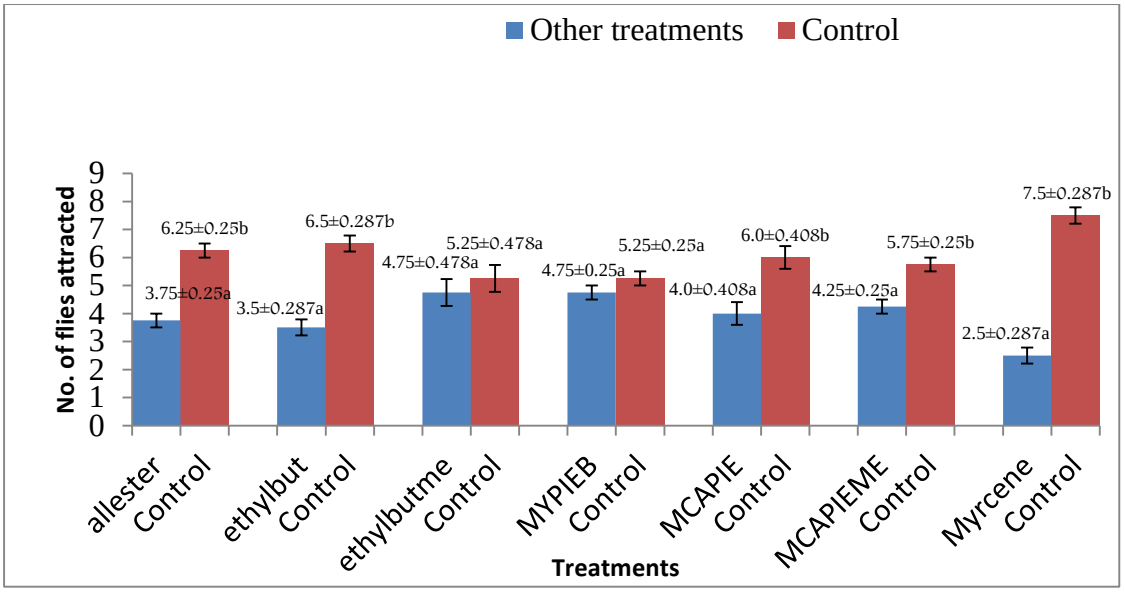


Figure 4.17: Number of *B. invadens* attracted by chemical blends, standard(s) and control in Laboratory conditions

Key: Allester = ethyl butanoate + ethyl octanoate + ethyl hexanoate + methyl salicylate, Ethylbut =Ethylbutanoate, ethylbutme = ethylbutanoate + methyl eugenol, MYPIEB = Myrcene + α-pinene + ethyl butanoate, MCAPIE = myrcene + δ-3-carene + α-pinene + ethyl butanoate, MCAPIEME = myrcene + δ-3-carene + α-pinene + ethyl butanoate + methyl eugenol, control = methyl eugenol.

4.3.5. Bioassay of blends of esters chemical standards in the field

The data showing the number of flies caught by the different blends of four esters (ethyl butanoate, ethyl octanoate, ethyl hexanoate and methyl salicylate) in a 6×6 completely randomized Latin square block experimental design (Appendix IX) is shown in Appendix X. The results for bioassay of the esters in field following ANOVA and LSD test (F=38.54, DF = 5, P =0.0001) are shown in Table 4.17 and appendix XI. The mean lure effect of all four esters (Almeths) (0.500±0.342) was not significantly higher than that of any other blend. However, all treatments of the ester blends were far much lower than that of the positive control (4.833±0.601) at P=0.0001).

Table 4.17: Number of *B. invadens* caught by traps baited with different blends of esters and methyl eugenol in the field

Treatment	Mean No. of flies caught ±SE
Control	4.833±0.601[a]
Allmeths	0.5000±0.342[b]
Allethoc	0.167±0.167[b]
Allethhe	0.167±0.167[b]
Allester	0.167±0.167[b]
Allethyl	0.0000±0[b]

Number of replicates and observations made were 6 and 36 respectively. Mean followed by the same letter are not significantly different at $p<0.05$.

Key: Control=methyl eugenol, Allester = ethyl butanoate + ethyl octanoate + ethyl hexanoate + methyl salicylate, Allmeths= ethyl butanoate + ethyl octanoaete + ethyl hexanoate, Allethoc = ethyl butanoate + ethyl hexanoate + methyl salicylate, Allethhe = ethyl butanoate + ethyl octanoate + methyl salicylate, Allethyl= ethyl octanoate + ethyl hexanoate + methyl salicylate

4.3.6 Bioassay of blends (myrcene, δ-3-carene, α-pinene and ethyl butanoate) in field

The data showing the number of flies caught by the different blends consisisting of three 3 monoterpenes (myrcene, δ-3-carene, α-pinene) plus 1 ester (ethylbutanoate) in a 6×6 completely randomized Latin square block experimental design (Appendix XII) is shown in Appendix XIII. The results following ANOVA and LSD test ($F=38.54$, $DF = 5$, $p =0.0001$) are shown in Table 4.18 and appendix XIV. The mean lure effect of MYPIEB (2.333±0.802) was highest although significantly not different ($p<0.05$) among the blends, which were however lower than that of positive control (4.500±1.088).

Table 4.18: Number of *B. invadens* caught by different blends of myrcene, δ-3-carene, α-pinene and ethyl butanoate in the field

Treatment	Mean No. of flies caught ±SE ±SE
Control	4.500±1.088[a]
MYPIEB	2.333 ±0.802[b]
CAPIEB	0.667±0.333[cb]
MCAPIE	0.667 ±0.211[cb]
MYCAEB	0.500 ±0.341[c]
MYCAP	0.167 ±0.167[c]

Key: Control= Methyl eugenol, MYPIEB =myrcene + α-pinene + ethyl butanoate, CAPIEB=δ-3-carene + α-pinene + ethyl butanoate, MCAPIE=myrcene + α-pinene + ethyl butanoate, MYCAEB=myrcene + δ-3-carene + ethyl butanoate, MYCAP =myrcene + δ-3-carene + α-pinene

4.3.7 Bioassay of individual pure compounds in natural field conditions

Raw data showing the actual number of flies attracted by the different individual pure compounds under a 8× 8 completely randomized Latin square block experimental design (Appendix XV) is shown in Appendix XVI. The analysed results for bioassay of individual pure compounds in the field after ANOVA and LSD test (F = 18.27, DF=7, P = 0.0001) are presented in Table 4.19 and Appendix XVII. Other than the positive control which had a mean catch of 4.125±0.953, the rest of the compounds did not lure any fruit fly (Table 4.19).

Table 4.19: Number of *B. invadens* caught by individual compounds in the field

Treatment	Mean No. of flies caught ±SE
Control	4.125 ±0.953[a]
Ethylbutanoate	0.125 ±0.125[b]
α-Phellandrene	00000± 0[b]
α-Pinene	00000±0[b]
δ -3-carene	0± 0[b]
Myrcene	0 ±0[b]
β- pinene	0 ±0[b]
Camphene	0±0[b]

CHAPTER FIVE

DISCUSSION

5.1 Essential oil chemistry of the mango leaf oils

The leaf essential oil composition of the six varieties of *Mangifera indica* is shown in the GC-MS total ion chromatograms (Figures 4.1- 4.6) and the chemical components are shown in Tables 4.1- 4.6.

A total of fifty different compounds were identified in the oils of the six mango varieties (Table 4.1- 4.6). Although some compounds were common in all or some of the six varieties, there were some differences in the individual composition in each variety. Whereas some compounds were identified in the essential oil of more than one mango variety, there were other compounds that were exclusively identified in the essential oil of particular varieties.

5.1.1 Essential oil composition of Ngowe leaf oil

In the Ngowe oil, 21 compounds were identified; representing 69.3% of the total essential oil. There were four compounds shown could not be identified. The identified compounds comprised non-oxygenated monoterpenes (18.7%), non-oxygenated sesquiterpenes (19.6%), oxygenated monoterpenes (3.5%), oxygenated sesquiterpenes (14.8%), non-terpenoid hydrocarbons (11.9%) and non-terpenoid oxygenated hydrocarbons (0.8 %).

The major compounds were spathulenol (**20**) (14.8%), β-pinene (**10)** (7.7%); α-pinene (**9**) (5.8%) and β-phellandrene (**31**) (4.9%). α-Pinene (**9**) and β-pinene (**10)** have also been reported as major compounds in the essential oil of Rosa mango variety (Ramos *et al*, 2014).

