BEHAVIORAL RESPONSES OF *Tuta absoluta* TO A WILD AND CULTIVATED TOMATO PLANTS AND CHARACTERIZATION OF THE MEDIATING SEMIOCHEMICAL BLENDS

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DECLARATION

Declaration by Candidate

This thesis is my original work and has not been presented for a degree in any other University

or any other award.

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DEDICATION

I dedicate this work to my late father, Mzee Miano Njogu, who inspired me throughout my entire school life, my mother, Eunice Njoki Miano, my siblings, my wife Mary Wanjiku and my children Gift, Joy, Vincent and Cleopas for their patience, love, prayers and moral support that they accorded me throughout my study time.

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Finally my thanks to my family and any other member of the community who have given me support, encouragement, love and prayer.

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ABBREVIATIONS AND ACCRONYMS

ANOVA…………………… Analysis of Variance

BVOCs…………………… Biogenic Volatile Organic Compounds

CAN……………………… Calcium ammonium nitrate

CC………………………… Monocrop of cultivated tomato

DAP……………………… Diammonium phosphate

DC………………………… Cultivated tomato volatiles trapped during the day

DCM……………………… Dichloromethane

DHT……………………… Dynamic Head Trapping

DW………………………… Wild tomato volatiles trapped during the day

EAG……………………… Electroantennography

FID………………………… Frame Ionization Detector

GC – EAD………………… Gas Chromatography – linked Electroantennographic Detector

GC – MS…………………… Gas Chromatography – linked Mass Spectroscopy

HIPVs…………………… Herbivorous Induced Plant Volatiles

HS – SPME……………… Headspace – Solid Phase Microextraction

ICIPE…………………… International Center for Insect Physiology and Ecology

IPM……………………… Integrated Pest Management

KARLO…………………… Kenya Agricultural and Livestock Research Organization

MANOVA………………… Multivariate Analysis of Variance

NC………………………… Cultivated tomato volatiles trapped during the night

NW………………………… Wild tomato volatiles trapped during the night

PCA……………………… Principal Component Analysis

PCPB…………………….. Pest Control Product Board

PI………………………… Preferential Index

RBD……………………… Randomized Brock Design

REML…………………… Residual Maximum Likelihood

SEM……………………… Standard Error

SNK……………………… Students Newmans Keul test

TB………………………… Monocrop of cultivated tomato surrounded by wild tomato

TC………………………… Intercrop of wild and cultivated tomatoes

TDDA …………………… (3E,8Z)–3,8–tetradecadien–1–yl acetate

TDTA …………………… (3E, 8Z, 11Z)–3, 8, 11–tetradecatrien–1–yl acetate

TT………………………… Monocrop of wild tomatoes

ABSTRACT

Tomato is rated the second most important horticultural crop after potato in most parts of the world. However, its cultivation is threatened by infestations of *Tuta absoluta* (Lepidoptera: Gelechiidae). The pest originated from South America and is now invading fields and greenhouse production sites in the world. *Tuta absoluta* was first officially reported in Kenya in March 2014 at Isiolo and has spread to all parts where tomato is grown. The pest has been nicknamed tomato 'Al–shabaab' as it leaves unimaginable damage of the crop after infestations. Chemical methods used to control the pest have led to high levels of residues, hence risking consumers, harming the ecology and the environment. The present study was based on field observations that a wild tomato, *Lycopersicon esculentum var. cerasiforme*, which grows in the tea zones of Mount Kenya region, Kenya, is not attacked by *T. absoluta*, unlike the cultivated commercial tomato varieties. It was hypothesized that the wild variety may be actively avoided by gravid females because of the presence of constituents that deter gravid *T. absoluta* females. The objective of the present study was to compare the behavioral responses of *T. absoluta* to wild and cultivated tomato plants and characterize their mediating semiochemical blends. The responses of gravid *T. absoluta* females to the wild tomato and cultivated tomato, *Solanum lycopersicum* L. (Rambo F1 variety) intact plants in a dual–choice olfactometer was conducted where the gravid females were attracted to the cultivated species but repelled by the wild species, PI = -45.45%, X^2 = 10.47, df = 1, p < 0.05. The levels of infestation of the pest in mono–crop and intercrops of the two varieties were also compared. There was significant reduction in the levels of infestation in the intercrop arrangements (P<0.001, at α=0.05). Gas chromatography–linked mass spectrometry (GC–MS) of the headspace volatiles collected from the two tomato species revealed large differences in their chemical profiles. A total of 162 compounds were positively identified and quantified: 85 from cultivated tomato's day volatiles, 73 from wild tomato's day volatiles, 68 from cultivated tomato's night volatiles and 64 from wild tomato's night volatiles. Principle component analysis (PCA) resolved the compounds into 12 distinct principle component (PC) clusters. Of these clusters, PC1 and PC2 captured over 79.0% of the total variation. MANOVA and ANOVA tests on PC1 and PC2 revealed that there were significant differences in the volatile compositions, P < 0.00001, α = 0.05. Gas chromatography–linked electroantennography (GC–EAD) showed a large proportion of electroantennography (EAG)–active compounds from the two species of tomato plants. Of these, hexanal, trans-3-hexenol, verbenene, 4-keto-isophorone, camphor, citronellal, isopulegol, limonene oxide, linalool propanoate, germacrene A, *β*-elemene, germacrene B, germacrene D, and *β*-bisabolene were unique to the wild tomato. A blend of available compounds, at the time of study, (*trans*–3–hexenol, camphor, citronellal and limonene oxide) showed dose-dependent repellence to gravid *T. absoluta* females in the dual–choice olfactometer. The study lays down some groundwork for exploiting semiochemical traits of the tomato species in novel management of *T. absoluta*.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Tomato and potato are among the most widely cultivated vegetables in the world. In Kenya, tomato is rated as the second most important crop after potato plant according to the most recent horticultural statistics (Gitau, 2014). In 2014, tomato was grown on 23,900 hectares of land and produced 494,036 tons of tomato fruits valued at Ksh 14.1 billion. Their cultivation has been threatened by the infestation of tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) (Gitau, 2014). *T. absoluta* is a nocturnal pest of solanaceous crops (Lietti *et al*., 2005). The pest originated from South America (Urbaneja *et al*., 2007) and was considered an important pest in all tomato growing regions of central and South America, including some Caribbean islands (Eiras, 2000). Since 2006, *T. absoluta* has spread across Europe, Mediterranean area and now it is in Africa (Jehle, 2015).

Tuta absoluta was first officially reported in Kenya in March 2014 (IPPC, 2014) at Isiolo where it is believed to have spread from Ethiopia and Sudan. The pest has spread to other parts of the country where tomato is grown. This has threatened the leading counties in tomato production (Ndung'u, 2014). *T. absoluta* has been reported to cause $50 - 100\%$ damage of tomato crop (Figure **1.1**), hence reduction in yields of tomato and also limiting the export of the product to different destinations.

Figure 1.1: Photograph of a totally infested tomato plants by T. absoluta

The minimum temperature for reproduction activity of *T. absoluta* is 9°C. The most destructive stage of the pest is the larva which feeds voraciously on tomato plants, right from seedlings to adult plant, producing large galleries in leaves, burrowing in stalks, and consuming apical buds as well as green and ripe fruits (Figure **1.2**). It has been nicknamed the tomato 'Al–Shabaab' as it destroys the whole plant (Cocco *et al.*, 2013; Ndung'u, 2014)

Figure 1.2: Photograph of damage caused by *T. absoluta* larvae on tomato leaves and fruits

In Kirinyaga County, Kenya, farmers lost over 80% of their crop in 2014 as a result of infestation by the pest (Ndung'u, 2014). In calling for solutions to cushion farmers from the pest, stakeholders have warned that the pest is a major threat to the country's food and economic security. Methods used in chemical control of the pest has always resulted in high levels of hazardous residues on the tomatoes hence risking the health of the consumers and the ecology in general (Shalaby *et al.,* 2012). Applications of non-selective insecticides have also interfered with biological control methods for other insect pests like aphids, thrips and white flies (Arno´ and Gabarra 2011; Biondi *et al*., 2012, 2013). *T. absoluta* has also been observed to develop resistance to most insecticides, hence in addition to their negative ecological effects, their use in pest control is not a suitable option (Siqueira *et al.,* 2001; Gontijo *et al*., 2013).

Naturally occurring biogenic volatile organic compounds (BVOCs), released by plants, have important atmospheric and ecological consequences (Ormeño *et al.,* 2011). They contain air borne semiochemicals that promote or deter interactions between plants and herbivorous insects (Bawin *et al*., 2014). Some varieties of cultivated tomatoes have shown that electroantenographic (EAG) responses for monoterpenes, in particular *β*–phellandrene, limonene, and 2–careen, and the sesquiterpene (*E*)–*β*–caryophyllene, were relatively high, suggesting that they could be playing a major role in attraction and oviposition of *T. absoluta* females (Zhang *et al.,* 2008; Proffit *et al.,* 2011; Bawin *et al.,* 2014). BVOCs released to the atmosphere by plants accounts for about 30% of the photosynthetically fixed carbon. These metabolites may act as plant defenses against herbivores and facilitate the foraging behavior of natural enemies of herbivores, and protect leaf cells from a variety of abiotic stress (Ormeño *et al*., 2011).

Wild tomatoes have been observed to have a high resistance to a variety of diseases, pests and fungi. Tomato leaf solvent extracts from wild tomato accessions of *Lycopersicon hirsutum* plant that are not consumed by humans have been found to contain three sesquiterpene hydrocarbons (*α*–curcumene, *trans*–caryophyllene, and *α*–zingiberene) and two methyl ketones (2–undecanone and 2–tridecanone), which have repellency and toxicity towards 2–spotted spider mites, *Tetranychus urticae* Koch and show potential for controlling the pest (Antonious and Snyder, 2006). Leaf extract from cultivated tomato (*Solanum lycopersicum* L.) have shown strong antifungal activity against *Botryotinis fuckeliana*, *Glomerella cingulate* and *Fusarium oxysporum* f. sp. *mlonis,* which are plant pathogenic fungi (Kobayashi *et al.,* 2012). According to a laboratory research done in Venezuela, *T. absoluta* was found to prefer tomato cultivar "Rome Gigante" as an oviposition host compared to potato, tobacco, eggplant, *Physalis angulate*, *Solanum hirtum*, *Solanum americanum*, and tomato variety Cerasiforme (Fernandez and Montagne, 1990b).

This project was based on the hypothesis that *T. absoluta* actively avoids the wild tomato, *L. esculentum var. cerasiforme* (Kinyanya) that grows wildly in the tea zones of Mount Kenya region, Kenya. This was tested by (i) comparing infestation levels of mono–crops of cultivated tomato, *S. lycopersicum* L (Rambo F1 variety), and the wild tomato, as well as intercrops of the two varieties in the open field; (ii) the behavioral responses of mated *T. absoluta* females to the two tomato plant species in a dual–choice olfactometric bioassay using mature intact tomato plants; and (iii) collection of the tomato volatiles using dynamic headspace and super Q as adsorbents. These volatiles were then analyzed using gas chromatography–linked mass spectrometry (GC–MS) and gas chromatography–linked electroantennography detector (GC–

EAD) (Cossé *et al.,* 1995; Marion-poll and Thirry, 1996; Tholl *et al.,* 2006; Kobayashi *et al.,* 2012; Durant *et al.*, 2013).

1.9 Problem statement and justification of the study

T. absoluta is a pest that leaves unimaginable loss after attacking cultivated tomato crop. Uncontrolled application of any agro-chemical leads to high level of residues on the tomato fruits, thus putting the lives of consumers and the general ecology at risk. *T. absoluta* have shown resistance to synthetic insecticides and spreads very fast. Other host plants of *T. absoluta* include *Solanum tuberosum* (potato), *Solanum melongena* (eggplant), *Capsium annuum* (pepper), *Nicotian atabacum* (tree tobacco), *Nicotian aglauca* (black night shade), *Solanum viride* (Green nightshade), *Solanum elaeagnifolium* Cav. (Silverleaf nightshade) and *Amaranthus viridis* (slender amaranth). Others are *Datura ferox* L. (Fierce thorn apple), *Lycium chilense* Bertero, *Datura stramonium* L. (Jimsonweed), *Solanum lyratum* Thunb, *Lycopersicon hirsutum* (Donal), *Solanum habrochaites* (Wild tomato), and *Solanum muricatum* Ait. (Pepinodulce) (Garcia and Espul, 1982).

In 2009, the world produced about 152 million tons of tomato from a production area of about 4.4 million hectares. In the year 2011 about 1.0 million hectares were infested by *T. absoluta,* thus reducing tomato production significantly (Muniappan, 2013). When *T. absoluta* invades a farm, either open field, screened house or green house, the damage is irreversible. In some regions, the cost of producing tomatoes increased 15 times per season after infestation of *T. absoluta*. It is estimated that when this pest invades tomato growing regions of the world, its management cost may go up by about $500 million per year (Muniappan, 2013).

Since its introduction in Kenya, the pest has spread to other parts of the country where tomato is grown. This has threatened the leading Counties in tomato production (Ndung'u, 2014). In Kirinyaga County, Kenya, farmers lost over 80% of their crop to this pest. This does not only affect the yields but also the local diet, the economy, and also limit the export of tomato products to different destinations. Climatic conditions in Africa in general provide a perfect home for it to multiply in massive numbers. Wild tomato, *L. esculentum var. cerasiforme*, which grows in the tea zones of Kirinyaga County, does not require application of insecticides to control this pest and many other insect and fungi pests. Elucidation of the semiochemical basis of avoidance of this wild tomato may open up novel methods of controlling the pest in "push-pull" tactics that have proved very efficient and environment–friendly method of controlling insect pests in crop farming and animal rearing (Hassanali *et al.,* 2008; Hinz, 2009; Khan *et al.,* 2011; Suckling, 2012; Markovi, 2013; Belmain *et al.,* 2013).

1.10 Hypotheses

(i) *T. absoluta* actively avoid the wild tomato variety but is preferentially attracted to cultivated tomato variety.

(ii) Wild tomato plants emit volatile constituents that repel *T. absoluta,* unlike cultivated tomato variety   which is attractive to the pest.

1.11 Objectives

1.11.1 General objective

To determine the behavioral responses of *T. absoluta* towards wild and cultivated tomato plants and characterize their mediating semiochemical blends.

1.11.2 Specific objectives

i. To determine the behavioral responses of mated *T. absoluta* females towards wild and cultivated tomato in the open field cultivation and in a dual–choice wind–tunnel experiments.

ii. To trap headspace volatiles released by the tomato plants, analyze, identify and quantify their constituents using gas chromatography–linked mass spectrometry (GC–MS), and undertake gas chromatography–linked electroantennograph (GC–EAD) to identify the EAG–active constituents.

iii. To undertake dose response bioassays of blend of available EAG–active constituents unique to wild tomato.

1.12 Significance of the study

This project sought to shed some light on the behavioral responses of adult female *T. absoluta* to the two tomato plant varieties and i) compare the chemical profiles of their headspace volatiles and ii) identify the active blend that is responsible for deterring *T. absoluta* in the wild tomato. The insights are expected to lay down some groundwork on the development of new tools (green chemicals) and tactics (green chemistry) to manage the pest.

1.13 Scope and limitations of the study

The tomato plants were grown at Mwea planes in Kirinyaga County. Comparison of plants from different climatic and geographical zones and other varieties was not done. Limited number of synthetic standards of EAG-active constituents that were specific to the wild tomato variety were available at the time of the study. The comprehensive blend of the compounds could not be evaluated.

CHAPTER TWO

LITERATURE REVIEW

2.1 *Tuta absoluta* morphology

Tuta absoluta belongs in the phylum Arthropoda, class Insecta, order Lepidoptera, suborder Grossata, superfamily Gelechioidea, family Gelechiidae, subfamily Gelechiinae, tribe Gnorimoschemini and genus Tuta (Clarke, 2005). The pest adult is a moth, which is grey–brown in color, approximately 6 mm in length and has a wing–span of about 10 mm (Figure **2.1**).

Figure 2.1: Photograph of female adult *T. absoluta* and the eggs

The male is somehow darker and has a narrower abdomen than the female (Koppert, 2008). These adults are mostly active during the night and hide between leaves during the day and flies low. Both males and females mate several times with the first mating occurring a day after adults emerge from pupa (Vargas, 1970) and it usually occurs at dawn between 6.00 a.m. to 11.00 a.m. (Miranda-Ibarra, 1999). Males have a life span of between 18 to 43 days while the females have a life span of between 17 to 38 days (Fernandez and Montagne, 1990a). Virgin males and females live longer than mated ones. The female can lay 250 – 300 eggs in her life time. The

females lay eggs singly and rarely in batches on all vegetative parts above ground of the host plant. The pest can breed between 10 – 20 generations a year with a life cycle of 21 – 34 days from hatching to adult depending on temperature. The most destructive stage of the pest is the larva which feeds voraciously on tomato plants, right from seedlings to adult plant, producing large galleries in leaves, burrowing in stalks, and consuming apical buds as well as green and ripe fruits (Ndung'u, 2014). Overwintering can take place during egg, pupa or adult moth but not in lava stage (EPPO, 2005).

2.2 Life cycle of *T. absoluta*

Tuta absoluta has a high rate of reproduction. It can complete between 10 – 20 generations in a year depending on environmental conditions, its minimum action temperature being 9°C (EPPO, 2005). Adult males and females mate a day after emerging from the pupa (Figure **2.2**).

Figure 2.2: Photograph of mating *T. absoluta* adults (Koppert, 2008)

Mating occurs once a day at the beginning of photophase (Vargas, 1970). Pre-oviposition period is on average 2.4±0.61 days (Fernandez and Montagne, 1990a). Oviposition takes place throughout the day with peak occurring at night (Vargas, 1970) and in the first and second day after adults mate (Imenes *et al*., 1990; Uchoa-Fernandes *et al*., 1995).

Eggs are oval-cylindrical, 0.4 mm in length and 0.2 mm in diameter (Muniappan, 2013). They are creamy white and then turn yellow–orange on further development. The egg takes 4-6 days to hatch at temperature of 24.6°C (Fernandez and Montagne, 1990a). They then turn dark and finally hatch (Estay, 2000; Vargas, 1970). The eggs normally hatch in the morning. Larvae produced penetrate into plant tissue and begin to feed (Muniappan, 2013). The larva has 4 instars. Early instars are cream or white with a black head. They later turn pink or green. The average duration for the larva to fully grow is 8 days (Muniappan, 2013). The larvae mines the leaves, shoots, flowers, young fruits, and the stem. They feed forming irregular galleries that become winder and longer as feeding continues and leave a dark frasses behind (Figure **2.3**) (Clarke, 2005).
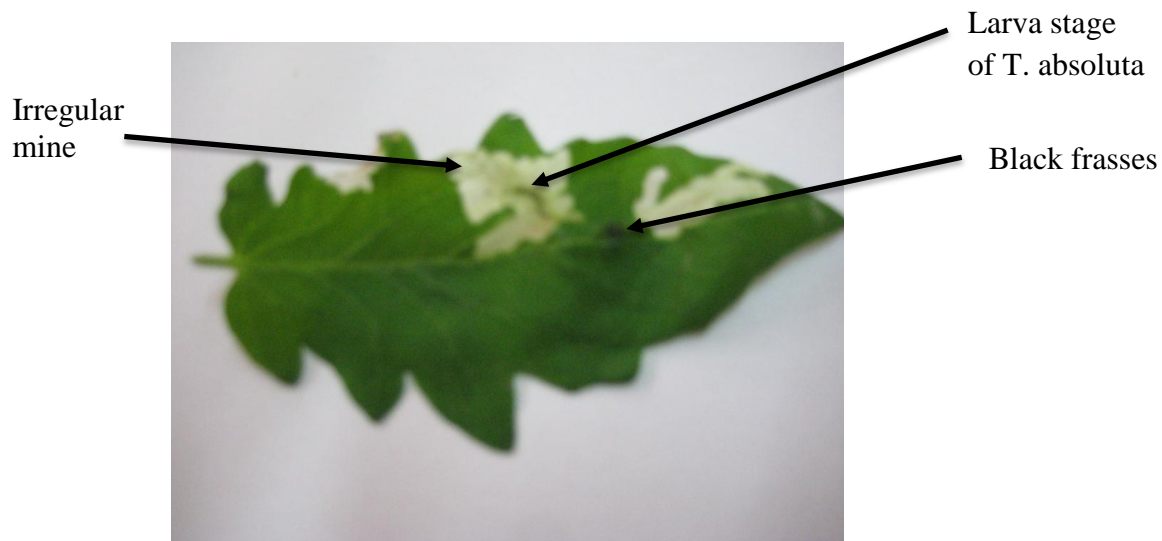
Figure 2.3: Photograph of irregular mines of *T. absoluta* larva with black frasses inside**.**

This is the most destructive stage of *T. absoluta*. They then drop to the soil to pupate (Muniappan, 2013). Pupae are brown in color and are about 4.3 mm in length and about 1.1 mm in width. Pupation can either take place in the soil or on dried leaves or stems. It takes a duration

of between 6 – 8 days on average for the adult to emerge (Muniappan, 2013). Males emerge in 7 – 8 days while females emerge after 6 – 8 days (Fernandez and Montagne, 1990a). Figure **2.4** shows the life cycle of *T. absoluta*.
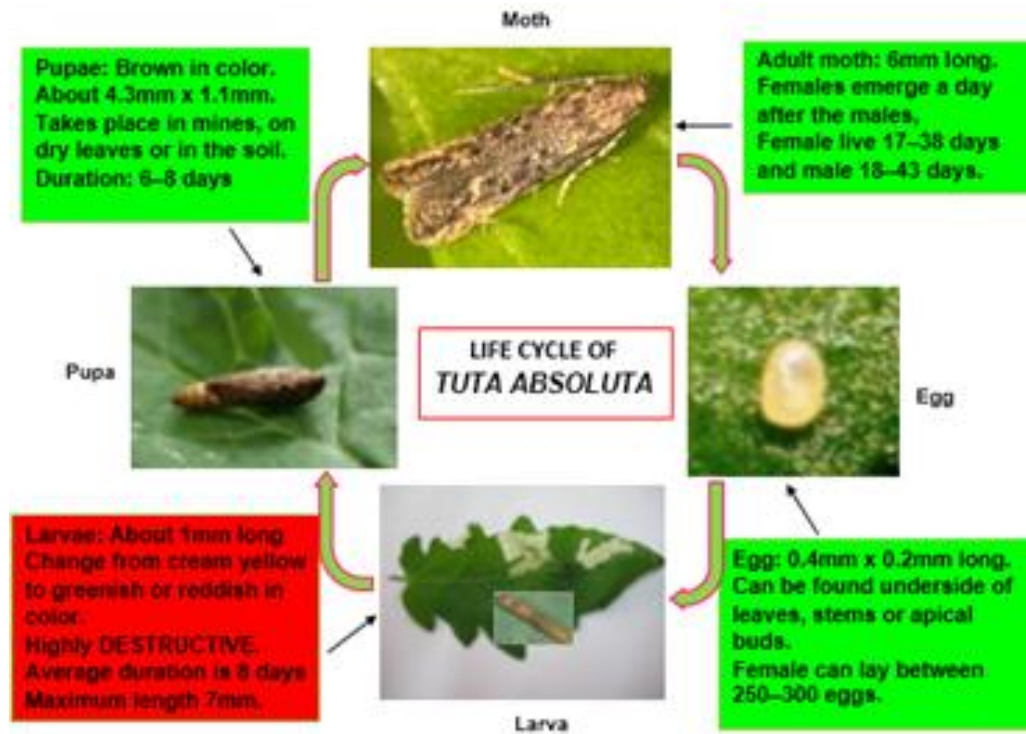
Figure 2.4: The live cycle of *T. absoluta*

Other than host plant, the life cycle of *T. absoluta* depends on temperature, with the minimum temperature for activity being 9°C. At 14°C, it takes 76 days while at temperatures above 20°C, it takes 24 days (Muniappan, 2013). This implies that Africa becomes a new conducive home for *T. absoluta*. *T. absoluta* occurs on plants of the Solanaceae family like datura, *Solanum nigrum*, potatoes, eggplants, sweet pepper, tomatoes among others (Koppert, 2008). Among the tomato plants, *solanum cultiva* is preferred for oviposition but not the cerasiforme (Fernandez and

Montagne, 1990b).The male and female *T. absoluta* can be identified and isolated in the laboratory using their reproductive organs at the pupa stage.

2.3 Migration and distribution of *T. absoluta* in Africa

T. absoluta is believed to have originated from South America, and spread through Europe, Middle East, Mediterranean region and then to Africa (Koppert, 2008). In Sudan, Ethiopia, Niger and Senegal, *T. absoluta* was confirmed present in the year 2012 (Muniappan, 2013). In Kenya *T. absoluta* was confirmed present in the year 2014. It was also reported in Tanzania in late 2014 but unlike in Kenya, sustainable and affordable biological solutions were developed and launched by Russell IPM, to empower the farmers against the damages caused by the pest, with a promising success (Agripest, 2015). The pest has spread to Mozambique, Malawi, Zimbabwe, Zambia, Botswana and South Africa. Arbitrary application, over use and application of banned insecticide products has escalated the problem of *T. absoluta*, causing early resistance development, harming the environment, and risking the health of consumers (Agripest, 2015). In Nigeria, *T. absoluta* was officially reported in May 2016. It destroyed 80% of Nigeria tomato farms causing a loss of over one billion naira (equivalent to about US$3.5 million) (**www.scidev.net/sub-saharan-africa/farming/news/surveillance-critical-halting-deadly-tomato-pest.html**). Figure **2.5** show how *T. absoluta* was predicted to spread in Africa with most of the regions having already been infested by the end of 2016.
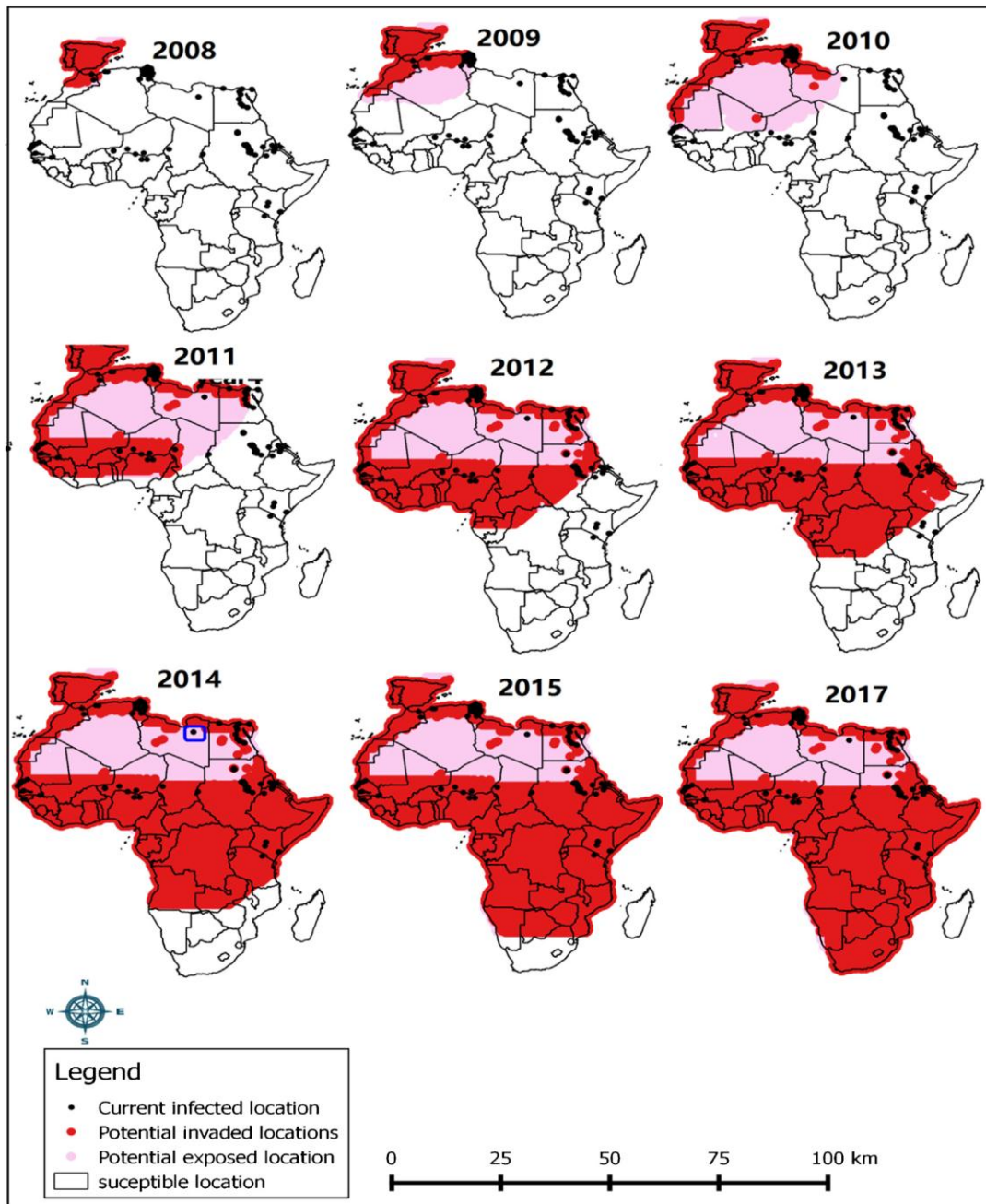
Figure 2.5: Predicted spread of *T. absoluta* in Africa, Guimapi *et al*. (2016)

Africa has a conducive climatic condition for *T. absoluta* survival as shown in Figure **2.6**, where

the pest can breed between 10-20 generations a year with a life cycle of between $21 - 26$ days

from hatching to adult (Ndung'u, 2014).
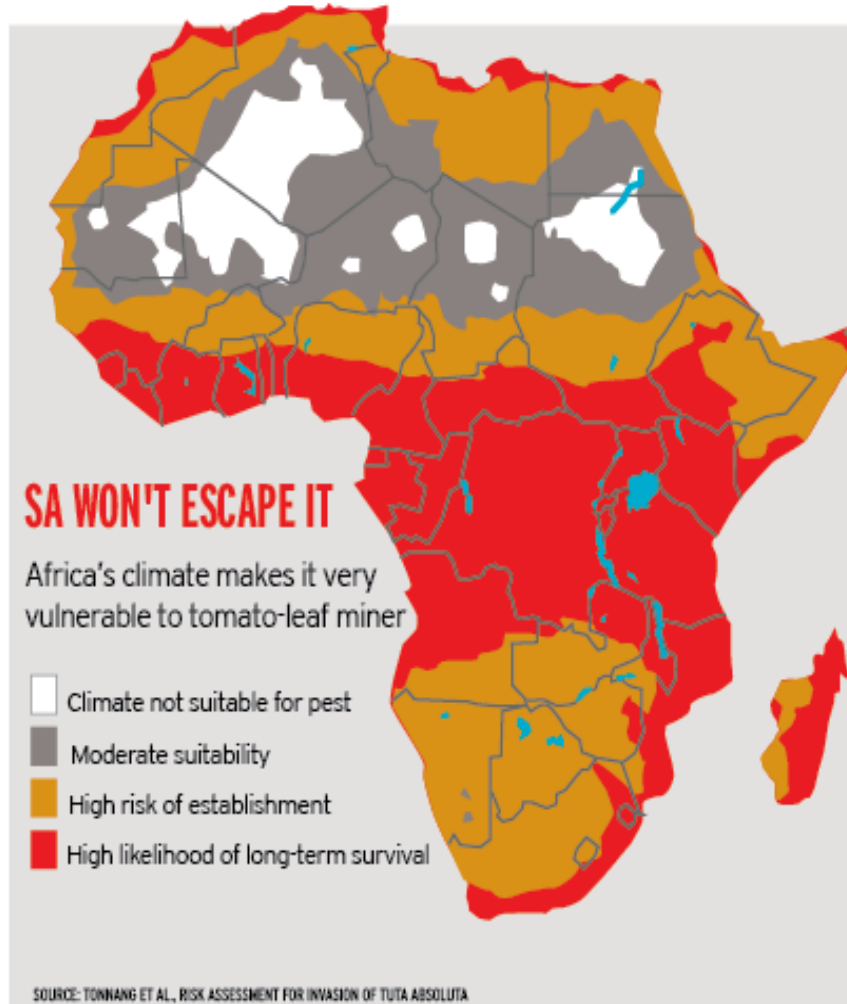
Figure 2.6: Africa climatic conditions and how they would affect the spread and distribution of *T. absoluta*. **(http://cdn.bdlive.co.za/FM/images/16Africamap.png, Wednesday, October 19, 2016 12:16:28 PM**)

To evaluate the presence and the levels of infestation, several methods have been proposed. These include; physical counting of eggs on leaves, counting of larvae, mines on the leaves, or adult males captured on pheromone traps (Gomide *et al*., 2001). The most effective of these methods are counting of larvae and mines in the leaves (Benvenga *et al*., 2007). *T. absoluta* moth has an ability of flying a distance of 0.4 km overnight (Salama *et al*., 2015)

2.4 Control methods of *T. absoluta*

2.4.1 Cultural methods

These involve (a) physical removal of the pest and the infested parts of the crop or the whole crop and either burning it or burying it deep in the soil, (b) effective weed control before and during crop season especially of all other alternative host plants such as black nightshades, potatoes, datura, solanum and nicotiana (Gitau, 2014; Ndung'u, 2014), (c) crop rotations with non-host crops help manage the pest (Markovi, 2013), (d) if a greenhouse has been invaded by the pest, it is advisable to remove the crop and close it to avoid the adults from migrating to the open field, and (e) ploughing, over–head irrigation, soil solarisation, use of pest free seedlings and manuring (Cocco *et al.,* 2012; Balzan and Moonen, 2012; Jehle, 2015).

2.4.2 Biological methods

Predators of *T. absoluta* include mired bugs (*Nesidiocoris tenuis*) and *Macrolophus pygmaeus*. They are commercially available and widely used in Europe and North Africa (Balzan and Moonen, 2012). Insecticide formulations based on *Bacillus thuringiensis*is are used in control of *T. absoluta* in their native and invaded fields. They are mostly used in control of the first – instar larvae and has no side effects on beneficial arthropods (Mollá *et al*., 2011). Neem formulations, Azadirachtin, are also effective in controlling *T. absoluta*. It acts as both systemic and contact insecticide for *T. absoluta* (Goncalves-Gervasio and Vendramin, 2007). *Metarhizium anisopliae* and *Beauveria bassiana* are amongst fungal species that have been reported to attack the eggs, larvae and adults of *T.absoluta* (Pires *et al*., 2009, 2010). Natural enemies of *T. absoluta* moth include *T. exiguum*, *Trichogramma pretiosum*, *Pristomerus*, *Dineulophus phthorimaeae*, *Cremastus*, *Copidosoma* and *Apanteles* (Vargas, 1970; Pacora, 1978; Larrain,

1986; Navarro, 1988; Berti and Marcano, 1991). These are used as parasitoids. Predators of the moths include *Chilocorus* (Vasicek, 1983) spiders, carabids, earwigs, hemipterans, wasps, ants, lace wings and *Steinernema carpocapsae* (Prada and Gutierrez, 1974; Jimenez *et al.,* 1989). In Kenya predators have not been identified yet due to excess use of insecticides (Ndung'u, 2014; Jehle, 2015). This implies that *T. absoluta* monitoring programs must be established where local natural enemies survey will be conducted and the effective ones identified (Muniappan, 2013).

2.4.3 Chemical methods

Insecticides recommended for the management of *T. absoluta* are of low to moderate effectiveness due to the cryptic nature of the larvae and the high biotic potential of the insect. They include pyrethrin, carbaryl, deltamethrin, spinosin, indoxacarb, abemectin, emamectin benzoate and cyromazin. Cases of insecticide resistance have been reported on organophosphates, pyrethroids, abamectin, cartap, permethrin and spinosad (Siqueira *et al*., 2001; Haddi *et al*., 2012, Ndung'u, 2014).

In Kenya two brands of insecticides, Belt SC 480 (Flubendiamide 480 g/L) (Kambo *et al.,* 2014a) and Tihan OD 175 (Flubendiamide 100 g/L + Spirotetramat 75 g/L) (Kambo *et al.,* 2014b) from Bayer East Africa Limited have been recommended by Kenya Agriculture and Livestock Research Organization, KALRO, for approval and registration by Pest Control Product Board, PCPB Kenya. Neem oil is recommended for preventing tomatoes from *T. absoluta* and can be applied to cover all parts of the plant since the pest feeds on any part. Today the most effective insecticide used in Kenva against *T. absoluta* is Corragen[@]205C (3-bromo-N-[4-chloro-2-methyl-6-(methylcarbamoyl)   phenyl]-1-(3-3chloropyridin-2-yl)-1H-pyrazole-5-

carboxamide) from Syngenta. Others are Warrant (Imidacloprid), Radiant (Spinosad), Tracer (Spinosad), and Thunder.

In all regions where *T. absoluta* has been introduced, the immediate consequence has been increased application of different types of insecticides and increase in the number of times these chemicals are applied. This has resulted in risking the health of the tomato consumers, destruction of the ecosystem in general, high cost of production, increased tomato prices, banning of tomato products trade and disruption of integrated pest management programs of other tomato pests (Ndungu, 2014).

2.4.4 Semiochemicals

Sex pheromones have widely been used to forecast, monitor and/or control moth pests' populations (Prasad and Prabhakar, 2012). They are chemical signals released by mostly female adult organisms to attract the same species of the opposite sex for mating (Cocco *et al.*, 2013; Megido *et al.*, 2013). Before 1995, virgin *T. absoluta* females were used to trap and capture males, and only about 100 males were captured per trap per day (Quiroz, 1978). However, characterization of the female pheromone has opened up an effective tactic to trap males. This is because males emerge earlier than females and females mate several times (Garzia *et al.*, 2012). Components of *T. absoluta* female pheromones are (3E, 8Z, 11Z)–3, 8, 11–tetradecatrien–1–yl acetate or TDTA (**1**) and (3E, 8Z)–3, 8–tetradecadien–1–yl acetate or TDDA (**2**) (Megido *et al.*, 2013).
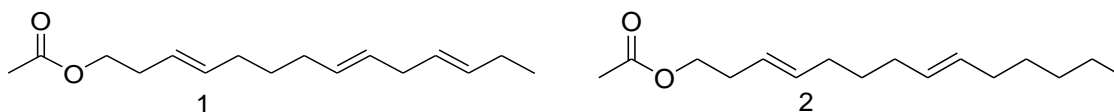
The synthetic pheromone blend has effectively been used in:

a) pheromone-baited traps,

b) mating disruption based on atmospheric saturation of the synthetic pheromone to reduce mating chances, and/or

c) lure and kill techniques using a combination of a low amount of the synthetic sex pheromone of *T. absoluta* and an insecticide to reduce the male population (Witzgall *et al*., 2010; Cocco *et al*., 2013).

In Kenya, a pheromone-based commercial product, Tutrack (Rusell IPM, 2012) has been recommended for monitoring and controlling *T. absoluta*. It consists of the pheromone lure and a trap. Most commercial lures in the market contains 0.5 mg of the pheromone and are recommended for greenhouses. Tutrack contains 0.8 mg of the pheromone, and can catch 3 times as many moths compared to the 0.5 mg of pheromone lure. They are recommended for open fields (Hassan and Al-Zaidi, 2010; Russell IPM, 2012). In hot desert climates, lures containing 3.0 mg are recommended (Hassan and Al-Zaidi, 2010).The number of Tutrack lures to be used per acre depends on the number of moths caught on one trap per week. If the number is between 3 and 30 moths, 10 traps are recommended per acre, and if it exceeds 30 moths, 20 traps are recommended. The traps need to be replaced after every 4 − 6 weeks. Tutrack are placed not more than 60 cm from the ground, since *T. absoluta* is a low flying insect (Coelho and Franca, 1987; Uchôa-Fernandes *et al*., 1995; Ferrara *et al*., 2001; Laore, 2010). Four factors that need to be considered when using the traps include colour of the trap (dark coloured traps catch more insects than lighter colours) (Uchôa-Fernandes *et al*., 1995); height of the trap; position of the trap with respect to vegetation; and the density of the traps in a given unit area (Howse *et al*.,

1998). In addition, completely open traps can increase the number of insects caught per trap (Ferrara *et al*., 2001).