Some compounds were identified only in the Ngowe essential oil and not in the other five varieties. They included the ketone 4-hydroxy-cryptone **(81)** and the sesquiterpene *cis*- calamenene (**82)**. Also present were the non terpenoid hydrocarbons heptadecane, nonadecane, heneicosane, docosane and tetracosane.

5.1.2 Essential oil composition of Apple leaf oil

A total of 24 compounds were identified in Apple essential oil; representing 76.1 % of total essential oil. The identified compounds comprised non-oxygenated monoterpenes (35.5%), non-oxygenated sesquiterpenes (32.6%), oxygenated monoterpenes (4.6%), oxygenated sesquiterpenes (1.9%) and non-terpenoid oxygenated hydrocarbons (1.5%). The major compounds in the Apple oil were β-phellandrene (**31**) (12.1%), α-pinene (**9**) (10.3%); α-gurjunene (**11**) (9.7%) and δ-cadinene (**69)** (8.4%). The α-gurjunene (**11**) was also reported as a major compound of the essential oil of Brazillian *M. indica* var. coquinho (Gebara *et al*., 2011) and Nigerian *Mangifera indica* L (Ana *et al.*, 2014).

Some compounds were identified only in the Apple essential oil. They included the alcohol 3Z- hexenol (**36)**; the monoterpenes δ-2-carene **(83**), α-phellandrene (**42**), E-β-ocimene (**45**) and *p*-cymenene (**84**); the ester *3Z*-hexenyl valerate **(85)**; the sesquiterpenes ϒ-gurjunene (**41**), bicyclogermacrene (**86),** and the alcohol ledol **(87**). These compounds were not present in the other five varieties under study.

5.1. 3 Essential oil composition of Keitt leaf oil

Keitt essential oil had a total of seventeen compounds identified; representing 76.2% of total essential oil. Four compound represented by peaks could not be identified. Among the identified compounds were monoterpenes (25.8%), sesquiterpene (45.1%), oxygenated monoterpenes (1.2%) and oxygenated sesquiterpenes (4.1%).The major compounds in the Keitt essential oil were δ-3-carene **(8)** (19.4%), α-gurjunene (**11**) (17.4%), viridiflorene (**72)** (7.7%) and germacrene D (**39**) (5.3%). The δ-3-carene **(8)** has also been reported in the oil of Brazillian (Gebara *et al*., 2011) and Nigerian mango varieties (Ana *et al*, 2014).

There was no single unique compound that was exclusively identified in the Keitt variety but absent in the rest under this. The leaf essential oil of this variety may therefore not be expected to exhibit unique characteristics.

5.1.4 Essential oil composition of Boribo leaf oil

In Boribo oil, a total of twenty two compounds were identified representing 73.3% of total essential oil components. Three compounds could not be identified. The identified

compounds comprised monoterpenes (36.3%), sesquiterpene (23.5%), oxygenated monoterpenes (2.4%), oxygenated sesquiterpenes (9.3%) and non-terpenoid oxygenated hydrocarbons (1.8%).

The major compounds were α-pinene **(9)** (10.9%), β-pinene**) (10)** (21.9%)**,** α-gurjunene (**11**) (8.7%), 4-allyloxyimino-2-carene (**88**) (4.6%) and 13-hydroxy valencene (**74**) (4.6%) The β-pinene (**10**) has also been reported as a major compound in the Brazillian mango variety Espada (Ramos *et al*, 2014).

The compounds identified only in the Boribo variety included the oxygenated monoterpene alcohol *trans*-pinocarveol (**89**), the aldehyde myrtenal (**90**), the sequeterpene *trans*- calamenene (**91**), the oxygenated sesquiterpenes humulene epoxide II **(92**) and 13-hydroxy-valencene (**74**).

5.1. 5 Essential oil composition of Tommy Atkins leaf oil

Seventeen compounds were identified in Tommy Atkins oil comprising 83.6% of the total oil. The identified compounds comprised monoterpenes (61.2%), sesquiterpene (21.7%) and oxygenated monoterpenes (0.7%).

The major compounds were δ-3-carene **(8**) (29.2%), α-pinene (**9**) (24.5%); α-gurjunene (**11**) (10.3%) and β-selinene (**21)** (3.0%). There was no single compound that was exclusively identified in the Tommy Atkins variety. Like Keitt, the essential oil of this variety may therefore not be expected to exhibit unique characteristics from the other varieties. All the four compounds, δ-3- carene **(8),** α-pinene (**9**), α-gurjunene (**11**) and β-selinene (**21)** were also found in Nigerian mango variety (Ana *et al*, 2014).

5.1.6 Essential oil composition of Van Dyke leaf oil

In Van Dyke leaf oil a total of sixteen (16) compounds were identified which comprised 89.2% of the total essential oil. Two compounds could not be identified. The identified compounds comprised monoterpenes (44.4%), sesquiterpene (43.9%) and non-terpenoid hydrocarbons (0.9%). The major compounds identified included the monoterpenes α-pinene (**9**) (18.0%), β-pinene (**10**) (4.3%), sylvestrene (**75**) (2.5%) and δ-3- Carene (**8**) (17.9%). The major sesquiterpenes included α-gurjunene (**11**) (16.7%),

E-caryophyllene (**13**) (3.7%), α- humulene (**12**) (3.9%) and β-Selinene (**21**) (9.9%). Previous studies show that essential oil of *M. indica* var. Rosa (EOMiR) is characterized by high amounts of β-pinene (Ramos *et al.,* 2014). The α-pinene (**9**), δ-3- Carene (**8**), E-caryophyllene (**13**) have also been found to be among principles constituents of the essential oils of the peel of Egyptian mango cultivars (Bishr *et al.,* 2016).

5.1.7 Comparative essential oil chemistry of the six leaf oils of mangoes

Some major compounds were identified in the leaf essential oils of the six mango cultivars. α-Pinene (**9**) was found in all of the six mangoes. β-Pinene (**10**) was found as major compound in Ngowe and Boribo. The β-phellandrene (**31**) was found in Ngowe and Apple. α-Gurjunene (**11**) was identified in Ngowe, Apple, Kett, Boribo, Tommy Atkins and Van Dyke. The δ-cadinene (**70**) was found in Apple. The δ-3-Carene (**8**) was found in Keitt, Tommy Atkins and Van Dyke mangoes. Viridiflorene (**72**) and germacrene D (**39**) were identified in Keit. The 13-Hydroxy valencene (**74**) was found in Boribo while β-Selinene (**21**) was found in Tommy Atkins and Van Dyke.

Oxygenated sesqueterpenes and non-terpenoid oxygenated hydrocarbons were not identified in the leaf oils of most of the six mango varieties. Ngowe oil however contained the oxygenated susquiterpene spathulenol (**20**) and non-terpenoid oxygenated hydrocarbons, 3-(4-methyl-3-pentenyl)-furan (**79**), which was also identified in the Boribo leaf oil.

Some compounds which were found in significant but varying amounts (0.3-21.9%) in the leaf essential oils of all six varieties were α-pinene **(9**), camphene (**65**), β-pinene (**10)**, α-copaene (**37**) , β-elemene (**70**), α-gurjunene (**11**) and α-humulene (**12**). The α-gurjunene (**11**) identified in the Apple, Keitt, Boribo, Tommy Atkins and Van Dyke varieties has also been reported as a major compound in the mature leaf and fruit essential oil of Brazillian *M. indica* var. *coquinho* (Anacardiaceae) (Gebara *et al.,* 2011) and Nigerian variety (Ana *et al*., 2014). The α-pinene (**9**) identified in Ngowe, Keitt, Boribo, Tommy Atkins and Van Dyke has also been reported as major in Nigerian *M. indica* variety essential oil (Ana *et al*., 2014).

The typical mango-like odor is due to presence of α-copaene (**37**), which has also been found as a minor component in a number of fruits, including citrus, guava, litchi, and

peach (Macleod et *al.,* 1988). α-Humulene (**12**) was also reported as major in *Mangifera indica var.* coquinho (Gebara *et al,* 2011). The Egyptian Mango has also been recenlty reported to contain α-copaene (El-Hawary and Rabeh, 2014).

In general, the chemical profiles of all the essential oils of the six varieties were qualitatively and quantitatively different. The δ-3-carene (**8**), which was identified in Keitt, Tommy Atkins and Van Dyke leaf oils as a major compound, has also been reported previously as a major leaf oil constituent of mango (Nigam *et al*., 1962). It has been found to be among other important terpenes contributing to mango flavor (Macleod and Snyder, 1985; Sakho *et al*, 1985; Bartley and Schwede, 1987; Winterhal, 1991). Similar results were also reported in cultivars from Sao Paulo (Franco *et al*., 2004) Cuba (Pino *et al*, 1989), Venezuela (Franco *et al*., 2004), Brazil (Andrade *et al*., 2000), Colombia (Quijano *et al*., 2007) and Indian cultivars (Ansari *et al*., 2004).