Other traps for *T. absoluta* contain a pheromone lure that is suspended above a sticky surface, which is removable, for trapping the attracted insects (Figure **2.7** (a) and (b)) (USDA APHIS, 2011).
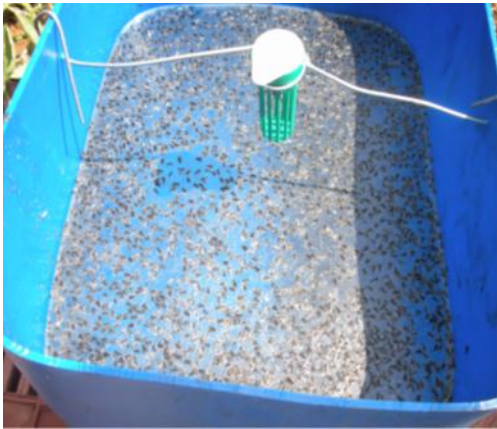
Figure 2.7: (a) Pan Trap (Russell IPM Ltd)   (b) Delta trap (Russell IPM Ltd)

Traps containing water and detergent instead of sticky surfaces are also used. *T. absoluta* males are attracted to the lure and then fall into the water and drown. Water traps capture high number of adult males without becoming saturated with insects (Clarke, 2005). Pheromone based traps are only limited to the male *T. absoluta,* which according to the research done does not target tomatoes. Mated *T. absoluta* females should be the major concern since they are the ones that locate oviposition sites.

2.4.5 Sterile males

The use of sterile males has been proposed as an alternative method for controlling *T. absoluta* (Cagnotti *et al*., 2012). The method is based on the assumption that *T. absoluta* reproduces only through amphimixis. Polyandry in females may hinder this method and may also have implications on integrated pest management (IPM) programs that uses pheromone–based techniques (Clarke, 2005). Polyandry affects fecundity, fertility, genetic variation and other pest attributes (Torres-Vila *et al*., 2004).

2.4.6 Light Traps

Both sexes of *T. absoluta* have a strong photo-tactic response (Vargas, 1970). Light traps can be used to capture these pests during sundown and sunset in greenhouses (Bolkmans, 2009; Laore, 2010). Ferolite-TUA is a light trap that uses a combination of a specific light frequency that is highly attractive to *T. absoluta* and pheromone lures (Russell IPM, 2009b). Light traps are more effective compared to standard pheromone traps by about $200 - 300$ percent (Cocco *et al*., 2012). They attract both males and females. In Kenya, and Africa in general, distribution of electricity is a hindrance to light trap usage.

2.4.7 Host – Plant Resistance

Host–plants with high contents of zingiberene (a repellent) and/or acylsugar (inhibits full development of the larval stages) are currently being evaluated for protection against the tomato pest. This could result in the development of tomato varieties that do not attract females for oviposition and/or that will not allow the full development of the larvae (de Azevedo *et al*., 2003; Maluf *et al*., 2010).

2.4.8 Integrated Pest Management Strategy, IPM

In South America, where the pest originated, IPM employs a combination of different methods of monitoring and controlling *T. absoluta*. These include:

  i. massive trapping before planting,

 ii. clearing off the host crop residues,

iii. application of imidacloprid in the irrigation water 8 – 10 days after planting,

 iv. application of either Spinosad or Indoxacarb if occasional individuals of *T. absoluta* are observed, and

  v. elimination of the remnants of the crop immediately after the last fruits have been harvested (Robredo *et al*., 2008).

Massive trapping using pheromone-baited water traps provide an environmentally friendly, effective, sustainable, and safe control method (Robredo *et al*., 2008).

2.5 Economic importance of *T. absoluta*

Tomato and potato are key staple foods for many subsistence farmers. They are the most widely cultivated horticultural crops in the world. They are both attacked by *T. absoluta* which can have serious nutritional consequences to the entire community (Agripest, 2015). The larvae stage of *T. absoluta* attacks stems, leaves, buds, calyces, flowers, young and ripe tomatoes (USDA, 2008). It can lead to destruction of between 90 – 100% losses of open field produced tomatoes (Estay, 2000; Vargas, 1970). In all regions where *T. absoluta* have been introduced, pesticides have been applied more than 15 times per season in the first year. It is estimated that when *T. absoluta* spread throughout the world, the cost of its management will go up by $500 million per year (Muniappan, 2013). This will impact on the health of the tomato consumers, destruction of the

ecosystem in general, high cost of production, increased tomato prices, banning of tomato products trade, disruption of integrated pest management programs of other tomato pests and the general human diet (Zappalà *et al*., 2012; Zlof and Suffert, 2012).

Tuta absoluta has been reported to attack potato (*Solanum tuberosum*) leaves (EPPO, 2005) and potato tubers (Pastrana, 1967; Russel IPM ltd, 2009b; Maiche, 2009). It has also been reported to attack eggplants, greenhouse peppers and beans (EPPO, 2009), Cape gooseberry (*Physalis peruviana*) (Garzia, 2009), *S. elaeagnifolium*, *Solanum nigrum, Datura stramonium* (jimson weed), *Lycopersicon puberulum*, *D. ferox* and *Nicotian aglauca* (black night shade), *Amaranthus viridis* (slender amaranth) (Garcia and Espul, 1982). Prizes for synthetic insecticides are usually high for small holder farmers to afford (Belmain *et al*., 2013)

2.6 Environmental impact

The immediate consequences of *T. absoluta* infestation have been increased application of a large number of insecticides and their frequency. In most cases farmers use trial and error methods, applying any agro-chemical, to control the pest which has led to high level residues on the tomato fruits hence endangering the lives and health of consumers and also harming the ecosystem (Ndung'u, 2014; Jehle, 2015). *T. absoluta* have shown resistance to synthetic insecticides and the pest spread very fast resulting to discovery of new pesticides and increased application without proper understanding of their environmental impact (Belmain *et al*., 2013). It has also resulted in disruption of integrated pest management programs for other tomato insect pests (Arno´ and Gabarra 2011; Biondi *et al*., 2012, 2013)

2.7 Chemical communication

Chemical communication is a behavioral characteristic of all living organisms. Semiochemicals are organic compounds that transmit chemical messages (Norin, 2007). Semiochemical is a term that is derived from the Greek word "semeon" which means "sign" or "signal". Volatile semiochemicals are detected directly by insects from the air through their olfactory receptors (Naturwissenschaften, 2010). These receptors are mostly located in the sensilla hairs of the antennae. Semiochemicals have been in use since 1880s when female insects were used to lure male insects into traps. Since 1950s more than 3,000 semiochemicals of different arthropods have been identified (Dicke and Sabeli, 1988a).

Semiochemical research involves continued molecular mapping, synthesis, studies of biosynthesis, understanding the neurophysiological sensory functions of insects, and how hormonal regulations in insects affects pheromone biosynthesis and release (Karlsson, 2011). Semiochemical research is based on development of means and methods of managing and controlling insect pests. It is placed high in the Pasteur's Quadrant of the Stokes model, because of its overall goal of using the tools discovered in pure research in chemistry downstream to develop eco-friendly practical solutions.

Chemical communications occur between species, host–predator, habitat–predator and different sexes within same kind of organisms (Karlsson, 2011). Chemical ecology is a science that deals with the chemical mediated interactions between organisms of different species (allelochemicals) or from the same species (pheromones) (Norin, 2007). Pheromone is derived from the Greek words "pherein" which means "to carry" and "horman" which means "to excite or stimulate".

Pheromones can be divided into five categories depending on their effects (Dicke and Sabeli, 1988b; Norin, 2007). These include

(i) Aggregation pheromones: these are compounds that increase the concentration of insects at the pheromone source;

(ii) Alarm pheromones: these are compounds that stimulate insects' escape or defensive tendencies;

(iii) Sex pheromones: these are compounds that help individuals of the same species to find their opposite sex mates;

(iv) Trail pheromones: they are mostly found among social insects like bees and ants, these compounds are used for example by workers to mark the way to a food source;

(v) Marking pheromones: they are compounds used by insects to mark the boundaries of their territory.

Allelochemicals are divided into three categories which are

(i) Synomones: (the name is derived from the Greek word "syn" which means "with" or "together") these are compounds that are beneficial to both the sender and the receiver.

(ii) Kairomones: (it is derived from the Greek word "kairos" which means "opportunistic") these are compounds that benefit predators and bugs to their prey or potential host (Norin, 2007).

(iii) Allomones: they are compounds that benefit the producer but not the receiver. They are part of chemical defense.

Both allelochemicals and pheromones have molecular weights ranging from 17 to 880 g/mol and they are usually volatiles. The known semiochemicals have lengths of carbon chain varying from one to forty–five carbons. The number of double bonds in these chains also varies from zero to thirteen. Other features include *cis–trans* isomerism and positional and optical isomerism. Most of these compounds have small and simple molecules while others have complicated structures. The largest numbers of the structural categories of semiochemicals are hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids, esters and amines (Dicke and Sabeli, 1988b).

Research involving semiochemicals is becoming an important field, where both chemistry and biology expertise converge. Semiochemicals are used in insect pest management and control both in cultivated land and stored products (Ayasse and Gross, 2006). In traps, semiochemicals are used to control insect pests where pheromones that attract either sex of a given insect are used hence affecting their reproduction. Semiochmicals can also be increased in the environment to interfere with insect communication hence impeding reproduction (Belmain *et al*., 2013)

Allelochemicals and pheromones are used by organisms to improve on food location, reproduction and predator avoidance. Since they are highly specific, they have been used in traps and in 'push and pull' tactics (Ayasse and Gross, 2006; Hassanali *et al.,* 2008; Hinz, 2009; Suckling, 2012; Markovi, 2013). Semiochemicals that are naturally occurring are released in trace amounts, thus difficult to collect and determine their absolute configuration by convectional means. They are volatile liquids and therefore acquisition in large quantities is a problem (Ayasse and Gross, 2006; Ormeño, 2011).

Plants also interact with their environment using semiochemicals. They can either act as intra–specific, allowing plant–plant communication, or interspecific thus allowing attraction of pollinators and herbivores (Barsics *et al*., 2016). Headspace–solid phase micro extraction (HS–SPME) is highly recommended for trapping volatiles released by living plants (Ormeño *et al.,* 2011; Mardarowicz *at al*., 2004). Traditionally HS–SPME is used to gain insights into the emission blends of a given plant (Augusto, 2002; Tholl *et al.*, 2006) and can also be used to study the emission rates of volatiles released by a living plant (Bouvier–Brown *et al.*, 2007; Yassaa *et al*., 2010).

Dynamic headspace trapping (DHT) techniques (Figure **2.8**) is rarely used to estimate the volatile contents of a living plant. It is mostly employed where volatile rates of emission are accurately required (Helmig *et al*., 2004; Joo *et al*., 2010)
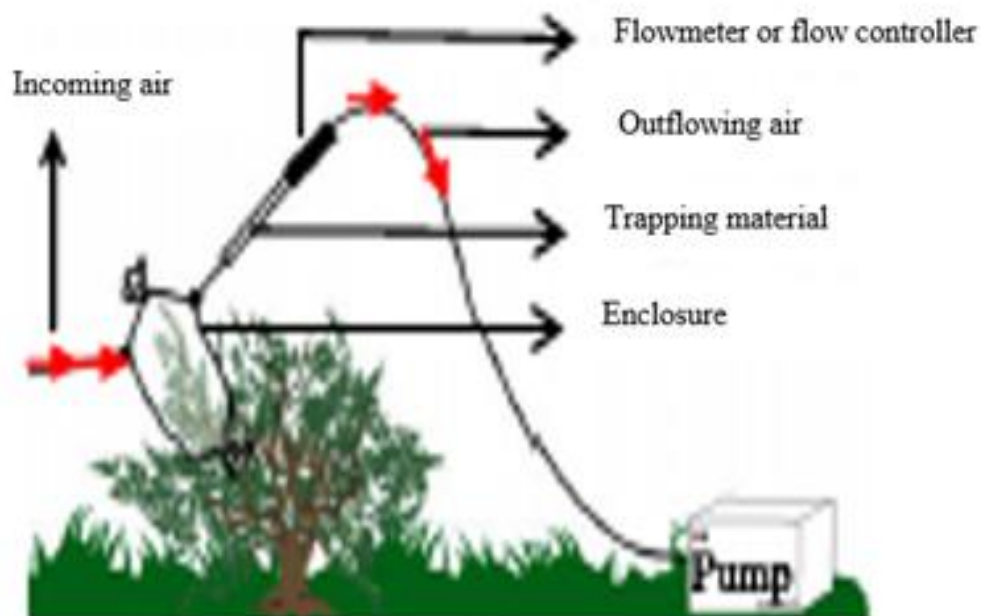
Figure 2.8: Dynamic headspace trapping (DHT) system and collection of volatiles using an appropriate adsorbent (Shu *et al*., 2010).

2.8 Wild tomato (*Lycopersicon esculentum var. cerasiforme*) and cultivated tomato, (*Solanum lycoperscon L.,* Rambo F1)

They belong to the order – *Solanales*, family – *Solanaceae* and genus – *Lycopersicon.* In most parts of Kenya all varieties of edible tomato plants are generally called nyanya. Wild tomatoes grow wildly and are mostly spread by birds. They grow to heights of 90 – 240 cm. Their fruits are small (grape/cherry varieties) and weighs 15 g per fruit on average. They are easy to grow organically than other tomatoes as they have a high tolerance to diseases and pests and can be grown successfully both under greenhouse and open field conditions. The recommended spacing is 60 – 90 cm. They can survive in more than two seasons under necessary conditions. Their economic importance is that their fruits are used as human food since they are an important source of vitamins. Wild tomatoes are also used as a source of genes to develop disease and drought resistant cultivated tomatoes. A wide variety of cultivated tomatoes, *Solanum lycoperscum L.,* are available in the Kenyan market which include Rambo F1 that was used in this project. Unlike the wild tomato, cultivated tomato cultivation is affected by a wide range of insect pests, fungi and diseases which have led to application of various chemicals that are not friendly to the consumer and the ecosystem in general (Shalaby *et al.*, 2012).

Leaf extracts from wild tomato accessions of *Lycopersicon hirsutum* plant that are not consumed by humans were found to contain phytochemicals that can be used to control two-spotted spider mite, *Tetranychus urticae Koch*. The extracts from the leaves were found to contain methyl ketones; 2–tridecanone (**3**) and 2–undecanone (**4**) and three sesquiterpene hydrocarbons; *(E)*–caryophyllene (**5**), *α*–curcumene (**6**), and *α*–zingiberene (**7**). They have repellency and toxicity towards two–spotted spider mites (Antonious and Snyder, 2006).

$H_3C-(CH_2)_{10}-\overset{\overset{\text{O}}{\|}}{C}-CH_3$

3

$H_3C-(CH_2)_8-\overset{\overset{\text{O}}{\|}}{C}-CH_3$

5

4

6

7
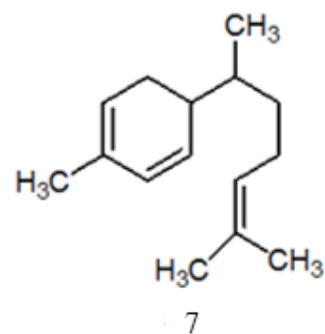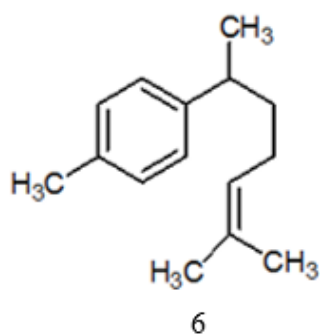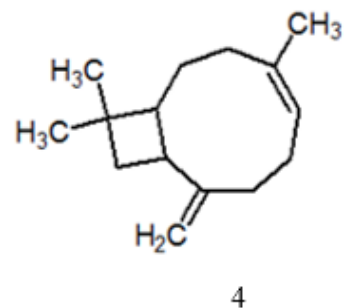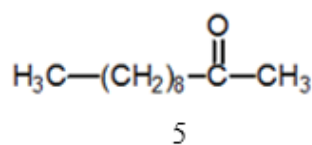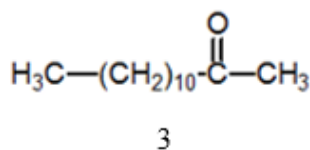
Volatiles from cultivated tomato leaves (*Solanum lycoperscum L.*) have shown strong antifungal activity against three types of plant pathogenic fungi of *Botryotinis fuckeliana*, *Glomerella cingulate* and *Fusarium oxysporum* f. sp. *melonis* (Kobayashi *et al.*, 2012). A study on attraction and oviposition of *T. absoluta* females in response to some varieties of cultivated tomatoes has also been conducted and it was found that amongst other blends, monoterpenes, in particular *β*-phellandrene (**8**), limonene (**9**), and *δ*-2–carene (**10**), and the sesquiterpene *(E)–β–*caryophyllene (**5**) and their percentages in volatiles play a major role (Proffit *et al.*, 2011). These monoterpenes account for more than 70% of total tomato headspace foliage.
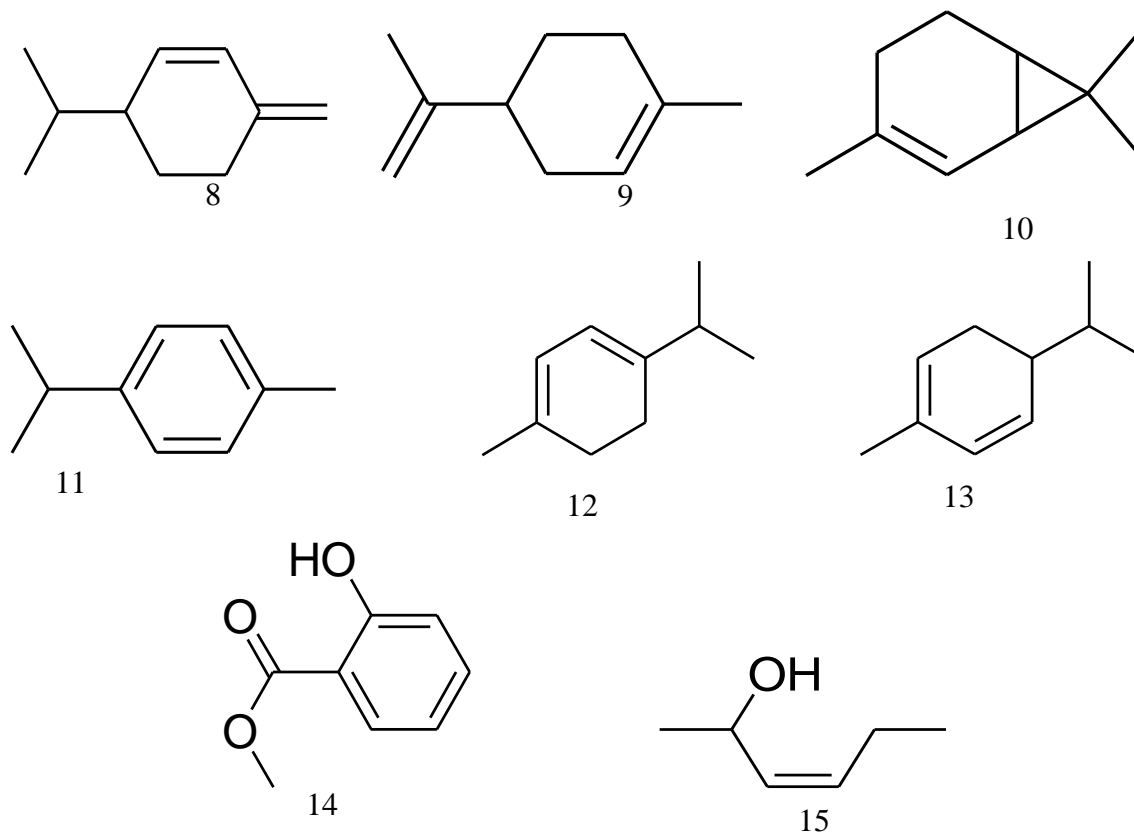
Specific tomato volatiles have been reported to have a great role in tomato–whitefly (*Bemisia tabaci*) interaction (Oa *et al*., 2009). These volatiles, that include sesquiterpenes, α-zingiberene (**7**) and α-curcumene (**6**) and the monoterpenes *p*–cymene (**11**), *α*–terpinene (**12**), and *α*–phellandrene (**13**), had the strongest deterring effects in free–choice bioassays. *α*-Zingiberene (**7**) and curcumene (**6**) have been observed to have toxic effects on a majority of insect pests (Carter *et al*., 1989; Weston *et al*., 1989; Eigenbrode *et al*., 1994; Freitas *et al*., 2002; de Azevedo *et al*., 2003).

Aphidiuservi (Hymenoptera: Braconidae) is an aphid parasitoid of *T. absoluta* that depends on stressed tomato plant volatiles caused by herbivorous insect pests like aphid *Macrosiphum euphorbiae*. It has been demonstrated that *(E)–β*–caryophyllene (**5**), methyl salicylate (**14**), and *(Z)*–3–hexen–1–ol (**15**) produced by stressed tomatoes plays a major role in attracting this

parasitoid (Pickett and Guerrieri, 2009). The higher the stress caused by the herbivorous insect pest, the more the expression of defensive genes resulting to higher release of volatiles that attract the aphid parasitoids. Herbivorous induced plant volatiles (HIPVs) are synthesized and released only after a plant is damaged (Raghava *et al*., 2009). These HIPVs either directly deter insect pests from oviposition (Kessler and Baldwin, 2001), or by attracting the natural enemies of the herbivorous pest (Dicke and Sabelis, 1988a). De Backer *et al*., 2014, observed that HIPVs, attracts *Macrolophus pygmaeus* (Heteroptera, Miridae), which is a natural enemy of *T. absoluta*. On oviposition preference of *T. absoluta* on tomatoes, pepper and eggplants, it was observed that *T. absoluta* preferred tomatoes followed by eggplants and pepper is last (Birgücü and Karaca, 2015). *T. absoluta* oviposition choice is influenced by the level of infestation or number of larvae on affected part of tomato plants (Bawin *et al*., 2014).

The stem and leaves of Wild tomato *Lycopersicon esculentum var. cerasiforme* have little aroma but when disturbed they produce a strong flavor. It is not fed on by *T. absoluta* like cultivated tomato (Rambo F1), which is highly damaged at all stages of its growth and development and on all part of the plant (Ndung'u, 2014). Cultivated tomato have been observed to be preferred for attack by *T. absoluta*, whiteflies, and red spider mites, amongst other tomato insect and fungi pests unlike the wild tomato. The ability of *T. absoluta* to localize and develop on wild and cultivated solanaceous plant species have been conducted with recommendation on studying how host plant choice is influenced by plant volatile organic compounds (Bawin, *et al.*, 2014).

According to Andersson *et al.* (1980) and Buttery (1987), a wide range of cultivated tomatoes are reported to have terpene hydrocarbons such as β–phellandrene (**8**), δ–2–careen (**10**),

limonene (**9**), α–phellandrene (**13**), terpinolene (**16**), α–terpinene (**12**), myrcene (**17**) and α–pinene (**18**); sequiterpene hydrocarbons like, β–caryophyllene (**5**) and humulene (**19**); C_6 compounds like hexanal (**20**), *(Z)*–3–hexenal (**21**), (E)–2–hexenal (**22**), Hexanol (**23**) and (Z)–3–hexen-1-ol (**15**); Oxygenated monoterpenoids, sesquiterperpnoids and aromatic compounds. Colby *et al*. 1998 reported additional sesquiterpenes from cherry tomato leaves which include germacrene A (24), germacrene B (**25**), germacrene C (**26**), germacrene D (**27**), *β*–elemene (**28**) and Guaia–6, 9–diene (azulane) (**29**). De Backer *et al*., (2014) observed that there could be partial attraction mediation of *Macrolophus pygmaeus* (Heteroptera, Miridae)*,* a natural enemy of *T. absoluta,* by herbivore induced plant volatiles (HIPVs) which varied with levels of infestations.
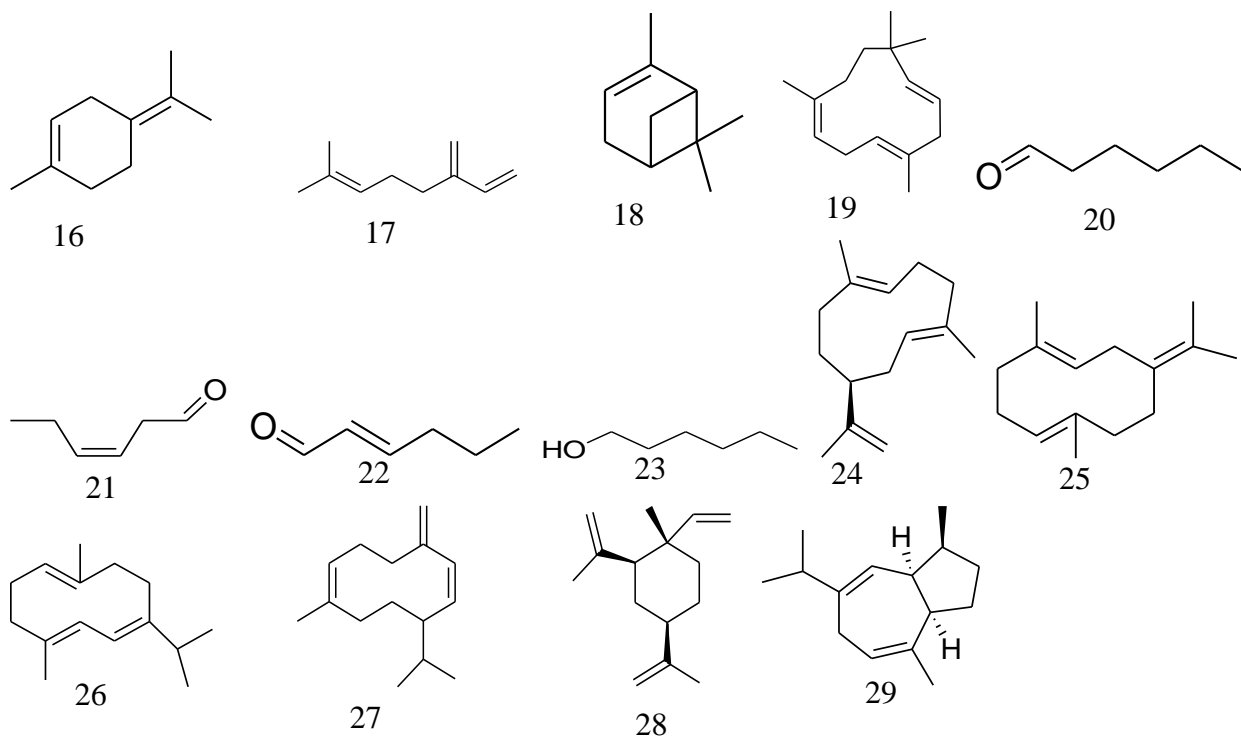
CHAPTER THREE

MATERIALS AND METHODS

3.1 Wild tomatoes and cultivated tomatoes plants

Solanum lycopersicon L. (Rambo F1) seeds from Royal seed Company were purchased from New Down Town; Angro–pest Fighter shop in Wang'uru town, while the seeds of Wild tomato *Lycopersicon esculentum var. cerasiforme* (Kinyanya) were acquired from a farmer from Kiangai, Kirinyaga County. The tomato seeds were grown in nursery beds, at Wang'uru in Mwea planes (S 00'4137.4"; E 037°22'12.3"), 4 m GPS, and 1158 m above sea level, of Kirinyaga County, Kenya, using a mixture of loam soil and composed manure from goats and chicken. After three weeks, 15 seedlings of each variety were transplanted into five portable plastic pots each containing about 4 kg of the soil mixture and 15 g of diammonium phosphate (DAP). The potted plants were placed in a screened house to prevent them from attack by herbivores pests. Watering was done twice per week. After three weeks top dressing with 15 g of calcium ammonium nitrate (CAN) was done. After eight weeks the plants, at flowering and production of young fruits stage, were transported to ICIPE, Nairobi, Kenya, for laboratory work.

The rest of the seedlings were transplanted in an open field at the same location of nurseries, where the effects of mono–cropping and intercropping of the two species were monitored. The seedlings were planted in a mixture of loam soils, composed manure from goats and chicken and 15 g of DAP. Watering was done using farrow irrigation twice per week for the first 3 weeks followed by ones per week. After 3 weeks the soil was top dressed with CAN. No pesticides were applied on the tomatoes during growth and development.

3.2 Source of *T. absoluta* colony

The adult *T. absoluta* pests used in the laboratory work were obtained from colonies in the insectary at ICIPE, (S01°13.140'; E036°53.440'), Nairobi, Kenya which was established in early 2015 and supplemented with larvae from the infested open fields tomato plantations in the country. Adult males and female *T. absoluta* were reared in cages, 65 cm x 45 cm x 45 cm, made of plexi–grass and fed with potted tomato plants, honey and water to oviposit. The plants were then put in similar cages where the eggs hatched. To obtain adults of the same age, pre–pupal stage larvae were placed in cages 20 cm x 15 cm x 15 cm, and provided daily with fresh tomato leaves until pupation. Newly formed pupae were placed in a cold chamber at 10°C until all the larvae had pupated. The pupae were then sexed according to their external morphology (Solomon, 1962). Male pupae were placed in a climatic chamber a day after the female pupae (the average emergence time for males is 7.8 ± 0.28 days, while that of females is 8.7 ± 0.22 days at 25 °C (Lee *et al.*, 2014)

Same age adult males and females were put into the same cage, 20 cm x 15 cm x 15 cm, for mating. They were provided with honey and water. The following morning, mating couples were separated into individual cages, 10 cm x 10 cm x 6 cm, for ease of separation using their body characteristics i.e. the male is somehow darker and has a narrower abdomen than the female (Koppert, 2008). Sets of ten mated female adults were placed in the same vial and provided with water and honey ready for behavioral assay experiments.

3.3 Field layout for mono-crop and inter-crop of the two tomato varieties

A four factor Randomized Block Design, RBD, was used on a ¼ acre piece of land at Wang'uru Mwea (S 00'4137.4"; E 037°22'12.3"), 4 m GPS, and 1158 m above sea level, of Kirinyaga County, Kenya). The land was divided into 16 equal portions of plots each measuring 4 m × 4 m to produce a two factor (treatment and blocking) RBD, 4 levels of treatments, namely, monocrop of the wild tomato (TT), monocrop of cultivated tomato surrounded by wild tomato (TB), intercrop of both varieties of tomatoes (TC), and monocrop of cultivated tomato (CC) arranged in 4 levels of blocking (A, B, C, D) factor. Each plot represents a replicate n = 1 while the total number of replications, N = 4 × 4 = 16. A path of 4 m in width was left between every two adjacent plots to overcome interference between neighboring plots (i.e. to maintain independence of responses) within blocks and between blocks as shown in Fig **3.1**.
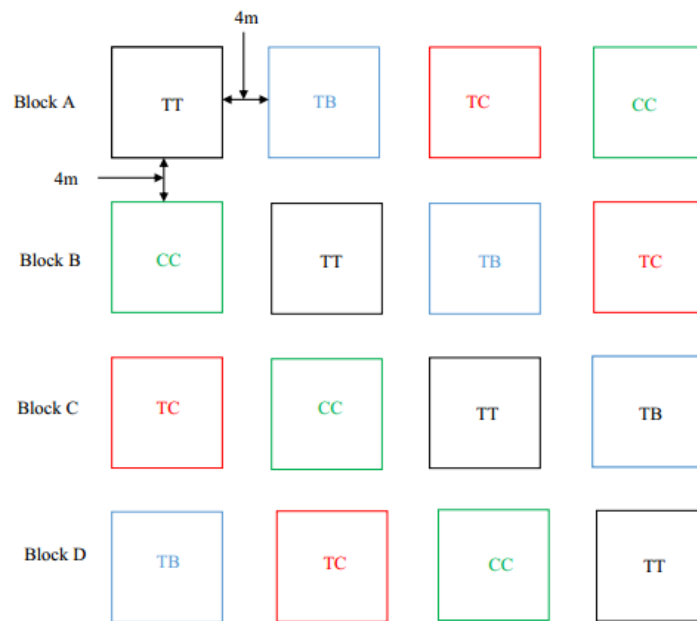
Figure 3.1: Field layout for the four treatments: monocrop of the wild tomato (TT), monocrop of cultivated tomato surrounded by wild tomato (TB), intercrop of both varieties of tomatoes (TC), and monocrop of cultivated tomato (CC) and 4 levels of blocking (A, B, C, D)

Blocking was used to remove the effects of a few of the most important nuisance variables like water moisture gradient, nutrient gradient, slope differences and soil composition, while randomization was used to reduce the contaminating effects of the remaining nuisance variables. For important nuisance variables, blocking would yield higher significance in the variables of interest than randomizing.

Early assessment and observation showed that there were no infestations on apical leaves of the tomatoes by *T. absoluta*. After one month of the tomatoes in the field, 2 leaves were randomly sampled from each plant of a given plot to get up to 20 pairs of leaves per plot and transported to ICIPE for laboratory work. Observations were done on the leaves and fruits for *T. absoluta* mines and larvae. This process was replicated every two weeks for 14 weeks to give 7 replicates.

3.4 Dual – choice wind tunnel (olfactometer) for behavioral responses of mated *T. absoluta* female to wild tomato and cultivated tomato

A dual–choice wind tunnel design was constructed using plexi–glass, joined using chloroform. Chloroform dissolves plexi–glass which on hardening forms a joint. The duo–choice wind tunnel is cuboidal, 152 cm x 21 cm x 21 cm, with pyramidal ends whose slanted edges are 21 cm in length. The plant cages are cuboidal, 61 cm x 35 cm x 35 cm, while the tight air removable pyramidal tops slanted edges are 36 cm in length. The two air tight windows on the pyramid part of the tunnel, 5 cm x 5 cm, were for introducing sample dispensers. The open window, 12 cm x 12 cm, at the middle of the tunnel was for introducing mated *T. absoluta* females and allowing air out. It has nylon netting that allows for opening and tie closing. Above the open window is a vacuum pump for sucking air at a given rate. A red fluorescent tube was suspended 1.5 m above the tunnel giving about 1000 lux incident red light. A strip of white paper marked in black, after

every 5 cm from the center (0) and on either side, was fixed on the lower side and lengthwise to the tunnel. This was to allow correct reading of insect fright distance. The distance on either side, from the center of the tunnel to the plexi–gauze, is 76 cm. The duo-choice wind tunnel was connected to the plant cages and the source of air using transparent flexible tubing each 150 cm long (Photo, figure **3.2** and sketch, figure **3.3).**
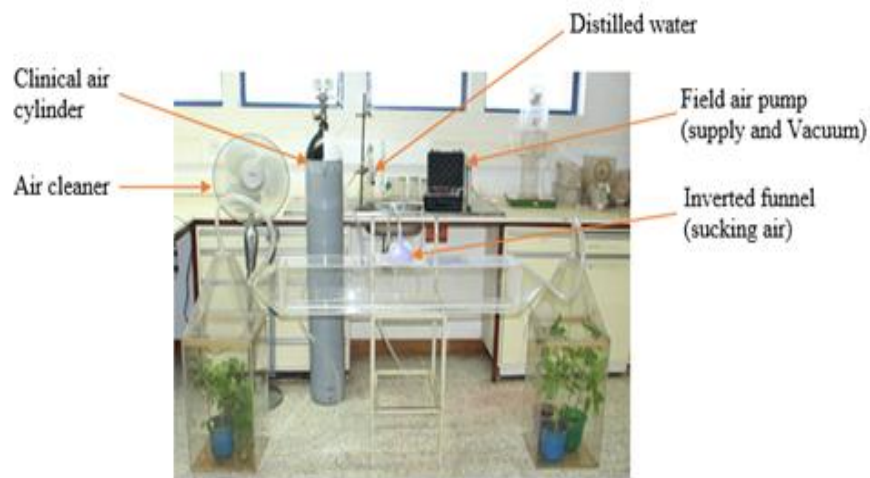
Figure 3.2: Photograph of a dual–choice wind tunnel
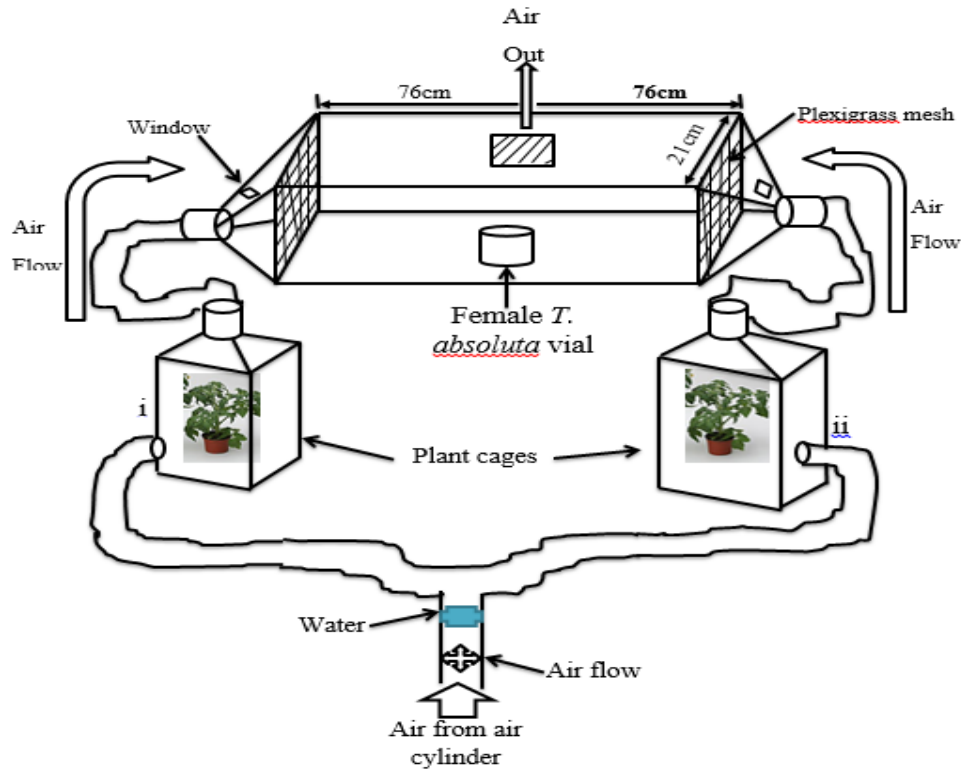
Figure3.3: Schematic diagram of a duo−choice wind tunnel (not drawn to scale)

3.5 Experimental procedure for the *T. absoluta* behavioral responses

3.5.1 Infestation of *T. absoluta* to the mono−crops and intercrops of cultivated and wild tomatoes

From each randomly selected tomato plant in a given plot, two apical leaves were randomly sampled to get a maximum of 40 leaves (Zouba et al., 2013). Leaves collected from each plot were preserved in separate labeled plastic containers. The leaves were then observed for *T. absoluta* mines and larva and recorded as number of mines and larvae per leaf per plot. This procedure was repeated after every two weeks to get 7 replicates. The same procedure was done on fruits. The data collected were then grouped and summarized in a concise, tabular format for easier analysis and reporting using the pivot tables in excel 2013. To find whether the averages

per treatment per harvest were equal, the data were first auto scaled using $\log_{10}x+1$ and then subjected to Student Neuman Keuls, SNK, using *Agricolae* package in R. The cleaned data were then analyzed using linear mixed model by REML ['lmerMod']. Analysis of variance, ANOVA, was performed using type II Wald chi–square tests. This was performed on the overall averages per treatment to find whether the ratios due to treatment, weekly harvests and weekly treatments were equal.