Non-terpenoid and aliphatic compounds including their esters, which were detected in the Kenyan varieties have been reported as the dominant aroma principles in different fruit cultivars from Cuba though obtained by solvent extraction (Pino and Mesa, 2006).

The African mango in Senegal had a sesquiterpene, eremophillene as the major constituent (Sakho *et al*., 1985). Two Indian mango fruit oils are characterized by β-ocimene and 2,5-dimethyl-4-hydroxyl-3(2H)-furanone (MacLeod and Pieris, 1984), α-pinene, caryophyllene oxide and humulene oxide (Ansari *et al*., 1999). The Srilankan cultivars are dominated by β-ocimene and terpinolene (Idsteom and Schreier, 1985; MacLeod and Pieris, 1984). The Egyptian cultivars are dominated by myrcene and limonene (Engel and Tressl, 1983), while the Brazilian cultivars by terpinolene (Andrade et *al*., 2000) and Florida cultivar by terpinolene and ethyl butyrate (MacLeod andTroconis, 1982). These differences could be attributed to variations in climate and soil type.

5.2 Fruit pulp volatile aroma chemistry of the ripe mango fruits

The results of chemical analyses of *M. indica* ripe fruit volatiles, is shown in the GC-MS total ion chromatograms. A total of 58 volatile compounds were identified in the fruits of the eight mango varieties. Monoterpenes and esters were dominant in the volatiles of Ngowe, Keitt, Tommy Atkins, Van Dyke and Kent varieties. Apple

volatiles was dominated by monoterpenes and hydrocarbons while Boribo comprised mostly of monoterpenes and sesquiterpene, Sabre was dominated by monoterpenes and oxygenated compounds.

5.2.1 Volatile aroma chemistry of ripe Ngowe mango fruit pulp

Twenty three (23) volatile compounds were identified in the Ngowe ripe fruit volatiles, representing 77.4% of the total volatile compounds. The identified compounds comprised monoterpene hydrocarbons (37.7%), oxygenated monoterpenes (0.3%), sesquiterpene hydrocarbons (2.2%), oxygenated sesquiterpenes (0.2%), non-terpenoid hydrocarbons (5.5%) and oxygenated non terpenoid compounds (31.5%). The major compounds were the monoterpenes α-pinene (**9**) (10.9%) and myrcene (**23**) (22.8%). Others were the ester ethyl butanoate (**54**) (15.1%), and the aromatic hydrocarbon toluene (**34)** (5.5%). α-Pinene (**9**), and myrcene (**23**) were also found as major compounds in Mexican Ataulfo variety (Sandoval *et al*., 2007).

5.2. 2 Volatile aroma chemistry of ripe Apple mango fruit pulp

A total of twenty two (22) volatile compounds were identified in the Apple mango ripe fruit volatiles, representing 78.4% of the total fruit volatiles. The identified compounds comprised monoterpene hydrocarbons (65.4%), oxygenated monoterpenes (0.5%), sesquiterpene hydrocarbons (1.4%), oxygenated sesquiterpenes (0.3%), non-terpenoid hydrocarbons (6.2%) and oxygenated non terpenoid compounds (4.6%). The major compounds found were the monoterpene α-pinene (**9**) (34.9%), myrcene (**23**) (24.4%) and β- Phellandrene (**31**) (4.0%). The chemical composition of the Kenyan Apple mango is different from that of Apple mango from Taiwan which comprised mainly δ-3-Carene, α-Copaene, α-guaiene, germacrene D, α-bulnesene, and γ-gurjunene (An *et al*., 2015a). The difference in composition between the two Apple varieties could be due to differences in soil type and climatic conditions.

5.2. 3 Volatile aroma chemistry of ripe Keitt mango ripe fruit pulp

A total of thirteen compounds were identified in the Keitt ripe fruit volatiles, representing 69.9% of the total volatile compounds. The identified compounds comprised monoterpene hydrocarbons (38.6%), sesquiterpene hydrocarbons (1.0%),

oxygenated sesquiterpenes (0.7%) and oxygenated non terpenoid compounds (26.6%). The major compounds identified were the three esters and one monoterpene. The esters present were ethyl butanoate (**56**) (4.4%), ethyl hexanoate (**59** (4.1%) and ethyl octanoate (**63**) (14.0%). The monoterpene present was α-pinene (**9**) (10.2%). The chemical composition of Kenyan Keitt variety is different from same variety from Cuba, which was found to contain mainly δ-3-carene (Pino *et al*., 2005). Keitt ripe fruit volatiles were found to contain the highest number of esters compared to the volatiles from the fruit of any other mango variety in this study.

5.2. 4 Volatile aroma chemistry of ripe Boribo mango fruit pulp

A total of twenty six (26) volatile compounds were identified in the Boribo ripe fruit volatiles, representing 77.9% of the total fruit volatiles. The compounds comprised monoterpene hydrocarbons (59.4%), oxygenated monoterpenes (1.1%), sesquiterpene hydrocarbons (11.9%), and oxygenated sesquiterpenes (1.5%) and oxygenated non terpenoid compounds (4.0%). The major compound was the monoterpene myrcene (**23**) (57.0%). Myrcene has also been found as a major compound in Philippines Carabao Mango (PCM) volatiles (An *et al*., 2015a).

5.2. 5 Volatile aroma chemistry of ripe Tommy Atkins mango fruit pulp

A total of thirty volatile compounds were identified in the Tommy Atkins ripe fruit volatiles, representing 73% of the total volatile compounds. The identified compounds comprised monoterpene hydrocarbons (41.4%), oxygenated monoterpenes (0.3%), sesquiterpene hydrocarbons (1.2%), oxygenated sesquiterpenes (6.4%), non-terpenoid hydrocarbons (0.2%) and oxygenated non terpenoid compounds (23.5%). The major compounds were the monoterpenes α-pinene (**9**) (7.4%) and δ-3-carene (**8**) (27.1%). Others were non-terpene oxygenated compounds comprising the ketone, 3-hydroxy-2-butanone (**52**) (4.8%), the alcohol 2, 3-butanediol (**77**) (3.7%) and ester ethyl butanoate (**54)** (6.7%). The presence of δ-3-carene as a major compound is in agreement with the same variety that grows in Colombia (Quijano *et al*., 2007), Mexican Ataulfo variety (Sandoval *et al*., 2007) and Haden, Irwin and Manila varieties from Colombian (Quijano *et al*., 2007).

5.2. 6 Volatile aroma chemistry of ripe Van Dyke mango fruit pulp

A total of seventeen volatile compounds were identified in the Van Dyke ripe fruit volatiles, representing 49.2% of the total fruit volatiles. The identified compounds comprised monoterpene hydrocarbons (33.9%), oxygenated monoterpenes (0.3%), sesquiterpene hydrocarbons (2.1%) and oxygenated non terpenoid compounds (12.9%). The major compounds were monoterpenes α-pinene (**9**) (3.9%) and δ-3-carene (**8**) (25.6%). Others were the esters ethyl butanoate (**54**) (3.4%) and (E)-2-butenoic acid, ethyl ester (**76**) (4.9%) The major compounds present in the Van Dyke variety were different from those found in the Colombian variety which had α-phellandrene as a dominant compound (Quijano *et al*., 2007).

5.2. 7 Volatile aroma chemistry of ripe Sabre mango fruit pulp

A total of twenty seven compounds were identified in the Sabre ripe fruit volatiles, representing 65.2% of the total volatile compounds. The identified compounds comprised monoterpene hydrocarbons (44.7%), Sesquiterpene hydrocarbons (3.1%), non-terpenoid hydrocarbons (1.4%) and oxygenated non terpenoid compounds (16.0%). The major compounds identified were the monoterpenes α-pinene (**9**) (7.8%) and δ-3-carene (**8**) (30.3%). The other compounds were the ester methyl salicylate (**79**) (3.8%) and the ketone *p*-ethyl-acetophenone (**80)** (6.6%). α-Pinene (**9**), and δ-3-carene (**8**) were also found as major compounds in Mexican Ataulfo variety (Sandoval *et al*., 2007).