3.5.2 *Tuta absoluta* behavioral responses towards cultivated and wild tomato intact plant volatiles in the duo–choice olfactometer

From preliminary experiments that were conducted to come up with the best time of the day when mated *T. absoluta* females are highly active, it was observed that activity was optimal between 0300 hr and 1130 hr East African time. Flight behavior of mated *T. absoluta* females to the target tomato was measured as either attractiveness or repellence in the dual–choice wind tunnel bioassay as previously described in Nyasembe *et al.,* (2012) and Bawin *et al.*, (2014) similar to the one described in Figure 3.3. The parameters of the bioassay tunnel were, temperature, $21 \pm 2°C$ and relative humidity, $65 \pm 5\%$. Medical air from air cylinder was used and was passed through air pump, fitted with an activated charcoal column filter, via gas flow meter and water chamber for moisturizing. Air-flow speed was 350 ml/min in each arm of the dual–choice olfactometer and was also pulled from the center of the bioassay tunnel at a rate of 700 ml/min using a vacuum pump. The distance between the center and the plexi–gauze was 76 cm. A red fluorescent tube was suspended 1.5 m above the tunnel giving about 1000 lux incident red light to illuminate the test arena evenly.

Mated *T. absoluta* females were assayed for the test–plant behavioral responses as follows: (a) each tomato species was assayed against a control (air), and (b) the two tomato species were assayed against each other. The position of test plant cages were randomized between runs. Between each run and before introduction of a new plant, humid air was allowed to pass through the set–up for 10 minutes to drive off volatiles. After introduction of the plants, humid air was allowed to pass through the control and the caged plant for 10 minutes to stabilize the flow of volatiles. In each assay, 10 freshly mated, 3 days old female *T. absoluta* were introduced at the center of the olfactometer using release–vial. Each bioassay lasted 20 minutes as demonstrated by earlier preliminary tests. *T. absoluta* females landing beyond 30 cm on either side of the center were considered to have responded to plant volatiles or to the control while those that remained between the release point and 30 cm on either side were considered non–respondents. The studies were conducted between 0500 hr and 1100 hr (East Africa time) as preliminary observations had indicated this as optimal activity time. Each assay was replicated 5 times.

3.6 Trapping of naturally occurring headspace volatiles and analysis of volatiles

3.6.1   Trapping of headspace volatiles from naturally growing plants

This was done in the open field at Wang'uru in Mwea planes (S 00'4137.4"; E 037°22'12.3"), 4 m GPS, and 1158 m above sea level, of Kirinyaga County, Kenya, where the plants were grown in their natural habitats. During the day, the temperature ranged at 23±6°C and humidity at 68±17%, and at night, the temperature ranged at 20±4°C and humidity at 70±19%. Dynamic head trapping (DHT) was used in the collection of volatiles from the aerial parts of the two tomato varieties. The aerial part of the plant (leaves, flowers and young fruits) were enclosed in a

polyacetate cooking bag (oven bag), 25 cm x 38 cm, that was connected to a clean air supply and a vacuum pump (Figure **3.4**).

Figure 3.4: A photograph of the setup used in the field for headspace collection of tomato volatiles.

Clean air from an activated charcoal column filter was passed through the collection chamber at 250 ml/min and drawn from the chamber together with volatiles at the same rate through adsorbent Super-Q (ethyl vinyl benzene–divinyl benzene polymer) traps (30 mg, Analytical Research System, Gainesville, Florida, USA) using a vacuum pump. Five replicates were collected from each plant and a control, with each replicate of each plant and control running at the same time per session. Volatiles collected during the day and night were henceforth referred to as DC (cultivated tomato day volatiles), DW (wild tomato day volatiles), NC (cultivated tomato night volatiles) and NW (wild tomato night volatiles). Elution of volatiles from Super–Q traps to grass vials was done using 200 µl GC/GC-MS grade dichloromethane (DCM) (Burdick

and Jackson, Muskegon, Michigan, USA) with nitrogen as the releasing gas. The elutes were stored at -80°C for use in GC–MS and GC–EAD (Cossé *et al.,* 1995; Marion-poll and Thirry, 1996; Cosse and Baker, 1996; Bleeker *et al.,* 2009; Ormeño *et al.,* 2011; Omolo *et al.,* 2013)

3.6.2 Analysis of headspace tomato volatiles using GC–MS and GC–EAD

Analysis of headspace tomato volatiles and identification of the components was carried out using gas chromatography–linked mass spectrometry (GC–MS). 1μl aliquots of the collected tomato foliage volatile extract were injected in split–less mode into Agilent technologies–7890 gas chromatography coupled to 5975C inert XL EI/CI mass spectrometer (EI, 70 eV, Agilent, Palo Alto, California, USA), equipped with a HP-5 column (30 m $\times$ 0.25 mm ID $\times$ 0.25 µm film thickness, Agilent, Palo Alto, California, USA) at an injection temperature of 280°C. Helium was used as the carrier gas at a flow rate of 1.2 ml/min. The oven temperature was held at 35°C for 5 min, then programmed to increase at 10°C/min to 280°C and maintained at this temperature for 10 min. Sample compounds from headspace tomato volatiles were identified by comparison of spectra with library data (Adams2.L, Chemecol.L and NIST08.L).

Those compounds that showed quality above 80% were considered to be present. The compounds present from the volatiles were quantified using their peak areas in comparison to those of authentic standards (α–pinene and α–humuline) at different concentrations. The data obtained were then subjected to principal component analysis (PCA), which is a statistical tool that uses orthogonal transformation to convert correlated variables into a set of linearly uncorrelated variables called principal components (PC). PCA helped in identifying patterns in data and highlighting their similarities and differences (Liao *et al.*, 2003; Durant *et al.*, 2013; *Scheidler et al.,* 2015). For the dependent variables, PC1 and PC2, multivariate analysis of

variance (MANOVA) was performed. Analysis of variance (ANOVA) and Student Newman Keuls Test (package *Agricolae*) were performed to PC1 and PC2 independently to identify the quantifiable differences.

For GC–EAD, 6 µl aliquot of each of the plant foliage volatile extracts, collected during the day and night, were injected into a Hewlett-Packard (HP) 5890 Series II gas chromatograph equipped with an HP-1 column (30 m × 0.25 mm ID × 0.25 µm film thickness, Agilent, Palo Alto, California, USA). Nitrogen was used as the carrier gas at 1 ml/min and volatiles analyzed in the split–less mode at an injector temperature of 280°C and a split valve delay of 5 min. The oven temperature was held at 35°C for 3 min, then programmed at 10°C/min to 280°C and maintained at this temperature for 10 min. The column effluent was split 1:1 after addition of make-up nitrogen gas for simultaneous detection by flame ionization detector (FID) and EAD. For EAD detection, silver-coated wires in drawn-out glass capillaries (1.5 mm I.D.), were filled with Ringer saline solution (Cossé *et al.,* 1995; Nyasembe *et al.,* 2012) that served as reference and recording electrodes.

Antennal preparations were made by first putting the adult mated *T. absoluta* female in ice using a glass vial to deactivate it. Then the base of the head and distal end of antenna were cut with a scalpel. The base of the head was then connected to the reference electrode while the tip of the antenna was connected to the recording electrode. The analog signal was detected through a probe (INR-II, Syntech, Hilversum, the Netherlands), captured and processed with a data acquisition controller (IDAC–4, Syntech, the Netherlands), and later analyzed with software (EAG 2000, Syntech) on a personal computer. Each plant volatiles were analyzed using fresh

female antennae. Each of the four crude headspace tomato volatiles were tested more than 6 times. At least 3 of the most consistent peripheral responses were used to determine the volatile constituents that elicited substantial electrophysiological responses. The compounds identified were then compared to get those that were unique to the wild tomato. Blends of different concentrations were then prepared using standards that were available and then used on testing for the behavioral response of mated *T. absoluta* females (Cossé *et al.,* 1995; Cosse and Baker, 1996; Tholl *et al.,* 2006; Bleeker *et al.,* 2009; Proffit *et al.,* 2011; Scheidler *et al.,* 2015).

3.7 Data analyses

3.7.1 Statistical analysis

3.7.1.1 For infestation for mono crop and intercrop of the two tomato varieties

Data collected from the field were grouped and summarized in a tabular format for easy analysis and reporting using the pivot tables in excel 2013. The cleaned data were then analyzed using linear mixed model by Residual Maximum Likelihood, REML ['lmerMod'] in R.

Linear mixed model, in matrix notation, can be represented as;

$$y = X\beta + Z\mu + \epsilon,$$

Where:

y is a known vector of observations, with mean $E(y) = X\beta$; β is an unknown vector of fixed effects; μ is an unknown vector of random effects, with mean $E(\mu) = 0$ and variance $-$ covariance matrix ; $var(\mu) = G$, ϵ is an unknown vector of random errors, with mean $E(\epsilon) = 0$ and variance $Var(\epsilon) = R$; and X and Z are known design matrices relating the observations y to β and μ, respectively.

To find whether the averages per treatment per harvest were equal, the data were first auto-scaled using $\log_{10}X+1$ before being subjected to Student Neuman Keuls, SNK, using *Agricolae* package in R to separate the means. Analysis of variance, ANOVA, was performed per weekly sample to find out whether treatments affected the number of mines and larvae in leaves and fruits. The data were then averaged per treatment level and ANOVA performed in type II Wald chi–square tests to find out whether treatment and weekly sampling had any effects on the number of mines and larvae.

3.7.1.2 For olfacto–metric assays using intact tomato plants

The preference indexes (PI) for olfactory assays were calculated using the formula:

$$PI = \{(SS - NSS) / (SS + NSS)\} \times 100$$

Where,

SS is the number of *T. absoluta* females that responded to control and NSS is the number of *T. absoluta* females that responded to test tomato odors.

The formula was also used to calculate the preference on comparison between cultivated and wild tomato intact plant volatiles where;

SS is the number of *T. absoluta* females that responded to wild tomato odors and NSS is the number of *T. absoluta* females that responded to cultivated tomato odors.

If PI = 0, then equal number of *T. absoluta* females migrated to either arms of the olfactometer. A positive PI value indicated that a majority preferred the test odors arm while a negative value indicated the converse. If all insects migrated to the same arm, then PI = ±100. For each group,

Chi–squire (X^2) goodness of fit test was used to compare whether the ratio of the observed distribution of *T. absoluta* in the two arms of the olfactometer at α=0.05 were in the ratio 1:1 using R software. Non–responsive insects were not included in this analysis.

For GC–MS

The compounds present from the volatiles were identified by comparison of spectra with library data (Adams2.L, Chemecol.L and NIST11.L). They were then quantified using their peak areas in comparison to those of external authentic standards (α–pinene and α–humuline) at different concentrations. The calibration curves linear equations used were a) y=2000000x-169263, R^2=0.9963, which was used to quantify compounds with retention time, RT, less than 16.00 minutes and peak area less than 11000000, b) y=1000000x+6000000, R^2=0.995 for RT less than 16.00minute and peak area equal or above 11000000 both from α–pinene and c) y=2000000x-5000000, R^2=0.9991 from α–humuline was used to quantify compounds with RT above 16.00min. (Supplemental graph Appendix **1**: Figure **4.9**). The mean masses in ŋg/hr ± standard error (SE) of the identified compounds were tabulated in increasing order of retention time. The data were then subjected to Principal Component Analysis (PCA) to determine if the volatile profiles can be resolved into distinct clusters.

3.7.1.3 For GC–EAD

From the wild tomato, compounds that were unique and elicited electrophysiological responses were *trans*–3–hexenol (alcohol), camphor, citronellal, isopulegol, limonene oxide (terpenoloids), linalool propanoate (ester), germacrene A, *β*–elemene, germacrene B, germacrene D, *β*–bisabolene (sesquiterpenes) and verbenene (terpene). Of these compounds, a blend of trans–3–hexenol, camphor, citronellal and limonene oxide was prepared from pure standards in

relative proportions found in the volatiles. Hexane was used as the solvent. Three doses, A, B and C of the blend were prepared and used in bioassays against control (pure hexane). Dose B contained 1.2 ng/µl of trans–3–hexenol, 2.0 ng/µl of camphor, 1.4 ng/µl of citronellal and 1.2 ng/µl limonene oxide i.e. in the ratio they were transmitted from the plant. Dose A and C contained of half and double of the concentrations in B respectively.

The dual–choice olfactometer described was used to test the behavioral response of mated female *T. absoluta* to the blend of synthetic standards. The blends and the control were dispensed by applying 200 µl each onto 1cm long Luna dental roll (Roeko®, Langenau, Germany), and left for 20 minutes at room temperature to dry. Each dose was tested against the control 5 times using freshly impregnated dental roll. Since all the dosages showed repellence, dose B was tested against intact cultivated and wild tomato plants 5 times.

The preference indexes (PI) for olfactory assays of blends were calculated using the formula:

$$PI = \{(SS - NSS) / (SS + NSS)\} \times 100$$

Where:

SS is the number of *T. absoluta* females that responded to the blend and NSS is the number of *T. absoluta* females that responded to control

The formula was also used to calculate the preference on comparison between blend B and intact plant volatiles of the two varieties where:

SS is the number of *T. absoluta* females that responded to blend odors and NSS is the number of *T. absoluta* females that responded to tomato odors.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Results

4.1.1 Infestations by *T. absoluta* on monocrop and intercrop of the two tomato varieties

Figure **4.1** (supplemental Appendix **1**: Table **4.1**) is a line graph that shows the variation in the means of mines per leaf per week for the 4 treatments. Means per week with different letters are significantly different while those with the same letters are not, at α=0.05. The results show that infestation in CC is significantly different in all the weekly leaf samples. The number of mines and larvae in leaves were in the order CC >> TC > TB ≥ TT. For the first six weekly leaf samples, the number of mines per leaf per week were significantly influenced by treatment (P<0.001, at α=0.05). In the seventh leaf samples, treatment did not significantly affect the number of mines (p<0.05, α=0.05).
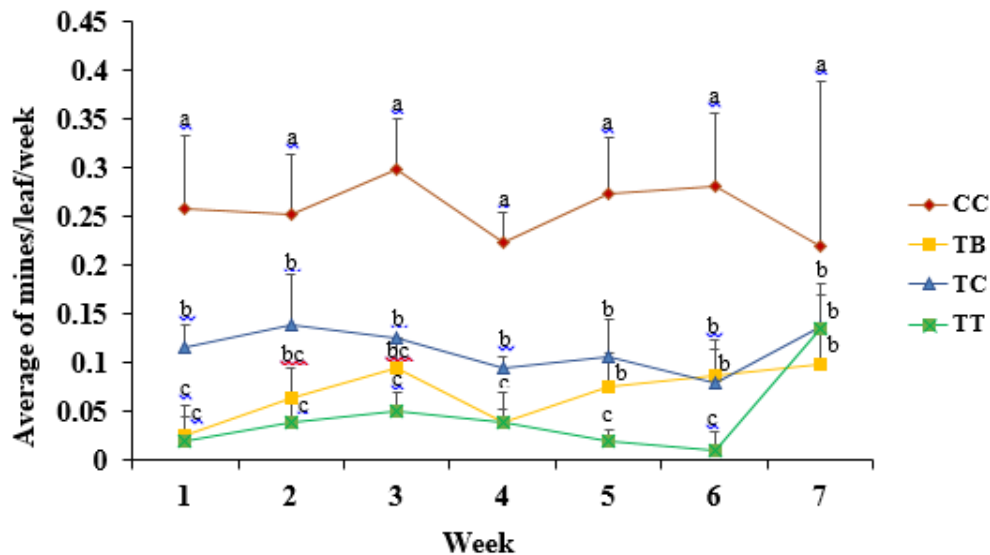
Figure 4.1: Mean mines per leaf per week for the 4 teatments and their standard errors in bars. Means per week with different letters are significantly different while those with the same letters are not at α=0.05. TT–monocrop of the wild tomato, TB–monocrop of cultivated tomato

surrounded by wild tomato, TC–intercrop of both varieties of tomatoes, and CC–monocrop of cultivated tomato

From figure **4.2** (supplemental Appendix **1**: Table **4.2**), the number of larvae per leaf per weekly leaf samples were also influenced by the treatment ($p<0.001$, $\alpha=0.05$) for the first 12 week of leaf sampling. For the 14th week, there was no significant influence of the treament ($p>0.05$, $\alpha=0.05$). For the first six weeks, the number of larvae per leaf samples were significantly different in CC. Means per week with different letters are significantly different while those with the same letters are not, at $\alpha=0.05$.
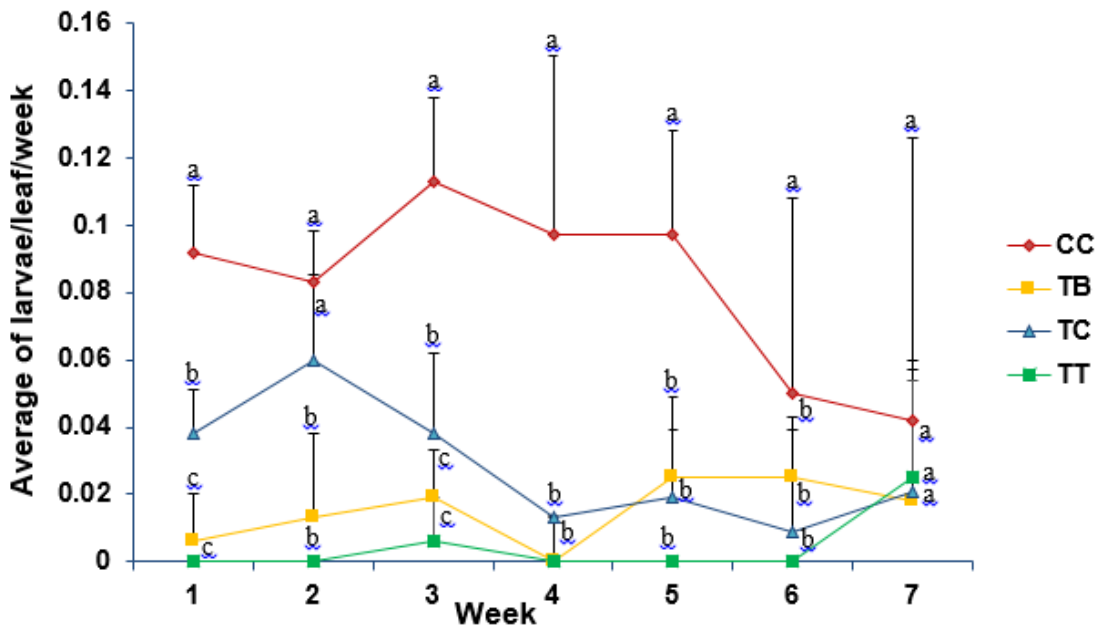
Figure 4.2: Mean larvae per leaf per week for the 4 teatments and their standard errors in bars. Means per week with different letters are significantly different while those with the same letters are not at $\alpha=0.05$. TT–monocrop of the wild tomato, TB–monocrop of cultivated tomato surrounded by wild tomato, TC–intercrop of both varieties of tomatoes, and CC–monocrop of cultivated tomato

Results on average of mines and larva per leaf per week were averaged over the treatment levels at 95% confidence level (Figure **4.3**, supplemental Appendix **1**: Table **4.3**). Tukey method for comparing a family of 4 estimates was used to adjust the P value at significant level, $\alpha = 0.05$.
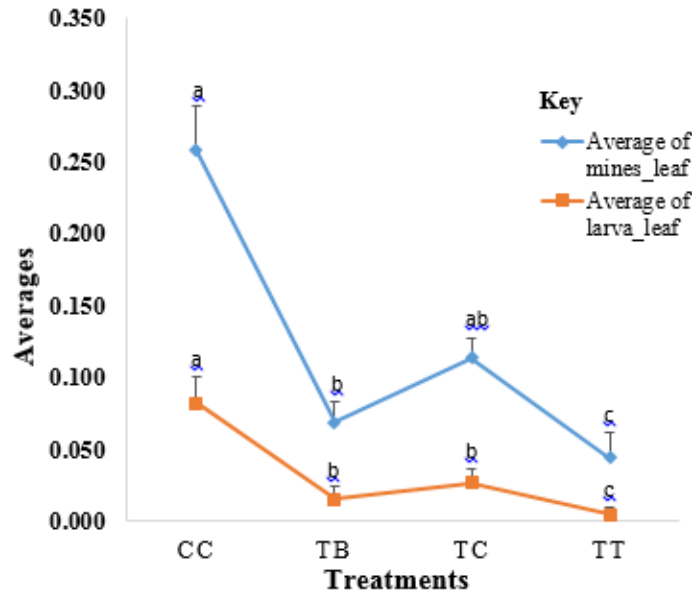
Figure 4.3: Average of mines and larvae per leaf per treatment. TT–monocrop of the wild tomato, TB–monocrop of cultivated tomato surrounded by wild tomato, TC–intercrop of both varieties of tomatoes, and CC–monocrop of cultivated tomato

The averages of mines and larvae per leaf per treatment were then subjected to Type II Wald Chi–Square test to see whether the ratio of probability of the effects due to treatments were of the same ratio at different weeks. From mines per leaf, the following results were obtained; treatment ($X^2=261.2$, DF=3, p<0.001) and treatment per week ($X^2=5.86$, DF=3, p>0.05). From larva per leaf; treatment ($X^2=73.04$, DF=3, p<0.001) and treatment per week ($X^2=13.44$, DF=3, p<0.001). From these results, treatment caused a significant difference on the average of mines per leaf while treatment and weekly leaf samples caused significant difference on the average of

larvae per leave. This could mean that as the number of leaves for infestation decreases, the gravid female then turn to the fruits as oviposition sites.

The number of mines and larvae in fruits were also significantly influenced by the treatment in the 5th and 6th week ($p<0.05$, $\alpha=0.05$) but not in the 7th week ($p>0.05$). For the first 2 weekly fruit sampling, there was significant difference in the number of mines per fruit. (Figure **4.4**, supplemetal Appendix **1**: Table **4.4**), shows the mean mines per fruit per weekly fruit samples.
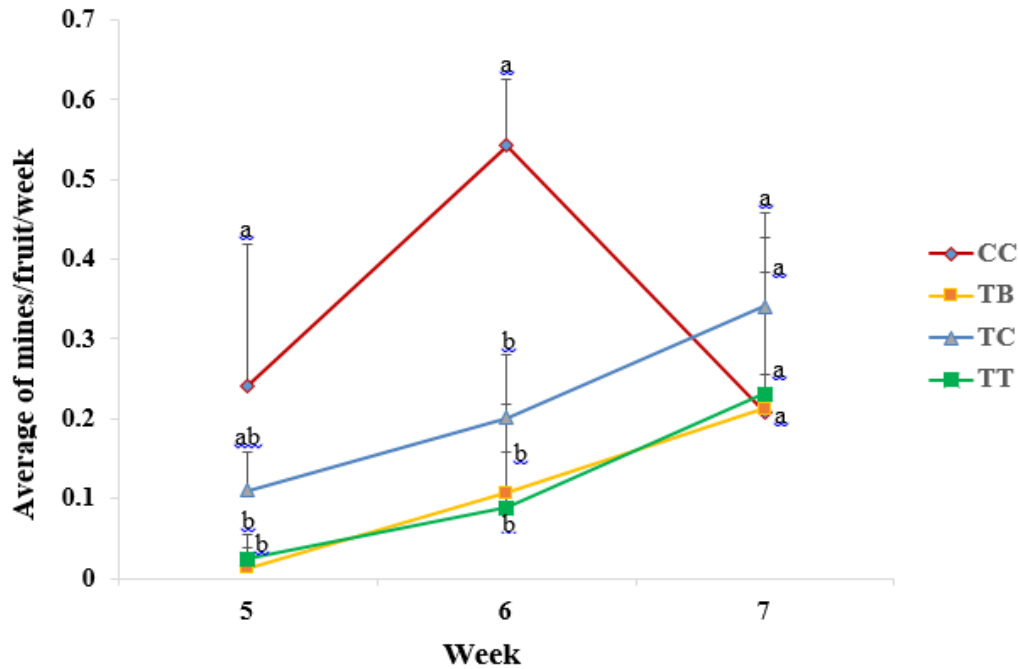
Figure 4.4: Mean mines per fruit per week for the 4 teatments and their standard errors in bars. Means per week with different letters are significantly different while those with the same letters are not at $\alpha=0.05$. TT–monocrop of the wild tomato, TB–monocrop of cultivated tomato surrounded by wild tomato, TC–intercrop of both varieties of tomatoes, and CC–monocrop of cultivated tomato

Figure **4.5**, (supplemental Appendix **1**: Table **4.5**), show the mean number of larvae per fruit per weekly fruit samples. Treatment significantly influenced the number of larvae per leaf. There was significant difference in the number of larvae on weekly harvests for CC.
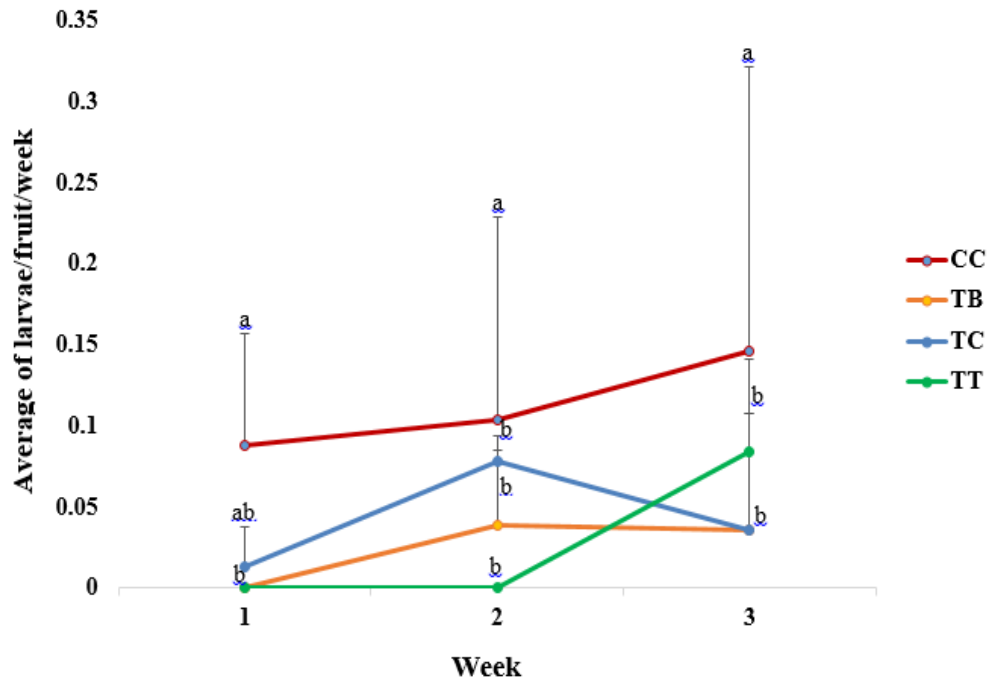
Figure of 4.5: Mean larvae per fruit per week for the 4 teatments and their standard errors in bars. Means per week with different letters are significantly different while those with the same letters are not at α=0.05. TT–monocrop of the wild tomato, TB–monocrop of cultivated tomato surrounded by wild tomato, TC–intercrop of both varieties of tomatoes, and CC–monocrop of cultivated tomato

Results on average of mines and larva per fruit per week were also averaged over the treatment levels at 95% confindence level (Figure **4.6**, supplemental Appendix **1**: Table **4.6**). Tukey method for comparing a family of 4 estimates was used to adjust the P value at significant level,α = 0.05.
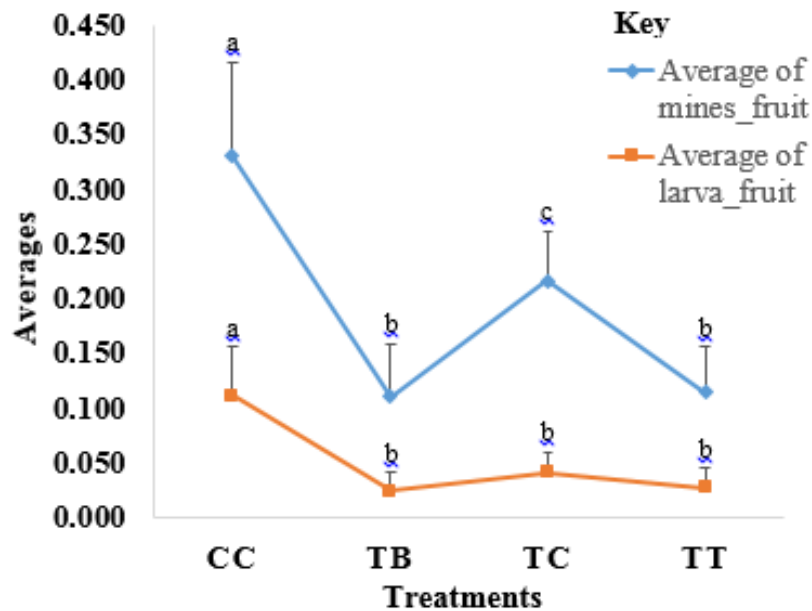
Figure 4.6: Averages of mines and larvae per fruit. TT–monocrop of the wild tomato, TB–monocrop of cultivated tomato surrounded by wild tomato, TC–intercrop of both varieties of tomatoes, and CC–monocrop of cultivated tomato

The averages of mines and larvae per fruit per treatment were subjected to Type II Wald Chi–Square test to see on whether the ratio of probability due to treatments and weekly fruit samples were 0.5. From mines per fruit, the following results were obtained; treatment (X^2=19.43, DF=3, p<0.001) and weekly fruit samples (X^2=4.59, DF=3, p>0.05). From larva per fruit; treatment (X^2=11.13, DF=3, p<0.05) and weekly fruit samples (X^2=0.78, DF=3, p>0.05). Treatments caused significant difference on the average of mines and larvae per fruit.

There is significant difference in the means of mines and larvae in both leaves and fruits from the mono–crop of cultivated tomato (Rambo F1) compared to the others. The mean of mines per leaf from TC is significantly difference from TT and TB which are not significantly different. This

implies that preference for infestation by *T. absoluta* in the four treatments was in the order CC

$\gg$ TC $>$ TB $\geq$ TT.

4.1.2   Olfacto–metric bioassays (Chi–Square goodness of fit test) for intact tomato plants

 The cultivated tomato (Rambo F1) was significantly more attractive to mated *T. absoluta*

females (control: -56.86%, X^2 = 15.37, df = 1, p < 0.0001) while wild tomato was significantly

more repellent (control: 12.20%, X^2 = 0.390, df = 1, p > 0.05) compared to the control (Bar

graph 4.1). Comparison of the two tomato varieties   showed significant repellent effect of the

wild tomato (-45.45%, X^2 = 10.47, df = 1, p < 0.05) (Bar graph **4.1**, supplemental Appendix **1**:

Table **4.7**).
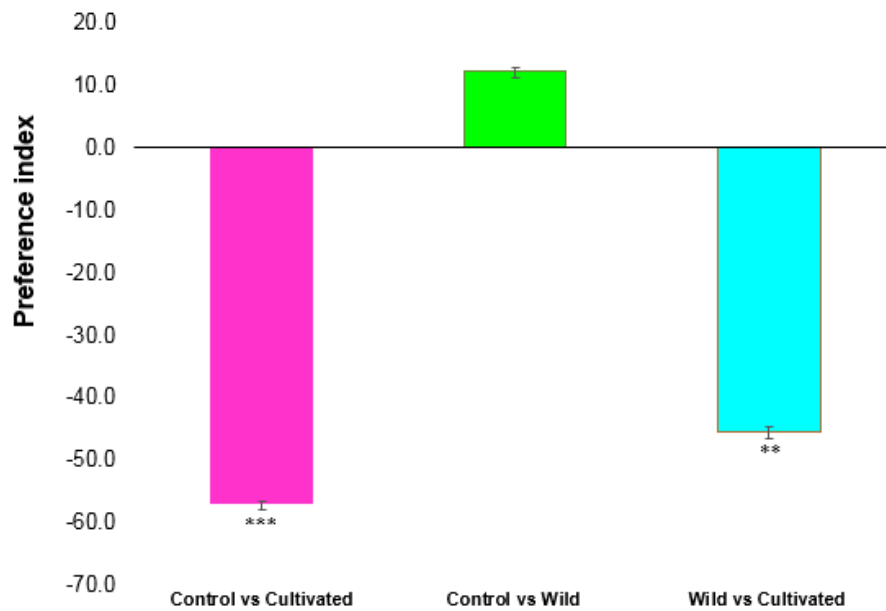
  Bar graph 4.1: Olfacto–metric responses of mated female *T. absoluta* to intact cultivated and
wild tomatoes against control and cultivated tomato against wild tomato expressed as mean PI ±
SE. Positive PI indicates preference for the control (air) while negative PI indicates preference to
the test tomato variety, while for the comparison between the two tomato varieties, negative PI
indicates preference for the cultivated tomato. The asterisks indicate the significance levels with
* = significant at 0.05, and *** = significant at 0.001

4.1.3 GC–MS FID results for volatiles collected from the two tomato plant species during the day and at night

The two tomato species produced distinct chemical profiles during the day and at night. A total of 162 compounds were identified, 85 from cultivated tomato's day volatiles, 73 from wild tomato's day volatiles, 68 from cultivated tomato's night volatiles and 64 from wild tomato's night volatiles (Appendix **1**: Table **4.8**). These compounds were present in varying amounts ranging from below the detection threshold of the mass spectrometer to about 45260ng/hr. Of the common compounds identified during the day from the wild and the cultivated tomato headspace volatiles, *α*-pinene (**18**), *o*-cymene (**30**), *δ*-2-careen (**10**), *β*-phellandrene (**8**), *δ*-elemene (**31**) and *E*-caryophyllene (**5**), accounted for over 70% by mass. Sabinene (**32**) was not present in the night volatiles of both varieties. *α*-Phellandrene (**13**) was present in higher proportions in the wild tomato volatiles, figure **4.7**. Higher quantities of these compounds were produced by the cultivated tomato during the day and at night compared to the wild tomato. However, in both tomatoes the night volatiles contained substantially less amounts of the common compounds. The two tomato varieties also produced a substantial number of different compounds that were either unique to the variety or to the time of volatile collection.
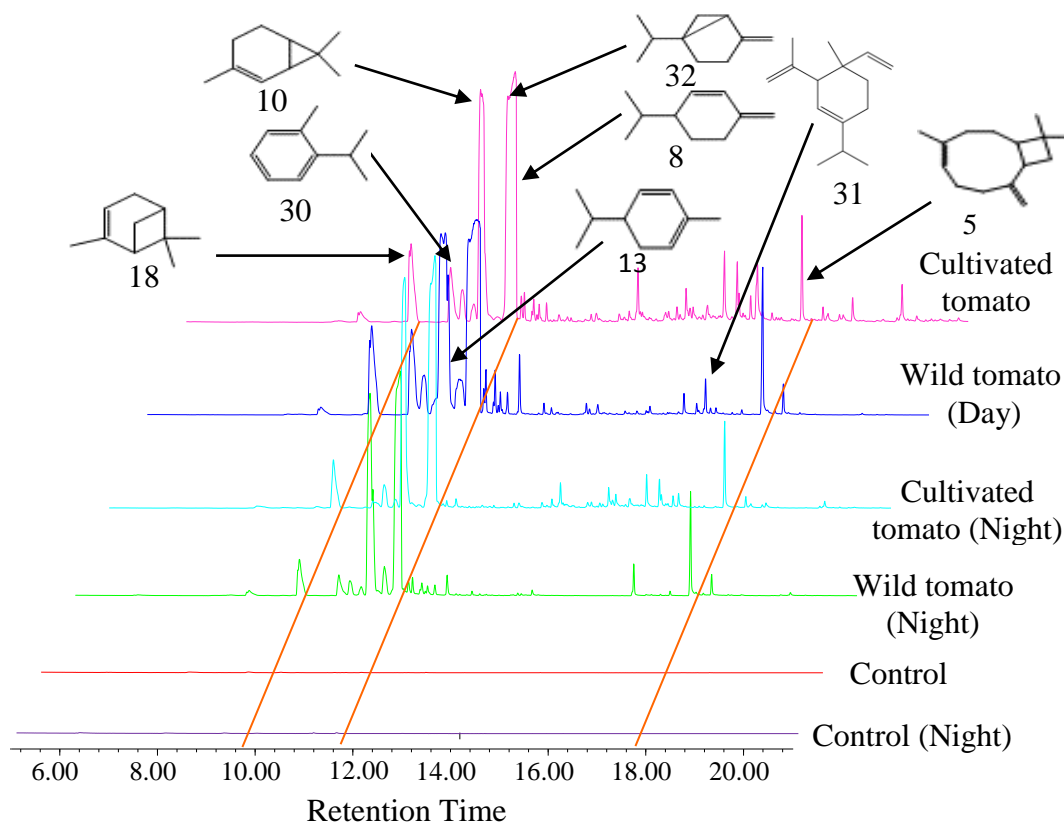
Figure 4.7: Offset representative of tomato headspace volatile profiles as measured by gas chromatography–linked mass spectrmetry (GC–MS)

To study the relation between variables and the data structure of the compounds, Principal Component Analysis, PCA was applied. The data was first auto scaled using $\log_{10}$ to avoid the effects by the variable sizes. PCA was aimed at studying the data structures in reduced dimensions with retention of maximum amount of variability present in the data. PCA separated the compounds into 12 principal components (PCs). Over 97.64% of the observed variation in the chemical profiles were explained by the first three PCs. PC1and PC2 explained over 79.0% and hence were used for further statistical analysis and production of a two dimension plot (Figure **4.8**) for visualization.
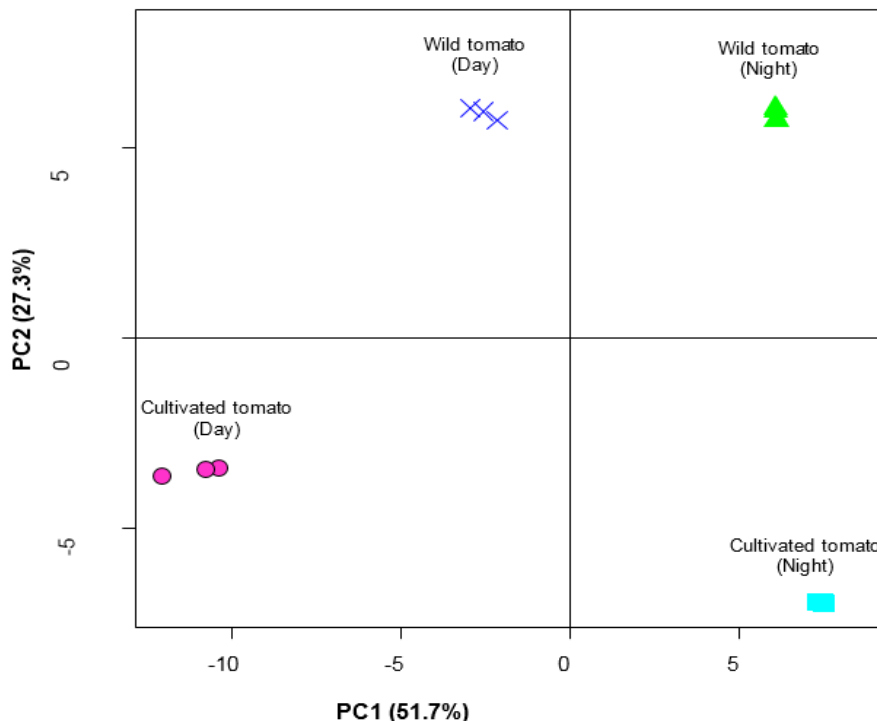
Figure 4.8: Two-dimension cluster plot based on PC1 and PC2 after resolving headspace tomato volatiles into discrete clusters. PC1 explained 51.7% of total variation while PC2 explained 27.3%. The two principle components were subjected to multivariate analysis of variance (MANOVA) and analysis of variance (ANOVA)

Loading values equal or above 0.15 were used to identify the loading factors that were most influential in the separation of tomato headspace compounds and as indicators of significant contribution to the determination of each PC. For PC1 which explained 51.7% of the overall variation, all compounds selected impacted negatively, i.e. sabinene (terpene), *p*–mentha–1,8–dien–6–ol and *α*–terpineol (alcohols), *α*–thujenal (aldehyde), and 3–methyl–6–(1–methylethyl)–7–oxabicyclo[4.1.0]heptan–2–one, *p*–ethylacetophenone and 2–acetylcyclopentanone (ketones). PC2 resolved 27.3% of the total variation. With the exception of *β*–elemene, all selected sesquiterpenes, namely *trans*–*α*–bergamotene, *E*–caryophyllene and bicyclogermacrene contributed positively, dauca–5, 8–diene, contributed negatively. *Trans*–4–hydroxyl–3–methyl–

6–(1–methylethyl)–2–cyclohexen–1–one (terpinoid) and 3–(1–adamantly) sydnone contributed positively, while allo–aromadendrene epoxide (sesquiterpinoids) contributed negatively. Multivariate analysis of variance (MANOVA) test was performed on PC1 and PC2 to find the statistical significance of the differences among the tomato volatile chemical profiles. The Wilks MANOVA test indicated that the chemical profiles were significantly different from each other ($P < 0.00001$, $\alpha = 0.05$). Analysis of variance (ANOVA) on PC1 and PC2 separately indicated significant difference ($p < 0.00001$, $\alpha = 0.05$) on the chemical profiles in DC, DW, NC and NW.