5.2. 8 Volatile aroma chemistry of ripe Kent mango ripe fruit pulp

A total of twenty volatile compounds were identified from in Kent ripe fruit volatiles, representing 57.3% of the total volatile compounds. One compound could not be identified. The identified compounds comprised monoterpene hydrocarbons (41.0%), oxygenated monoterpenes (0.4%), sesquiterpene hydrocarbons (2.4%), non-terpenoid hydrocarbons (2.7%) and oxygenated non terpenoid compounds (10.8%). The major compounds identified were δ-3-carene (**8**) (35.0%) and methyl salicylate (**80**) (9.3%). δ-3-Carene was also found to be dominant in the Cuban varieties Haden, Manga amarilla, Macho, Manga blanca, San Diego, Manzano, Smith, Florida, Keitt, and Kent (Pino *et al*., 2005).

5.3 Comparative volatile chemistry of ripe mango fruits

The various classes of compounds identified in the volatiles of the ripe fruit pulps of the mango varieties under this study varied both qualitatively and quantitatively. This section discusses these differences and similarities.

5.3.1 Monoterpenes

There were some similarities in qualitative distributions of monoterpenes in the volatiles of ripe fruit pulp of some mango varieties in this study. Major Monoterpenes found in the ripe fruits volatiles were α-pinene (**9**) (identified in Ngowe, Apple, Boribo, Tommy Atkins, Van Dyke and kent), myrcene (**23**) (identified in aroma fruit volatiles of Apple and Boribo), δ-3-carene (**8**) (identified in Tommy Atkins, Van Dyke, Sabre and Kent) and β-phellandrene (**31)** (identified in all eight varieties).

In addition some monoterpenes were found in the volatiles of ripe fruit pulp of a number of fruits in minor but significant quantities. The ripe fruit volatiles of all the eight mango varieties in this study were found to contain two monoterpenes, α-pinene **(9**) and β- phellandrene (**31**). Tommy Atkins, Sabre and Kent were all found to contain camphene (**65**), o-cymene (**93**), α-phellandrene (**42**) and terpenolene **(7**). Camphene **(65**) and terpenolene **(7**) were also found in Apple fruit volatiles. There was also similarity between Keitt, Boribo and Van Dyke as, other than containing few monoterpenes, they had β-Pinene (**10**) as a common compound.

There were differences in quantitative distributions of the monoterpenes in the eight varieties with three major aroma groups being identified. The first group was rich in α-pinene **(9**) and was present in the volatiles of all the eight varieties, with Ngowe (10.9%), Apple (34.9%), Tommy Atkins (7.4%) and Van Dyke (3.9%) having significant amounts of the compound. The second group, rich in myrcene (**23**), was found in Ngowe (22.8%), Apple (24.4%) and Boribo (57.0%). The third group was rich δ-3-carene **(8)** and was observed in Keit (25.8%), Tommy Atkins (27.1%), Van Dyke (25.6%), Sabre (30.3%) and Kent (35.0%).

The monoterpene α-pinene **(9**) has also been reported as dominant in the Colombian varieties, Hilacha and Vallenato, which were found to be rich in α-pinene (Quijano *et*

al., 2007). Myrcene (**23**) was identified in Brazilian varieties namely, Cavalo, Rosa, Espada and Paulista (Andrade *et al*., 2000) and also in the Alphonso and Jalna varieties from India and Sri Lanka, respectively (MacLeod and Pieris, 1984; Sakho *et al*., 1985). The δ-3-carene **(8)** was dominant in Haden, Irwin, Manila and Tommy Atkins varieties from Colombian (Quijano *et al*., 2007) and a Venezuelan mango fruit (MacLeod and De Troconis, 1982). Other varieties with δ-3-carene **(8)** were Tommy Atkins and Keitt varieties from Florida (MacLeod and Snyder, 1985). Additional varieties with δ-3-carene include M'Bingue, Téte de Chat and Palmer mangoes grown in Africa (Ollé *et al*, 1998), Haden, Rubi, Tommy Atkins and Keitt varieties from Brazil (Andrade *et al*., 2000; Lopes *et al*, 1999) and 10 Cuban varieties, Haden, Manga amarilla, Macho, Manga blanca, San Diego, Manzano, Smith, Florida, Keitt and Kent (Pino and Mesa, 2006).

These findings also show great similarities between the Kenyan Keitt, Tommy Atkins and Kent compared to the same varieties from the other parts of the world with respect to δ-3-carene **(8)** as a dominant compound. The Kenyan Keitt variety agrees with the Florida, Brazillian and Cuban varieties while Tommy Atkins is in agreement with the same variety from Colombia, Florida and Brazil and Kent with the Cuban variety in terms of the aroma volatile components of the major monoterpenoid compounds. The δ-3-carene **(8)** is considered as the most important aroma constituent, due to the high percentage in some volatile fractions (MacLeod and Pieris, 1984; Andrade *et al*., 2000).

Terpinolene (**7),** which was present in the Kenyan varieties Tommy Atkins, Van Dyke, Sabre and Kent, has also been reported in considerable quantity in Willard and Parrot varieties from Sri Lanka (MacLeod and Pieris, 1984). It was also present in Bowen, Kensington Pride and Florigon varieties from Australia (Bartley and Schwede, 1987; Bartley, 1988) and in Brazilian Espada mango (Lopes, *et al*., 1999).

The terpene hydrocarbons are considered to be important contributors to the flavour of Brazilian and Venezuelan mango varieties, (Engel, and Tressl, 1983) as well as Florida mango varieties, such as Keitt, Kent and Tommy Atkins (Malundo *et al*, 1997) and 20 varieties of Cuban mangoes (Pino *et al*., 2005; Pino and Mesa, 2006). The aroma typical of mango was reported to be correlated to monoterpenes (Lopes *et al*. 1999).

5.3.2 Oxygenated monoterpenes

The oxygenated monoterpene 6, 7-epoxymyrcene (**94**) was present in Apple and Boribo while camphor (**95**) was present in Ngowe, Van Dyke and Kent. p-Mentha-1, 5-dien-8-ol (**96**) was present in Boribo and Tommy Atkins.

5.3.3 Sesquiterpenes

The sesquiterpene α-copaene **(37**) was present in Ngowe, Boribo Tommy Atkins, Van Dyke and Sabre. Others were α-guaiene (**38)**, present in Apple and Boribo; α-humulene (**12**) found in Ngowe, Boribo, Tommy Atkins, Van Dyke, Sabre and Kent. ϒ-Gurjunene (**41**) was in Boribo, Van Dyke and Sabre; germacrene D (**39**) was found in Keitt, Boribo and Sabre; α-bulnesene (**40**) in Boribo, Sabre and Kent; *cis*-calamenene (**82**) was in Ngowe, Boribo and Sabre.

There were qualitative and quantitative differences in the aroma of ripe fruit volatiles. Sesquiterpenes. *(E)*-caryophyllene (**13**) was identified in all the varieties (0.5-1.6%), This differed with Hilacha, Haden, Irwin, Manila, Tommy Atkins and Yulima varieties in Springfield mango where *trans*-α-bergamotene was found to predominate, and the Vallenato variety was rich in ϒ-gurjunene (Andrade *et al*., 2000). Oxygenated sesquiterpene Caryophyllene oxide (**97**) was present in Ngowe, Apple, Keit and Boribo.

5.3.4 Non-terpene hydrocarbons and other oxygenated compounds

The non-terpene hydrocarbon ethylbenzene (**98**) was present in Tommy Atkins, Sabre and Kent while *p*-xylene (**99**) was found in Sabre and Kent.

Among the other oxygenated compounds, eight carbonyls were identified with a total contribution of 4.4% to the total volatile compounds. 3-Hydroxy-2-butanone **(52)** was identified in Ngowe, Apple, Tommy Atkins and Van Dyke in varying amounts of between 1%- 4.8%, while *p*-ethyl acetophenone was in Apple, Tommy Atkins, Sabre and Kent (0.3-6.6%) . *p*-Acetylacetophenone was found in Tommy Atkins (0.4%) and Sabre (1.1%). The rest of the carbonyls had less than 1% of the volatiles in all of the mango varieties in this study. Six alcohols were identified with 2, 3-butanediol (**77)** with the percentage composition of 3.7% being the highest amount in Tommy Atkins.

Considerable amounts of oxygenated volatile compounds, such as esters, furanones and lactones have also been identified in some Australian varieties (Bartley, 1988).

5.3.5 Esters

The major esters found in the ripe fruit pulp of the mango varieties were ethylbutanoate (**56**) which was identified in Ngowe, Apple, Keitt, Boribo, Tommy Atkins and Van Dyke, ethyl hexanoate (**59**) and ethyl octanoate (**63**) which were identified in Keitt and methyl salicylate (**79**) that was identified in Tommy Atkins, Sabre and Kent.