4.1.4   GC – EAD results for the volatiles collected from the two tomato plant species during the day and at night

After establishing that *T. absoluta* is repelled by the wild tomato and preferentially attracted to cultivated tomato, gas chromatography linked electroantenographic detection (GC–EAD) was conducted to investigate the sensory physiological bases of attraction and repulsion. The EAGs (Figure **4.9**) demonstrated that most of the tomato headspace volatile constituents elicited electrophysiological responses of different intensities.
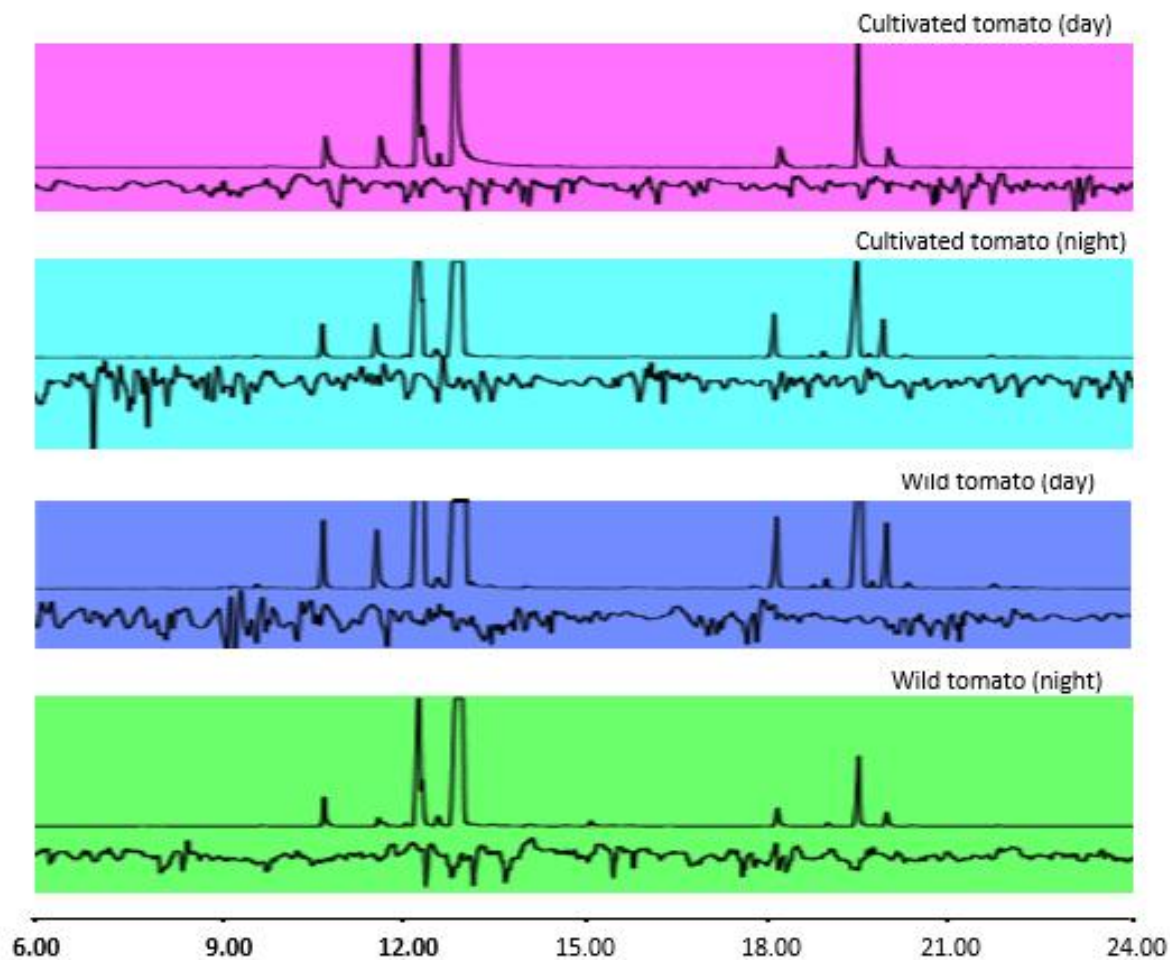
Figure 4.9: Offsets of representative antennal response profiles generated by gas chromatography–linked electro–antennographic detection (GC–EAD) method.

Those compounds that elicited electrophysiological responses of high intensities were hexanal, *trans*–3–hexenol, 2,4-dimethylheptane, *p*–xylene, *α*–pinene, *β*- Pinene, myrcene, *δ*–2–careen, *α*-phellandrene, *α*-terpinene, *β*- phellandrene, 3,5-dimethylene-1,4,4-trimethyl-cyclopentene, *E-β*-ocimene, *γ*-terpinene, verbenene, *p*-cymenene, linalool propanoate, limonene oxide, camphor, 4–keto–isophorone, limonene, limonene oxide, citronellal, isopulegol, tridecane, *δ*-elemene,

germacrene A, *β*-elemene, *E*-caryophyllene, germacrene B, 6,9-guaiadiene, *α*-humulene, germacrene D, isoledene, *β*-bisabolene, caryophyllene oxide. Compounds that were unique to wild tomato and elicited electrophysiological responses of considerable intensities and their PCA loadings, were hexanal, (0.058, PC3, **20**) (aldehyde); *Z*–3–hexen-1-ol (0.115, PC2, **15**) (alcohol); *β*-pinene (-0.245, PC3, **32**), verbenene (0.109, PC3, **33**), 4–keto–isophorone (-0.127, PC2, **34**), camphor (-0.105, PC3, **35**), citronellal (0.126, PC2, **36**), isopulegol (0.078, PC3, **37**), limonene oxide (-0.137, PC3, **38**), *p*-cymenene (-0.100, PC1, **39**), (monoterpenoids); linalool propanoate (0.102, PC3, **40**) (ester); germacrene A (-0.033, PC1, **24**), *β*–elemene (-0.204, PC2, **28**), germacrene B (-0.012, PC1, **25**), germacrene D (-0.123, PC2, **27**), and *β*-bisabolene (-0.119, PC2, **41**) (sesquiterpenes).
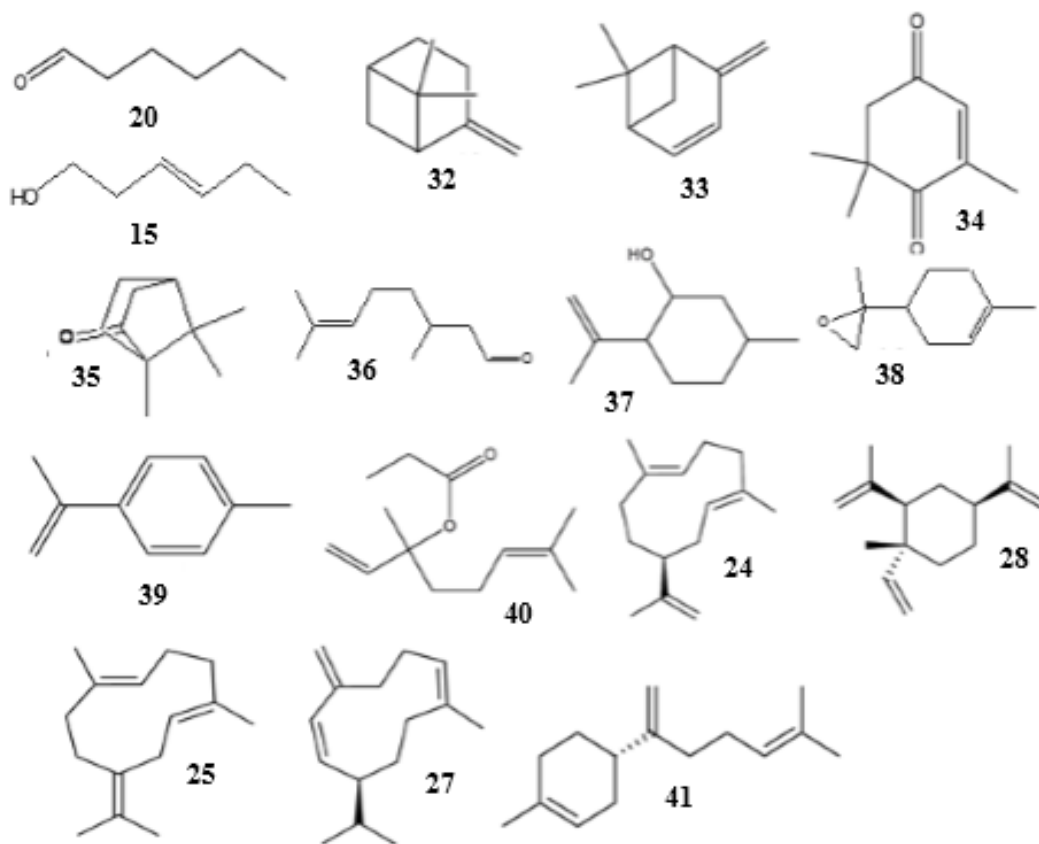
Of these compounds, a blend of *trans*–3–hexenol, camphor, citronellal and limonene oxide were prepared from pure synthetic compounds using their proportions in the volatiles.

4.1.5   Olfacto–metric bioassays (Chi–Square goodness of fit test) for intact tomato plants

A blend of *trans*–3–hexenol, camphor, citronellal and limonene oxide was prepared from pure synthetic compounds that were available in the laboratory by the time this study was donne. Hexane was used as the solvent. Three doses A, B and C of the blend, which contained half, equal and double respectively of quantities relative to those in volatiles naturally produced by the wild tomato, were prepared and used in bioassays against control (pure hexane). The dual–choice olfactometer (Figure **3.3**) was used to test on the behavioral response of mated *T. absoluta* females to the doses of synthetic standards. The doses and the control were dispensed by applying 200 µl each onto 1 cm long Luna dental roll (Roeko®, Langenau, Germany), and left for 20 minutes at room temperature to dry. Each dose was tested against the control 5 times using freshly impregnated dental roll. Since all the dosages showed repellence, dose B was tested against intact cultivated tomato and wild tomato plants to get 5 replicates. The data obtained were used to calculate the preference indices (Bar graph **4.2**, Supplemental Appendix **1**: Table **4.10**), for repellence.
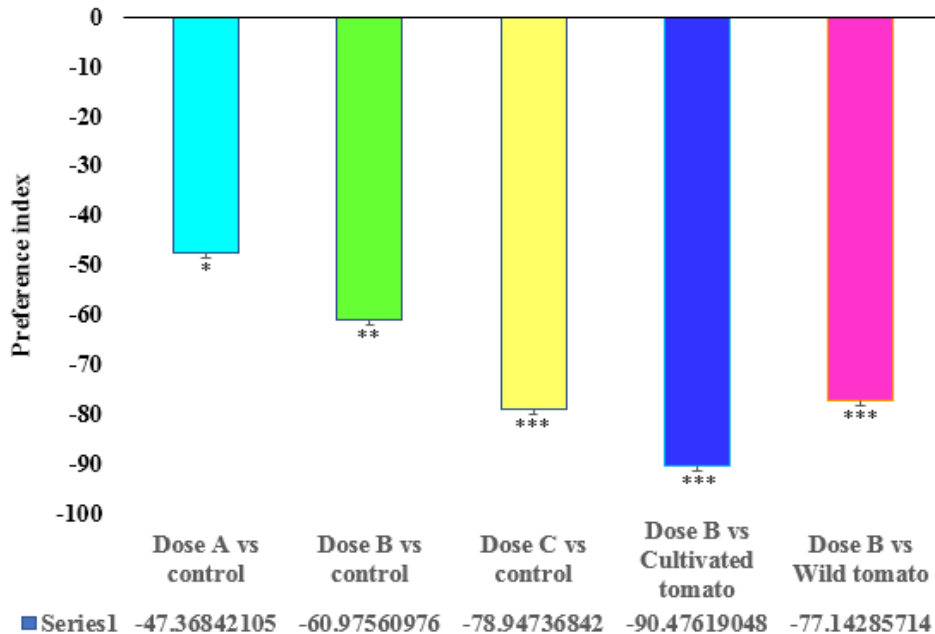
Bar graph 4.2: Olfactometric responses of mated *T. absoluta* females to blends A, B, C against control and blend B against intact cultivated and wild tomatoes expressed as mean PI ± SE. Negative PI indicates preference for the control (hexane) or the test plant. The asterisks indicate the significance levels with * = significant at 0.05, ** = significant at 0.01 and *** = significant at 0.001 (Supplemental Appendix 1: Table 4.10).

The olfacto–metric bioassay showed that female *T. absoluta* responded negatively to all the three dosages of blend against control (A: -47.37%, x^2 = 7.6, df = 1, p<0.01; B: -60.98%, x^2 = 14.05, df = 1, p<0.001; C: -78.95%, x^2 = 22.13, df = 1, p<0.0001). The same applied to both varieties of tomatoes after running them against B, (B against cultivated tomato: -90.48%, x^2 = 32.59, df = 1, p<0.0001; B against wild tomato: -77.14%, x^2 = 19.31, df = 1, p<0.0001).

4.2 Discussion

Volatiles of plants that host herbivorous insects play a major role in attracting or deterring gravid females and in mediating their oviposition (Cosse & Baker, 1996; Cosse *et al*., 2002; Tasin *et*

al., 2006; Knudsen *et al*., 2008; Zhang *et al.,* 2008; Pickett & Guerrieri, 2009; Proffit *et al.,* 2011; Bawin *et al.,* 2014; Biasazin *et al*., 2014; Schäpers *et al*., 2015; Scheidler *et al*., 2015). In the present study, preliminary observations in the field indicated that wild tomato, *Lycopersicon esculentum var. cerasiforme,* is not attacked by *T. absoluta* like the cultivated tomato, *Solanum lycopersicum* L. (Rambo F1 variety). We hypothesized that the wild tomato variety may be actively avoided by gravid females because of the presence of constituents that deter or have negative effects on the development of the young stages of the pest. In the present study, we compared the responses of gravid *T. absoluta* females to headspace volatiles emitted by the two intact tomato plants in a dual–choice olfacto–metric bioassays (Fig. 3.1). The cultivated tomato plant was found to be very attractive to the insects, while the wild tomato plants were significantly repellent. This was confirmed in the open semi-field study with mono-crops and intercrops of the two tomato varieties, which showed that infestation levels of *T. absoluta* in intercrops were significantly reduced relative to that of the mono-crop of the cultivated tomato [Figure **3.2 (a)** and **(b),** and Figure **3.3**].

GC-MS of dynamic headspace volatiles collected from mature flowering and fruiting plants during the day and at night showed emissions of very rich profiles of compounds (Figure **3.4**) from both tomato varieties, of which 162 compounds were positively identified (Table 1). There were significant differences in the chemical compositions, both quantitative and qualitative. Principal component analysis, PCA, of the compositions of the compounds associated with the two tomato varieties gave unique clusters. This was illustrated by the two-dimension cluster plot (Figure **3.5**), MANOVA and ANOVA tests based on PC1 and PC2, which confirmed significant difference in the chemical profiles of the two tomato headspace volatiles. A number of previous

studies have revealed that *T. absoluta* is sensitive to small variations in headspace compositions emanating from different varieties of tomatoes and other host plants which lead to different levels of attraction (Fernandez & Montagne, 1990b; Kang *et al*., 2010; Proffit *et al*., 2011; Birgücü & Karaca, 2015). However, in our study the difference in the volatile compositions emanating from the two varieties have opposite effects.

Although many volatile constituents from the two varieties elicited electrophysiological responses of different intensities (Figure **3.6**), EAG–active compounds that were unique to the wild tomato included hexanal, *Z*–3–hexen-1-ol, verbenene, 4–keto–isophorone, camphor, citronellal, isopulegol, limonene oxide, linalool propanoate, germacrene A, *β*–elemene, germacrene B, germacrene D, and *β*–bisabolene. Other compounds that were unique to the wild tomato but that elicited mild electrophysiological responses were *β*-pinene, *p*–cymene, *p*-cymenene, 6-camphenone, camphene, 2-allyl-phenol, veratrole, *E*-isocitral, *trans*-4-caranone, *trans*-sabinol, *p*-cymen-7-ol, α-funebrene, and butylated hydroxytoluene (Figure **3.7**). Moreover, a blend of available compounds (*Z*–3–hexen-1-ol, citronellal, camphor and limonene oxide) showed dose-dependent repellence to mated *T. absoluta* females in the dual–choice olfactometer. Interestingly, hexanal, *Z-3*-hexen-1-ol and verbenene, as well as methyl salicylate and *δ*-elemene that are emitted in higher relative amounts by the wild tomato, are produced by cultivated tomato plants that are stressed after oviposition by *T. absoluta,* and their levels increase with increased oviposition (Buttery *et al*., 1987; Proffit *et al*., 2011; Balayiannis *et al*., 2015). These compounds have been associated with attraction of predators of different stages of *T. absoluta* like *Aphidiuservi* (Hymenoptera: Braconidae) and *Macrolophus pygmaeus* (Heteroptera, Miridae) (De Backer *et al*., 2014; Balayiannis *et al*., 2015). Moreover, the cultivated tomato was observed

to produce high levels of *β*-phellandrene during the day and night (45268.2±3869 ng/hr and 15812.0±464 ng/hr, respectively) compared to the wild tomato (16535.4±832 ng/hr and 8184.2±619 ng/hr, respectively). Balayiannis *et al*. (2015) found that there is significant reduction in the amount of emitted *β*-phellandrene resulting from oviposition by *T. absoluta* on cultivated tomato varieties, which suggests that the terpenoid may be an important component of the attractant blend of *T. absoluta*. In addition, *α*–terpinene and *α*–phellandrene, and *p*–cymene were produced in higher relative amounts by the wild tomato compared to the cultivated tomato. These compounds have shown strong deterring effects on other insect pests like the tomato–whitefly (*Bemisia tabaci*) (Oa *et al*., 2009).

Thus, our results show that the wild tomato emits volatile compounds that does not only repel gravid *T. absoluta* females but may also attract parasitoids that prey on different stages of the pest. Moreover, the avoidance of the wild tomato by *T. absoluta* suggests that the insect may not be able to develop fully in this tomato variety and it will be interesting to study the development of the larval stages of the pest on tomato plants treated with extracts from the wild tomato. It will also be interesting to compare the metabolomic and genomic profiles of the two tomato varieties and identify those features that may be associated with preference and avoidance respectively. The present study also lays down some groundwork for downstream development of a 'push-pull' strategy to control *T. absoluta* that integrates deployment of intercrops of the two tomato varieties and traps or agro-nets baited with controlled-release optimized attractant blend.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The field experiments showed that intercropping of commercial and wild species of the tomato plants, significantly reduces the levels of infestations of *T. absoluta*. Moreover, in addition to the inter-crop arrangement, planting a boundary of wild tomatoes around the cultivated tomatoes further reduces the levels of infestations. These results show that the presence of wild tomato species has a negative effect on *T. absoluta.* This is confirmed by behavioral responses of the pest in olfacto–metric bioassays using intact tomato plants, which showed that mated *T. absoluta* females actively avoided the wild tomatoes but were attracted to the cultivated tomatoes.