Other esters found in minor but significant quantities include (*E)*-2-Butenoic acid, ethyl ester (**76**) and (Z)-2-Butenoic acid, ethyl ester (**78**) which were present in Ngowe, Apple, Keitt, Boribo, Tommy Atkins and Van Dyke. Isobutyl butanoate (**100**) was found in Tommy Atkins and Kent. Butyl butanoate (**101**) was found in Tommy Atkins and Van Dyke.

The esters formed the second dominant class of compounds after the monoterpenes and had a total of 10 compounds in the studied varieties. They formed 19.7% of the total volatile compounds. Among them, ethyl butanoate (**54**) was present in all varieties except Apple, Sabre and Kent, with Ngowe (15.1%) having the highest amount. Large amounts of ethyl butanoate were observed in Colombian varieties, (Quijano *et al*., 2007), Baladi mango from Egypt (Pino and Mesa, 2006), Kensington Pride grown in Australia (Bartley and Schwede, 1987) and Cuban varieties (Pino *et al*., 2005). Ethyl butanoate was reported to be responsible for the fruity flavour of mango (Bartley, 1988; Pino and Mesa, 2006), and has been reported as among the compounds that attract females *Anastrepha striata* Schiner (Diptera: Tephritidae in guava (Diaz-Santiz *et al*., 2016).

5.4 Bioassays

5.4.1 Bioassay of mango leaf essential oils in laboratory conditions

Results of the bioassay of essentials oils showed that the fly responded differently to odor emanating from the different leaf oils. There were significant differences in the attractiveness of all oils and their respective controls. Except for Boribo oil whose attraction was not significantly different from the control (4.5 ±0.287 and 5.5±0.28,

respectively at P<0.05), the attractions of all the oils were not significantly different (3.0±0.408 - 4.5±0.287 at P<0.05). Boribo oil, with a mean attraction of 4.5±0.287 was the highest, while Keit oil with an attraction of 3.0±0.408 was the lowest.

These differences could be due to the qualitative and quantitative differences in the chemical composition of the oils. Although the chemical composition of essential oil mainly depends on soil and climatic conditions of the location, growing season and maturity stages (Laskar and Majumdar, 1988), the plants subjected to present study were cultivated at the same location under same soil and climatic conditions and harvested at the same age. Therefore, variability of essential oil composition could be considered as variation among morphotypes.

There is no previous work that has been reported on the attractive or repellent effect of mango leaf oil on the fruit fly *B. invadens*. However, oils from *hinoki* , *Eucalyptus,* cinnamon, *Litsea* and *Ocimum tenuiflorum* plants have been tested for their attractiveness towards the fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) (Diongue *et al.,* 2013; Dharmadasa, *et al.,* 2015).

Bioassay studies of the leaf oil *hinoki* , *Eucalyptus,* cinnamon and *Litsea* plants showed that significantly more number of female adults of *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) alighted on filter paper treated with *hinoki* oil and eucalyptus oil at 400 ppm of the oil dilution. However, cinnamon was found to inhibit or repel alighting of the female adults at a dosage of 200 ppm, 400 ppm and 800 ppm in the same study. A similar repellent activity was observed when filter paper was treated with essential oils extracted from *Litsea* leaves (Diongue *et al.*, 2013). In another study, the essential oil of *Ocimum tenuiflorum* was found to attract *B. dorsalis* (Dharmadasa, *et al.,* 2015).

5.4.2 Bioassay of mango leaf essential oil in field conditions

Following the analysis of raw data for attractiveness of the mango leaf essential oil to *B. invadens,* the mean number of flies lured to methyl eugenol (positive control) was the biggest (5.143 ± 1.171) and significantly different (F=9.07, DF=6. P<0.0001) from the means of all the other essential oils. However, there was no significant difference in the attractiveness among the oils. The same behavior was observed in the dual choice

olfactometric bioassay where there was significant difference ($P < 0.05$) between the essential oils and the positive control. Among the essential oils, Boribo oil showed some slight attractive effects though not statistically of any significant difference when compared with positive control of methyl eugenol. Ngowe lured 0 fly hence a potential repellent candidate leaf essential oil. This is the first report of the field bioassay on an indication of attractiveness of a mango leaf essential oil towards a mango fruit fly.

5.4.3 Bioassay of ripe mango fruit pulp in field conditions

According to ANOVA and LSD test, P= 0.0836, there were no significant differences in the number of flies attracted by the different juices and the positive control ($F=2.02$, $DF= 6$, $P > 0.05$). Although the positive control's mean of 5.857±1.724 was the highest, it was very close to that of Keitt juice (4.571±1.445) and therefore did not have any statistical significant difference ($P>0.05$). The attractions of the other juices were not significantly different. Keitt juice was therefore as good as the methyl eugenol in attracting the flies. The other juices were comparable in their activity which decreased in the order: Boribo, Ngowe, Apple, Tommy Atkins and Van Dyke.

The flies captured by the juices were all females. Both female and male *Bactrocera invadens* have been reported to show higher responses to odours from mature unripe and ripe mangoes, *M. indica*, marula (Kimbokota and Torto, 2013). *Bactrocera dorsalis* are highly attracted to odours from soft and ripe fruits. Preference of flies for mature and ripe fruits could be due to the presence of certain groups of compounds that are produced at these levels of maturity that are detected by the antennal olfactory receptors of the flies, thus facilitating location of their hosts (Kimbokota and Torto, 2013).

The presence of these compounds in the fruit volatiles may also associatively be an indication to the gravid flies of the soft texture of the mature fruit whose skin can easily be punctured with the ovipositor. The presence of a certain group of compounds may also signal the availability of enough resources for the survival of the larval stages of the insect up to the time of pupation (Kimbokota and Torto, 2013). The major hypothesis of the evolution of oviposition behaviour in insects is that, the females choose host plant species that maximize larval survival and development (Thompson and Pelmyr, 1991). Host plants also play an important role in the synthesis of sex pheromones of some phytophagous insects through the acquisition of bioactive

chemicals and the necessary chemical precursors for the pheromones via consumption, absorption or inhalation of host plant materials (Landolt and Phillips, 1997). What guides them are the chemical stimuli emanating from these plant species. This ensures that there is a high chance for mate location and mating and hence propagation of their generations (Landolt and Phillips, 1997).

5.4.4 Bioassay of the chemical standards in laboratory conditions

There was no significant difference in the attractiveness among the treatments other than the control. However, there was significant difference in the attractiveness of the positive control and other treatments of allester (ethyl butanoate, ethyl octanoate, ethyl hexananoate and methyl salicylate) ethylbut (ethylbutanoate), MCAPIE (myrcene, δ-3-carene, α-pinene and ethyl butanoate), MCAPIEME (myrcene, δ-3-carene, α-pinene, ethyl butanoate) and Myrcene. There were no significant differences in the attraction of the ethylbutme (blend of ethylbutanoate and methyl eugenol) (4.75± 0.478) and MYPIEB (4.75±0.25) as compared to the positive control (5.25±0.25). The two esters were as good as the positive control in the Laboratory bioassay

The aim of the use of chemical standards was to identify the pure compounds or the blends that were responsible for the observed activity, the combination of which was based on the functional groups or class of the compounds. The blends of all esters comprising ethylbutabnoate, ethyl hexanoate, ethyl octanoate and methyl salicylate showed a remarkable attractive effect of 37.5±2.5% when compared to the positive control. There was no significant difference between the all ester blend and ethylbutanoate (35±2.87%). This showed that ethylbutanoate significantly contributed to the attractive effect of the esters towards the fruit fly. The findings in this work are in agreement with the previous study which showed that ethyl butanoate and ethyl octanoate were among the 15 compounds from 'Chausa' mango variety that elicited EAG response of *B. dorsalis* (Jayanthi *et al*., 2012). The contribution of individual esters, ethyl hexanoate and ethyl octanoate could not be established in this work. The two compounds have however been found to elicit EAG response of *B. dorsalis* (Jayanthi *et al.*, 2012).

The blend comprising ethyl butanoate and three monoterpenes; myrcene, δ-3-carene and α-pinene (MCAPIE) improved the attractiveness of ethylbutanoate from 35±2.87

to 40±4.08%. However, the level of attractiveness remained the same at 40±4.08% even when δ-3-carene was removed from the blend (MYPIEB). This was an indication that δ-3-carene probably had no contribution in the attractiveness of the three monoterpenes and ethylbutanoate blend. In a previous study myrcene, identified from the 'Alphonso' mango variety and δ-3-carene from 'Chausa'mango variety, were found to elicit EAG response of *B. dorsalis* (Jayanthi *et al*., 2012). There was no significant difference between the attractiveness of the MYPIEB (4.75±0.25) blend and methyl eugenol as the positive control (5.25±0.25). A similar level of attractiveness (4.75±0.25) was observed in the blend comprising ethylbutanoate and methyl eugenol (ethylbutme).

5.4.5. Bioassay of blends of esters chemical standards in the field conditions

According to ANOVA and LSD test there were significant differences in the number of flies attracted by the blends of esters ($F=38.54$, $DF = 5$, $P < 0.05$). The control attracted the highest number of flies (4.833±0.601). There were no significant differences in the attraction of the other blends towards the mango fruit fly sp. However, the blend that had no methyl salicylate attracted more flies (0.5000±0.342) than any other blend. The blend having no ethyl butanoate was totally inactive, with the mean attraction of zero.

In terms of individual contribution of each component, methyl salicylate exhibited repellent effect while ethylbutanoate showed attractive property. The subtraction of ethyl hexanoate and ethyl octanoate has no effect since the resulting blends have the same attractiveness as the all-esters blend (0.167±0.167). Ethyl hexanoate and ethyl octanoate could therefore neither be classified as attractants nor repellents to the fruit fly in the field bioassay thus they have no effect at all.

However, the two esters (ethyl hexanoate and ethyl octanoate) have been reported to elicit EAG responses in *B. dosalis* (Jayanthi *et al*., 2012). Ethyl hexanoate emitted from guava and orange has been reported to elicit responses in both sexes of *Anastrepha ludens* (Malo *et al.* 2005). It has also been reported to affect the behavior of the oriental fruit fly, *B. dorsalis* (Siderhurst and Jang 2006).

Esters like Isoamyl acetate and isoamyl butyrate have been reported to elicit significant EAD responses with *B. invadens* (Biasazin *et al*., 2014). Esters are compounds typical

of ripening fruit and this may indicate a strong antennal sensitivity and sensory neuron abundance to these 'fruity odors', similar to drosophilids (Dekker *et al*. 2006), and/or signify the behavioral significance of these compounds for the species.

Field tests suggested that attraction is governed by a few particular compounds rather than specific odor blends. The strong influence of single compounds led to the search for novel attractive compounds, and to investigate the role of individual compounds in the overall attractiveness of the blends.

5.4.6 Bioassay of blends consisting of myrcene, δ-3-carene, α-pinene and ethyl butanoate

Ethyl butanoate, which had been found to be active, was blended with three non oxygenated monoterpenes; myrcene, α-penine and δ-3-carene in a subtraction bioassay. There was significant difference in the attraction among the various blends of the monoterpenes and ethyl butanoate in this test ($F = 7.81$, $DF = 5$, $P = 0.0001$). The control with a mean catch of 4.5±1.088 had greater attractive effect compared to all the other blends although there was no statistical significant difference among three blends, MYPIEB, CAPIEB and MCAPIE. The blend comprising myrcene, α-penine and ethyl butanoate (MYPIEB) attracted more flies than any other blend (2.333 ±0.802). It was however far less attractive when compared to the positive control.

The activities of the individual components of this blend were not manifested in any other blend in this test. This showed the synergistic effect of these compounds, which were only active as a blend. This blend did not contain δ-3-carene (**8)** and all the blends containing δ-3-carene exhibited different levels of reduction in the level of attraction. The δ-3-carene (**8**) was therefore a repellent candidate to the mango fruit fly in the field bioassay. This was in contrast to other studies that have shown that this compound is attractive to *B. dorsalis* (Jayanthi *et al*., 2012). Myrcene (**23**) and α-pinene **(9**) were part of three mixture components reported as attractive to West Indian fruit fly, *Anastrepha obliqua* (Macquart) (Diptera: Tephritidae) (Malo *et al*., 2012).

Some of the dominant monoterpenes have been previously reported to have activity against various species of insects. The α-pinene **(9**) is one of the compounds that have been reported to possess repellent properties against *Kilifia acuminate* (Signoret)

(Hemiptera: Coccidae) (Monzer *et al*., 2013) but attractive to *Temnochila chlorodia* (Zhou *et al*., 2001). A trap baited with a blend of myrcene (**23**), α-pinene **(9**), and trans-β-ocimene (**45**) has been used to capture male and female *Anastrepha obliqua* (Macquart) (Diptera: Tephritidae) (Malo *et al*., 2012). Likewise, δ-3-carene **(8)** has been reported to be attractive to *Epitragus sallaei* (Champion), a beetle which feeds on the flower of *Mangifera indica* var Ataulfo (Cruz-Lopez *et al*., 2001).

5.4.7 Bioassay of individual pure compounds under natural conditions

There were significant differences in the level of attraction of the individual compounds (chemical standards) in these experiments as determined by one-way ANOVA ($F = 18.27$, $DF=7$, $P < 0.0001$). Methyl eugenol (positive control) was most attractive (4.125 ±0.953). Except for ethy butanoate, which was mildly attractive (0 .125 ±0.125), all the other compounds showed no attraction to the fruit fly. This showed that the chemical standards applied in the field bioassay were only active as blends and were not sufficiently active to attract the flies as pure compounds in the field bioassay. Insects often are finely tuned to ratio and composition of mixtures of compounds. Likewise, the behavioral activity elicited by mixtures is typically stronger than that elicited by individual compounds (Bruce and Pickett, 2011).

There were remarkable differences in the attractiveness of the treatments in the laboratory Dual choice olfactometric bioassay and the field bioassay. There was a drastic drop in the level of attractiveness of the treatments in the field bioassay when compared to the positive control. In the Laboratory bioassay the blend comprising myrcene, α-pinene and ethyl butanoate was as good as the positive control (methyl eugenol) in the fruit fly attraction. This blend however attracted fewer flies in the field bioassay than the positive control. In this work the tests were done on a farm that extensively used chemical lures, especially Methyl eugenol to control the *B. invadens*. The population of the flies on this farm therefore had greatly been reduced. The factors such as the direction of the wind, humidity and temperature on a particular day may have had an impact on the number of flies attracted in the field bioassay (Jaffery *et al*., 2017).

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6. 1 Conclusions

- The major compounds found in the essential oils were α-pinene (in all the six mango varieties), β-pinene (in Ngowe), β-Phellandrene (in Ngowe and Apple), α-gurjunene (in Apple, Keitt, Boribo, Tommy Atkins and Van Dyke), δ-cadinene (in Apple), δ-3-carene (in Keitt, Tommy Atkins and Van Dyke), viridiflorene and germacrene D (in Keitt), 13-hydroxy valence (in Boribo) and β-selinene (in Tommy Atkins and Van Dyke). The major compounds found in the aroma volatiles of the ripe fruits were α-pinene (Ngowe, Apple, Boribo, Tommy Atkins, Van Dyke and Kent), myrcene (Ngowe, Apple and Boribo), ethylbutanoate (Ngowe, Keitt,Tommy Atkins and Van Dyke), β-phillandrene (Apple), ethyl hexanoate and ethyl octanoate (keitt), δ-3-carene (Tommy Atkins, Van Dyke, Sabre and Kent) and methyl salicylate (Sabre and Kent).
- Boribo mango leaf essential oil partially attracts *Bactrocera invadens* mango fruit flies. Ngowe mango leaf essential oil repels *B. invadens* mango fruit flies.The raw fruit juice aroma is as attractive as methyl eugenol towards *B. invadens* fruit fly, though the juice aroma contains no methyl eugenol. Keitt juice aroma is the most attractive but attracts mostly female fruit flies.
- Blends of esters containing ethyl butanoate, ethyl hexanoate, and ethyl octanoate and methyl salicylate show reasonable activity towards *B. invadens*. Ethyl butanoate promotes the attractiveness of the blends while mythyl salicylate is repellent towards *B. invadens*.
- Methyl salicylate is among the compounds that are responsible for the refractiveness or resistance of some mango cultivars to attack by *B. invadens* mango fruit fly. The sucsceptibility or attractiveness of the Keitt mango fruit towards *B. invadens* is due to the presence of, among other compounds, the esters ethyl butanoate, and ethyl hexanoate and ethyl octanoate.

6.2 Recommendations

- The attractiveness of the blends found between the treatments in this work and methyl Eugenol in the field bioassay could form an interesting study area in the investigation of the attractant for both male and female fruit fly *B. invadens*.
- The field study bioassay was carried out on the farm that had been exposed to extensive use of attractants and lures, studies should be done to investigate the effect of the same treatments when applied on the farms that have not been exposed to lures.
- The synthetic chemical standards used in the bioassay were selected from a list of major compounds found in the mango cultivars. Further work should be conducted to determine the bioactivity of other major compounds and some minor components that were not tested in this work towards *B. invadens*.
- The study focused on the chemical compounds from the leaves and fruits of the mango cultivars. Further work should be done to investigate the effect of the essential oils and the aroma volatiles compounds from the flowers of the same compounds towards *B. invadens* fruit fly.

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APPENDICES

Appendix I: Raw data of bioassay of leaf essential oils under laboratoty conditions

Treatment	rep	Y-tube	Flies	Mean±SE	control	rep	Y-tube arm	Flies	Mean±SE
MWAP	1	A	3		Control	1	B	7	
MWAP	2	B	3		Control	2	A	7	
MWAP	3	A	4		Control	3	B	6	
MWAP	4	B	3	3.25±0.25	Control	4	A	7	6.75±0.25
MWNG	1	A	4		Control	1	B	6	
MWNG	2	B	4		Control	2	A	6	
MWNG	3	A	4		Control	3	B	6	
MWNG	4	B	3	3.75±0.25	Control	4	A	7	6.25±0.25
MWKE	1	A	3		CONTOL	1	B	7	
MWKE	2	B	2		CONTOL	2	A	8	
MWKE	3	A	3		CONTOL	3	B	7	
MWKE	4	B	4	3±0.408	CONTOL	4	A	6	7±0.408
MWBO	1	A	4		CONTROL	1	A	6	
MWBO	2	B	5		CONTROL	2	B	5	
MWBO	3	A	5		CONTROL	3	A	5	
MWBO	4	B	4	4.5±0.288	CONTROL	4	B	6	5.5±0.288
MWTA	1	A	4		CONTROL	1	B	6	
MWTA	2	B	4		CONTROL	2	A	6	
MWTA	3	A	4		CONTROL	3	B	6	
MWTA	4	B	3	3.75±0.25	CONTROL	4	A	7	6.25±0.25
MWVD	1	A	3		CONTROL	1	B	7	
MWVD	2	B	3		CONTROL	2	A	7	
MWVD	3	A	4		CONTROL	3	B	6	
MWVD	4	B	3	3.25±0.25	CONTROL	4	A	7	6.75±0.25

Appendix II: A 7×7 completely randomized Latin Square bock experimental design for bioassay of the essential oils in field

	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	site 7
Day1	MWNG	MWAP	MWKE	MWBO	MWTA	MWVD	F
Day2	MWVD	MWKE	F	MWNG	MWAP	MWBO	MWTA
Day3	F	MWTA	MWVD	MWKE	MWBO	MWAP	MWNG
Day4	MWBO	MWNG	MWAP	F	MWKE	MWTA	MWVD
Day5	MWKE	MWVD	MWBO	MWTA	MWNG	F	MWAP
Day6	MWTA	F	MWNG	MWAP	MWVD	MWKE	MWBO
Day 7	MWAP	MWBO	MWTA	MWVD	F	MWNG	MWKE

Key: MWNG= Ngowe oil; MWAP=Apple oil; MWKE; Keit oi; MWBO=Boribo; MWTA=Tommy Atkins oil; MWVD=Van Dyke oil; F=methyl Euginol (Control).

Appendix III: Raw data for bioassay of leaf essential oils under natural field in field conditions

	Site 1	Site 2	Site 3	Site 4	Site5	Site 6	site 7
Day1	0	0	1	1	1	0	8
Day2	0	0	4	0	0	1	0
Day3	0	0	0	0	4	0	0
Day4	1	0	2	11	2	0	1
Day5	0	0	1	0	0	5	0
Day6	0	1	0	0	1	0	2
Day 7	0	0	0	0	7	0	0

Appendix IV: Statistical output for bioassay of leaf essential oi ls in field conditions showing ANOVA, LSD and means of the flies captured

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The GLM Procedure

Dependent Variable: flies

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	142.4897959	23.7482993	9.07	<.0001
Error	42	110.0000000	2.6190476		
Corrected Total	48	252.4897959			

R-Square	Coeff Var	Root MSE	flies Mean
0.564339	146.8500	1.618347	1.102041

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Treat	6	142.4897959	23.7482993	9.07	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treat	6	142.4897959	23.7482993	9.07	<.0001

The GLM Procedure
t Tests (LSD) for flies

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	42
Error Mean Square	2.619048
Critical Value of t	2.01808
Least Significant Difference	1.7457

Means with the same letter are not significantly different.

t Grouping	Mean	N	Treat
A	5.1429	7	CONTROL
B	1.4286	7	MWBO
B			
B	0.4286	7	MWKE
B			
B	0.2857	7	MWAP
B			
B	0.2857	7	MWVD
B			
B	0.1429	7	MWTA
B			
B	0.0000	7	MWNG

Appendix V: A 7×7 completely randomized Latin square bock experimental design for bioassay of the ripe mango fruit pulp in field

	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	site 7
Day1	NGJ	APJ	KEJ	BOJ	TAJ	VDJ	F
Day2	VDJ	KEJ	F	NGJ	APJ	BOJ	TAJ
Day3	F	TAJ	VDJ	KEJ	BOJ	APJ	NGJ
Day4	BOJ	NGJ	APJ	F	KEJ	TAJ	VDJ
Day5	KEJ	VDJ	BOJ	TAJ	NGJ	F	APJ
Day6	TAJ	F	NGJ	APJ	VDJ	KEJ	BOJ
Day 7	APJ	BOJ	TAJ	VDJ	F	NGJ	KEJ

Key: NGJ=Ngowe juice; APJ=Aple Juice; KEJ=Keit juice; BOJ=Boribo juice; TAJ=Tommy Atkins juice; Van Dyke juice; F=Methyl Euginol (positive control)

Appendix VI: Raw data for bioassay of ripe mango fruit pulp under natural field conditions

	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	site 7
Day1	1	0	4	0	0	0	5
Day2	0	2	15	4	3	3	3
Day3	1	1	1	2	2	1	2
Day4	2	2	5	5	4	1	2
Day5	1	2	4	3	1	6	2
Day6	0	2	4	4	5	12	6
Day 7	2	3	6	4	7	4	7

Appendix VII: Statistical output for bioassay of ripe mango fruit pulp in field conditions showing ANOVA, LSD and means of the flies captured

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The GLM Procedure

Dependent Variable: flies

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	90.4897959	15.0816327	2.02	0.0836
Error	42	312.8571429	7.4489796		
Corrected Total	48	403.3469388			

R-Square	Coeff Var	Root MSE	flies Mean
0.224347	85.72744	2.729282	3.183673

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Treat	6	90.48979592	15.08163265	2.02	0.0836

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treat	6	90.48979592	15.08163265	2.02	0.0836

The GLM Procedure

t Tests (LSD) for flies

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	42
Error Mean Square	7.44898
Critical Value of t	2.01808
Least Significant Difference	2.9441

Means with the same letter are not significantly different.

t Grouping	Mean	N	Treat
A	5.857	7	Control
A			
B A	4.571	7	Kejuice
B			
B	2.857	7	Bojuice
B			
B	2.571	7	NGJuice
B			
B	2.429	7	APJuice
B			
B	2.000	7	TAjuice
B			
B	2.000	7	VDJuice

Appendix VIII: Raw data of bioassay of chemical standards and methyl eugenol (positive control) under laboratoty conditions

Treatment	rep	Y-tube arm	Flies	Mean±SE	control	rep	Y-tube arm	Flies	Mean±SE
Allesters	1	A	3		Control	1	B	7	
Allesters	2	B	4		Control	2	A	6	
Allesters	3	A	4		Control	3	B	6	
Allesters	4	B	4	3.75±0.25	Control	4	A	6	6.25±0.25
Ethylbut	1	A	4		Control	1	B	6	
Ethylbut	2	B	4		Control	2	A	6	
Ethylbut	3	A	3		Control	3	B	7	
Ethylbut	4	B	3	3.5±0.287	Control	4	A	7	6.5±0.287
Ethylbutme	1	A	6		control	1	B	4	
Ethylbutme	2	B	5		control	2	A	5	
Ethylbutme	3	A	4		control	3	B	6	
Ethylbutme	4	B	4	4.75±0.478	control	4	A	6	5.25±0.478
MYPIEB	1	A	5		Control	1	B	5	
MYPIEB	2	B	4		Control	2	A	6	
MYPIEB	3	A	5		Control	3	B	5	
MYPIEB	4	B	5	**4.75±**0.25	Control	4	A	5	5.25±0.25
MCAPIE	1	A	4		Control	1	B	6	
MCAPIE	2	B	5		Control	2	A	5	
MCAPIE	3	A	4		Control	3	B	6	
MCAPIE	4	B	3	4±0.408	Control	4	A	7	6±0.408
MCAPIEME	1	A	4		Control	1	B	6	
MCAPIEME	2	B	4		Control	2	A	6	
MCAPIEME	3	A	5		Control	3	B	5	
MCAPIEME	4	B	4	4.25±0.25	Control	4	A	6	5.75±0.25
Myrcene	1	A	3		Control	1	B	7	
Myrcene	2	B	2		Control	2	A	8	
Myrcene	3	A	2		Control	3	B	8	
Myrcene	4	B	3	2.5±0.287	Control	4	A	7	7.5±0.287

Appendix IX: A 6×6 completely randomized Latin square bock experimental design for bioassay of blend of esters in field

	Site 1	**Site 2**	**Site 3**	**Site 4**	**Site 5**	**Site 6**
Day1	A	B	C	D	E	F
Day2	F	A	E	C	B	D
Day3	E	C	F	B	D	A
Day4	C	D	A	E	F	B
Day5	B	F	D	A	C	E
Day6	D	E	B	F	A	C

Key: A=All esters (Ethyl butanoate+methyl salicylate+Ethyl hexanoate+ Ethyl octanoate; B=All esters minus Ethyl ethanoate; C=All esters minus ethyl octanoate; D=All esters-methyl salicylate; E=All esters –Ethy butanoate; F=Positive control (methyl eugenol).

Appendix X: Raw data for bioassay of bend of esters under natural field conditions

	Site 1	**Site 2**	**Site 3**	**Site 4**	**Site 5**	**Site 6**
Day1	0	1	0	0	0	5
Day2	4	0	0	0	0	0
Day3	0	0	6	0	1	0
Day4	0	0	1	0	4	0
Day5	0	7	2	0	0	0
Day6	0	0	0	3	0	1

Appendix XI: Statistical output for bioassay of blends of esters in field conditions showing ANOVA, LSD and means of the flies captured

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                                    The GLM Procedure
Dependent Variable: flies
                                           Sum of
Source                      DF         Squares     Mean Square    F Value
Pr > F
Model                        5     108.1388889      21.6277778      38.54
<.0001
Error                       30      16.8333333       0.5611111
Corrected Total             35     124.9722222


                       R-Square     Coeff Var      Root MSE    flies Mean
                       0.865303      77.04756      0.749074      0.972222

Source                      DF       Type I SS     Mean Square    F Value
Pr > F
Treat                        5     108.1388889      21.6277778      38.54
<.0001
Source                      DF     Type III SS     Mean Square    F Value
Pr > F
Treat                        5     108.1388889      21.6277778      38.54
<.0001

                           The GLM Procedure
                     Student-Newman-Keuls Test for flies

NOTE: This test controls the Type I experimentwise error rate under the
complete null hypothesis but not under partial null hypotheses.
                              Alpha                          0.05
                              Error Degrees of Freedom       30
                              Error Mean Square        0.561111

 Number of Means              2              3              4
5              6
 Critical Range       0.8832417      1.0662034      1.1759549
1.2544496      1.3154235
                    Means with the same letter are not significantly
different.


                     SNK Grouping          Mean      N    Treat

                                A        4.8333      6    Control

                                B        0.5000      6    Allmeths
                                B
                                B        0.1667      6    Allethoc
                                B
                                B        0.1667      6    Allethhe
                                B
                                B        0.1667      6    Allester
                                B
                                B        0.0000      6    Allethyl
```

Appendix XII: A 6×6 completely randomized Latin square bock experimental design for bioassay of blends consisting of myrcene, δ-3-carene, α-pinene and ethyl butanoate

	Site1	**Site 2**	**Site 3**	**Site 4**	**Site 5**	**Site6**
Day1	G	H	I	J	K	F
Day2	F	G	K	I	H	J
Day3	K	I	F	H	J	G
Day4	I	J	G	K	F	H
Day5	H	F	J	G	I	K
Day6	J	K	H	F	G	I

Key: G = myrcene+δ-3-carene + α-pinene +ethyl butanoate; H= myrcene+δ-3-carene + α-pinene; I = myrcene+δ-3-carene + ethyl butanoate; J= myrcene+ α-penene +ethyl butanoate; K= δ-3-carene + α-penene +ethyl butanoate; F= mythyl eugenol (Control)

Appendix XIII: Raw data for bioassay blends *c*onsisting of myrcene, δ-3-carene, α-pinene and ethyl butanoate under natural field conditions

	Site 1	**Site 2**	**Site3**	**Site4**	**Site5**	**Site6**
Day1	1	0	0	4	1	4
Day2	6	1	0	2	1	4
Day3	0	0	1	0	4	1
Day4	0	2	0	0	8	0
Day5	0	6	0	1	1	1
Day6	0	2	0	2	0	0

Appendix XIV: Statistical output for bioassay of blends of myrcene, δ-3-carene, α-pinene and ethyl butanoate in field conditions showing ANOVA, LSD and means of the flies captured

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The GLM Procedure

Dependent Variable: flies

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	83.1388889	16.6277778	7.81	<.0001
Error	30	63.8333333	2.1277778		
Corrected Total	35	146.9722222			

R-Square	Coeff Var	Root MSE	flies Mean
0.565678	99.08086	1.458690	1.472222

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Treat	5	83.13888889	16.62777778	7.81	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treat	5	83.13888889	16.62777778	7.81	<.0001

The GLM Procedure

t Tests (LSD) for flies

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	30
Error Mean Square	2.127778
Critical Value of t	2.04227
Least Significant Difference	1.72

Means with the same letter are not significantly different.

t Grouping	Mean	N	Treat
A	4.5000	6	CONTROL
B	2.3333	6	MYPIEB
B			
C B	0.6667	6	CAPIEB
C B			
C B	0.6667	6	MCAPIE
C			
C	0.5000	6	MYCAEB
C			
C	0.1667	6	MYCAPI

Appendix XV: An 8×8 completely randomized Latin square bock experimental design for bioassay of individual chemical standards in field conditions

	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	site 7	site 8
Day1	Camp	Aphil	EB	Apin	MY	Bpin	Car	F
Day2	F	MY	Bpin	Aphil	Car	EB	Camp	Apin
Day3	EB	Apin	Car	MY	F	phil	Bpin	Camp
Day4	Car	Camp	APhil	EB	Bpin	Apin	F	MY
Day5	Aphil	Bpin	MY	F	Camp	Car	Apin	EB
Day6	Apin	F	Camp	Car	EB	MY	Aphil	Bpin
Day 7	Bpin	EB	Apin	Camp	Aphil	F	MY	Car
Day 8	My	Car	F	Bpin	Apin	Camp	EB	Aphil

Key: Camp=camphene; APhil=α-phellandrene; EB=Ethylbutanoate; Apin=α-pinene; MY=Myrcene; Bpin= β-pinene; δ-3-carene; F=methyl eugenol (control).

Appendix XVI: Raw data for bioassay of individual chemical standards under natural field conditions

	Site1	Site 2	Site 3	Site 4	Site 5	Site 6	site 7	site 8
Day1	0	0	0	0	0	0	0	3
Day2	6	0	0	0	0	1	0	0
Day3	0	0	0	0	8	0	0	0
Day4	0	0	0	0	0	0	4	0
Day5	0	0	0	0	3	0	0	0
Day6	0	2	0	0	0	0	0	0
Day 7	0	0	0	0	0	7		0
Day 8	0	0	0	0	0	0	0	0

Appendix XVII: Statistical output for bioassay of individual chemical standards in field conditions showing ANOVA, LSD and means of the flies captured

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The GLM Procedure

Dependent Variable: flies

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	118.1875000	16.8839286	18.27	<.0001
Error	56	51.7500000	0.9241071		
Corrected Total	63	169.9375000			

R-Square	Coeff Var	Root MSE	flies Mean
0.695476	180.9515	0.961305	0.531250

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Treat	7	118.1875000	16.8839286	18.27	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treat	7	118.1875000	16.8839286	18.27	<.0001

The GLM Procedure

t Tests (LSD) for flies

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	56
Error Mean Square	0.924107
Critical Value of t	2.00324
Least Significant Difference	0.9629

Means with the same letter are not significantly different.

t Grouping	Mean	N	Treat
A	4.1250	8	control
B	0.1250	8	ethylbut
B	0.0000	8	Aphiland
B	0.0000	8	Apinene
B	0.0000	8	Dcarene
B	0.0000	8	Myrcene
B	0.0000	8	Bpinine
B	0.0000	8	Camphene