Most of the compounds produced by the two varieties of tomatoes were EAG–active. However, the two tomato varieties showed significant qualitative and quantitative differences in the chemical compositions of volatiles emitted during the day and at night. Principal Component Analysis (PCA) of the identified compounds separated them into groups of 12 PCs. Of these, PC1 and PC2 resolved over 79.0% of the total variation. MANOVA and ANOVA tests on PC1 and PC2 indicated significant difference in the chemical profiles of the tomatoes.

EAG-active hexanal, *Z*–3–hexen-1-o, *β*-pinene, verbenene, 4–keto–isophorone, camphor, citronellal, isopulegol, limonene oxide, *p*-cymenene, linalool propanoate, germacrene A, *β*–elemene, germacrene B, germacrene D, and *β*-bisabolene were unique to the wild tomato. The blend of available compounds (*trans*–3–hexenol, camphor, citronellal and limonene oxide)

showed repellence to mated *T. absoluta* females, and it will be interesting to compare this with the full blend.

From these results it is evident that mated *T. absoluta* female adults actively avoid the wild tomato variety, but are strongly attracted to the cultivated tomato. It is probable that the larval stage of *T. absoluta* cannot develop fully in the wild tomato variety. This would explain why the females avoid ovipositing on this variety. The results obtained from the present study lays some groundwork for further studies and downstream development of new tools (green chemicals) and tactics (green chemistry) to manage *T. absoluta.*

5.2 Recommendations

i.   The wild tomato produces edible fruits that could be having high nutritional value and is free from insecticides and hence should be exploited as an alternative or a complement to cultivated commercial tomatoes.

ii. Inter–cropping wild and cultivated tomatoes can be practiced to reduce infestations by *T. absoluta.*

iii. The identified repellent compounds can be used to impregnate nets in agro–net technology for suppression of *T. absoluta*.

iv. Further studies need to be undertaken on:

a) Other chemo–factors associated with avoidance of the wild tomato variety by the pest;

b) The attractant profile of the cultivated tomato and the possibility of exploiting this in 'push-pull' set up to divert the pest from intercrops of the two varieties to baited traps;

c) Additional behavioral responses of *T. absoluta* on other wild tomatoes, cultivated tomatoes, and related *Solanaceae* plants available in Kenya and the plant chemo–factors associated with the responses;

d) Genotype comparison of the attracting and the repelling tomato varieties.

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APPENDICES

APPENDIX 1

Table 4.1 Average of mines per leaf per treatment per sampling.

	CC	TB	TC	TT
	CC	TB	TC	TT
week	Average of mines/leaf	Average of mines/leaf	Average of mines/leaf	Average of mines/leaf
1	0.259±0.075	0.025±0.020	0.115±0.023	0.019±0.038
2	0.252±0.063	0.063±0.032	0.139±0.051	0.038±0.025
3	0.299±0.052	0.094±0.031	0.125±0.000	0.050±0.020
4	0.223±0.031	0.038±0.032	0.094±0.013	0.038±0.014
5	0.273±0.058	0.075±0.035	0.106±0.038	0.019±0.013
6	0.281±0.075	0.086±0.028	0.079±0.044	0.010±0.019
7	0.219±0.171	0.099±0.032	0.137±0.045	0.136±0.033

Table 4.2 Average of larva per leaf per treatment per sampling.

	CC	TB	TC	TT
	CC	TB	TC	TT
week	Average of larva/leaf	Average of larva/leaf	Average of larva/leaf	Average of larva/leaf
1	0.092±0.020	0.006±0.013	0.038±0.014	0.000
2	0.083±0.015	0.013±0.025	0.050±0.025	0.000
3	0.113±0.025	0.019±0.024	0.038±0.014	0.006±0.013

4	0.097±0.053	0.000	0.013±0.014	0.000
5	0.097±0.031	0.025±0.020	0.019±0.024	0.000
6	0.050±0.058	0.025±0.030	0.009±0.018	0.000
7	0.042±0.084	0.018±0.036	0.021±0.042	0.025±0.029

Table 4.3 Average of mines and larva per leaf per treatment.

Treatment	Average of mines_leaf	Average of larva_leaf
CC	0.258±0.03	0.082±0.018
TB	0.068±0.014	0.015±0.009
TC	0.113±0.014	0.027±0.009
TT	0.044±0.017	0.005±0.005

Table 4.4 Average of mines per fruit per treatment per sampling.

	CC	TB	TC	TT
Week	Average of mines/fruit	Average of mines/fruit	Average of mines/fruit	Average of mines/fruit
5	0.242±0.177	0.013±0.025	0.110±0.049	0.025±0.029
6	0.542±0.083	0.107±0.052	0.201±0.079	0.089±0.129
7	0.208±0.250	0.213±0.171	0.341±0.087	0.231±0.024

Table 4.5 Average of larva per fruit per treatment per sampling.

	CC	TB	TC	TT
Week	Average of larva_fruit	Average of larva_fruit	Average of larva_fruit	Average of larva_fruit
5	0.088±0.069	0.000	0.013±0.025	0.000
6	0.104±0.125	0.039±0.046	0.078±0.016	0.000
7	0.146±0.175	0.036±0.072	0.036±0.072	0.084±0.057

Table 4.6 Average of mines and larva per fruit per treatment.

Treatment	Average of mines_fruit	Average of larva_fruit
CC	0.330±0.086	0.113±0.045
TB	0.111±0.048	0.025±0.018
TC	0.218±0.045	0.042±0.018
TT	0.115±0.043	0.028±0.030

Table 4.7 Number of *T. absoluta* migrating to either side of the olfactometer when intact plants were used.

Treatment	SS	NSS	SS-NSS	SS+NSS	PI	Std error
Control vs Cultivated	11	40	-29	51	-56.8627	0.37
Control vs Wild	23	18	5	41	12.19512	0.5
Wild vs Cultivated	15	40	-25	55	-45.4545	0.71

Table 4.8 Mean quantities ± SE in ng/hour of the compounds identified from cultivated tomato and wild tomato during the day and night. Headspace tomato volatiles produced distinct quantitative and qualitative chemical profiles.

<div align="center">Mean ŋg/hr ± SE</div>

	RT (min	Library/ID	DC	DW	NC	NW
1	6.30	Hexanal	*	42.8±9.1	*	36.4±10.8
2	6.37	3-Methyl-2-hexanol	10.3±1.8	*	*	*
3	7.31	3,4-dimethyl-1-Pentanol	*	*	3.9±0.0	*
4	7.87	Ethylbenzene	92.9 ± 18.4	*	18.8±7.6	*
5	7.88	*Z – 3 – Hexenol*	*	34.9 ± 11.1	*	9.5±2.3
6	7.94	*p*-Xylene	21.4±6.4	*	29.5±11.1	*
7	8.14	*n*- Hexanol	*	*	17.2±5.1	*
8	8.17	1-Ethyl-3,5-dimethyl-benzene	119.2±36.6	*	*	*
9	8.21	3-Ethyl-2-heptanol	*	*	*	28.5±3.1
10	8.44	*cis*-1-Ethyl-3-methyl-cyclohexane	*	*	2.8±0.8	*
11	8.53	3,7,7-Trimethyl-1,3,5-cycloheptatriene	130.1±54.6	38.5±21.6	25.2±9.2	20.5±11.6
12	8.56	Styrene 0r Phenylethylene	*	*	19.0±9.6	*
13	8.60	*m*-Xylene(1,3-dimethyl benzene)	*	*	24.9 ± 3.1	*
14	8.83	Nonane	17.0±2.1	*	8.8±0.9	*
15	9.36	Cumene	*	*	5.85±1.70	*
16	9.48	*α*- Thujene	51.7±11.7	28.6±8.6	11.4±0.7	11.9±1.2
17	9.58	*α*-Pinene	9613.4±1493.2	4573.6±1032.5	741.9±199.4	2036.2±455.8
18	10.26	2,7-Dioxa-tricyclo[4.4.0.0(3,8)]deca-4,9-diene	*	*	*	11.6±2.0
19	10.39	*p*-Cymene	*	11.6±1.7	*	*
20	10.42	*o*- Cymene	6010.2±1624.8	342.8±157.6	856.7±247.9	762.5±263.9
21	10.46	*β*- Pinene	*	200.6±70.7	*	51.7±14.9
22	10.66	*trans*- Isolimonene	3398.2±1230.2	1899.67±789.74	167.9±11.1	625.6±99.7
23	10.90	Myrcene	1374.4±526.4	289.1±138.9	92.7±10.2	226.0±98.0
24	11.04	*δ*-2-Carene	17270.8±1293.9	12273.5±2303.5	7453.1±787.3	12312.1±793.8
25	11.12	*α*- Phellandrene	456.1±93.2	4566.2±416.7	*	3000.0±144.5
26	11.20	Tricyclene	*	*	*	144.4±14.9
27	11.38	*α*- Terpinene	828.3±73.1	1855.2±83.8	495.7±44.6	1473.4±73.5
28	11.59	Sabinene	16203.2±1887.4	10831.7±1097.2	*	*
29	11.75	*β*-Phellandrene	45268.2±869.4	16535.4±832.3	15812.0±464.9	8184.2±619.1
30	11.87	3,5-Dimethylene-1,4,4-trimethyl-cyclopentene	901.6±141.3	278.7±121.1	86.2±21.1	207.4±95.4
31	11.93	(*E*)-*β*- Ocimene	808.2±166.1	377.3±55.8	78.7±18.2	250.9±82.7
32	11.93	*δ*-3- Carene	60.8±12.5	*	*	*
33	12.00	*γ*-Terpinene	708.1±174.5	423.4±190.8	77.3±26.3	241.6±13.0
34	12.18	Verbenene	*	43.6±8.4	*	36.4±10.4
35	12.31	*p*-Mentha-3,8-diene	71.2±16.2	*	*	*
36	12.33	m-Cymene	*	*	23.9 ±2.9	*
37	12.37	*trans-meta*-Mentha-2,8-diene	*	*	*	80.7±61.9

38	12.54	*m*-Cymenene	49.7±11.2	24.8±2.4	*	*
39	12.58	*p*-Cymenene	*	22.6±4.0	*	*
40	12.64	Terpinolene	653.0±377.2	467.2±403.4	101.2±27.6	410.2±21.5
41	12.72	(2-Methyl-2-propenyl)-benzene	62.1±15.7	*	*	*
42	12.73	6-Camphenone	*	82.6± 27.8	*	*
43	12.76	Camphene	*	38.1±3.3	*	*
44	12.77	Linalool propanoate	*	*	*	27.5±4.1
45	12.82	Linalool	*	*	68.4±30.4	*
46	12.89	*n*-Nonanal	74.7±26.1	47.7±8.3	37.7±20.8	37.0±16.8
47	13.03	*para*-Mentha-1,3,8-triene	48.9±5.9	24.6±6.9	17.2±8.1	22.9±2.0
48	13.07	Phenylethyl Alcohol	52.4±6.3	*	*	*
49	13.15	*iso*-Sylvestrene	73.9±31.2	120.8± 11.4	39.4±8.8	54.4±3.9
50	13.18	*trans*-1-Methyl-4-(1-methylethyl)-2-Cyclohexen-1-ol	*	149.4± 52.0	*	*
51	13.30	*neo-allo*-Ocimene	207.9±69.1	93.1±31.0	14.2±5.0	19.9±4.6
52	13.39	(1*S*)-4,6,6-Trimethyl-bicyclo[3.1.1]hept-3-en-2-one	*	27.7±2.6	*	*
53	13.40	3-(2-Furanyl)-3-Penten-2-one	255.0±71.7	*	*	*
54	13.41	2-allyl-phenol	*	85.8±27.4	*	*
55	14.43	Limonene	155.1±11.0	*	9.9±1.8	*
56	13.47	Limonene oxide	*	44.8±14.3	*	11.5±1.4
57	13.54	4-keto-Isophorone	*	32.8 ±9.1	*	*
58	13.58	1,2-Dimethoxy benzene (Veratrole)	*	28.0±9.7	*	*
59	13.59	Camphor	*	47.5±19.3	*	13.8±2.6
60	13.64	Citronellal	*	*	*	11.5±0.7
61	13.73	Isopulegol	*	14.5±1.4	*	4.5±0.7
62	13.80	2-Methyl-3-(1-methylethyl)-cyclopentanone	*	*	*	6.1±0.5
63	13.82	Bicyclo[3.3.2]decan-9-one	35.9±3.5	*	*	*
64	13.87	2-(1*E*)-Propenyl- phenol	118.3± 88.0	*	*	*
65	13.87	3-Ethyl-benzaldehyde	207.7±50.8	76.0±27.1	*	6.8±2.9
66	13.89	*p*-Mentha-1,5-dien-8-ol	*	*	11.1±2.8	*
67	13.93	Neopentylidenecyclohexane	*	*	*	26.0±5.1
68	13.94	1-(1,4-Dimethyl-3-cyclohexen-1-yl)-ethanone	*	*	12.3±2.6	*
69	13.97	6-Methyl-bicyclo[3.3.0]oct-2-en-7-one	158.8±23.8	*	*	*
70	14.02	*Z*-Myroxide	55.6±5.4	42.0±18.7	*	*
71	14.03	*trans*-2-Caren-4-ol	*	*	43.7±16.3	*
72	14.06	*E*-Isocitral	*	90.6 ±11.1	*	*
73	14.07	*(S)-cis*-Verbenol	*	*	*	48.1±19.0
74	14.08	4-(1-Methylethyl)-1-cyclohexene-1-carboxaldehyde	415.6±42.5	421.1±187.2	*	*
75	14.14	*p*-Mentha-1,8-dien-6-ol	*	*	*	25.75±6.05
76	14.20	Dill ether	*	*	28.56±8.34	19.98±3.58
77	14.26	Cryptone	1687.6±456.9	475.1±148.3	*	*
78	14.27	*α*-Terpineol	*	*	35.1±15.0	8.6±0.8

79	14.34	*trans*-4-Caranone	*	20.1± 2.2		
80	14.34	Dodecane	*	*	27.8±9.7	*
81	14.38	Methyl salicylate	23.6±3.4	76.9±12.8	*	44.7±22.8
82	14.45	*n*-Decanal	*	*	31.8±17.9	22.2±6.7
83	14.50	*trans*-Sabinol (*trans* for OH vs. IPP)	*	44.8±6.1	*	*
84	14.52	*trans*-Dihydro carvone	127.2±51.9	*	*	*
85	14.63	Umbellulone	226.9±83.3	44.3±8.4	*	*
86	14.76	Eucarvone	*	*	23.2±4.6	*
87	14.82	*o*-Cumenol	455.4±85.5	176.6±66.8	*	*
88	15.00	α-Campholenal	79.10± 33.7	36.2±12.3	*	*
89	15.04	Methyl ether carvacrol	*	*	12.7±3.5	*
90	15.06	4,6-Dimethyl-dodecane	*	*	*	6.1±2.2
91	15.06	Cumin aldehyde	415.9±90.0	137.4±57.8	*	*
92	15.12	α-Thujenal	259.7±42.5	123.8±66.0	*	*
93	15.13	2,8,8-Trimethyl-decane	*	*	*	7.7±1.6
94	15.24	6-Demethoxy-ageratochromene	*	*	*	25.2±11.6
95	15.24	Car-3-en-2-one	917.0±443.3	255.2±185.0	*	*
96	15.33	3-Methyl-6-(1-methylethyl)-7-oxabicyclo[4.1.0]heptan-2-one	516.7±77.4	232.2±70.3	*	*
97	15.39	*p*-Ethyl acetophenone	575.7±217.9	184.6±147.4	*	9.8±10.1
98	15.51	(-)-Myrtenol	126.1 ± 71.8	27.0±4.2	*	*
99	15.58	α-Terpinen-7-al	211.6±33.1	73.9±20.1	*	*
100	15.68	6-Methyl-3-(1-methylethyl)-7-oxabicyclo[4.1.0]heptan-2-one	506.48± 186.4	*	*	*
101	15.71	2-Caren-10-al	211.1± 13.9	*	*	*
102	15.79	4-(1-Methylethyl)-benzenemethanol	215.1±33.7	*	*	*
103	15.80	*p*-Cymen-7-ol	*	46.9±14.2	*	*
104	15.83	Tridecane	151.6±4.5	*	8.86±1.7	7.6±2.4
105	15.91	Carvacrol	84.9±56.1	68.1±9.2	*	*
106	16.03	7-Methyl-3-octen-2-one	2542.3±563.7	542.7±66.8	*	*
107	16.18	2-Acetylcyclopentanone	224.5±18.5	*	*	*
108	16.41	3-*oxo*-*p*-Menth-1-en-7-al	211.8±17.5	*	*	*
109	16.45	δ-Elemene	329.0±32.6	393.1±112.2	513.3±176.4	482.8±177.5
110	16.70	*E*-Isoeugenol	2416.0±239.3	*	*	67.3±6.2
111	16.77	Amorpha-4,7(11)-diene	*	*	93.0±1.5	*
112	16.85	*trans*-α-Bergamotene	*	*	88.3±1.5	*
113	16.89	*Z*-Isoeugenol	311.8±34.7	*	*	*
114	17.00	α-Copaene	380.4±12.2	89.4±4.4	113.4±8.0	58.1±4.0
115	17.06	Germacrene A	*	67.2±1.9	*	53.1±2.8
116	17.14	β-Bourbonene	175.8±4.8	69.8±13.5	*	54.74±3.97
117	17.19	Tetradecane	301.5±15.8	*	149.2±23.2	*
118	17.19	β-Elemene	*	145.9±6.3	*	117.1±27.6
119	17.40	*trans*-4-Hydroxy-3-methyl-6-(1-methylethyl)-2-cyclohexen-1-one	158.5±8.3	*	*	*
120	17.43	*Z*-Caryophyllene	142.1±5.3	85.2±9.6	84.1±3.4	*
121	17.48	α-Cedrene	*	*	96.5±5.5	*
122	17.48	α-Funebrene	*	78.9±9.2	*	49.5±1.1
123	17.62	*E*-Caryophyllene	2346.3±128.1	1533.6±231.8	1608.5±677.4	1471.9±430.8

124	17.68	Germacrene B	*	97.6±12.3	*	152.8±27.5
125	17.69	Bicyclogermacrene	*	*	184.2±27.4	*
126	17.72	*β*-Copaene	212.2±14.5	*	*	*
127	17.88	6,9-Guaiadiene	181.5±12.1	113.0±7.3	139.6±20.9	97.8±14.7
128	17.91	9-epi-(*E*)-Caryophyllene	*	*	*	57.4±0.3
129	17.93	*α*-Eeocallitropsene	*	*	107.7±6.3	*
130	17.96	(*E*)-*β*-Farnesene	146.4±9.9	*	*	*
131	17.96	*γ*-Amorphene	*	*	*	59.9±3.0
132	18.05	*α*-Humulene	358.9±45.4	282.3±66.0	427.8±161.4	351.7±102.4
133	18.15	cis-*α*-Bisabolene	*	*	*	147.7±3.4
134	18.17	(*Z*)-*α*-Bisabolene	*	*	100.7±4.4	*
135	18.26	*γ*-Muurolene	*	*	108.3±10.8	*
136	18.29	1,2,4*α*,5,6,8*α*-Hexahydro-4,7-dimethyl-1-(1-methylethyl)-naphthalene	*	*	*	61.9±1.6
137	18.34	.*β*-Cubebene	*	*	101.7±6.1	*
138	18.38	Germacrene D	*	102.2±14.1	*	74.3±5.6
139	18.39	3-(1-Adamantyl)sydnone	281.5±11.8	*	*	*
140	18.40	Isoledene	*	*	114.0±5.3	69.8±4.2
141	18.46	Pentadecane	356.4±30.6	*	*	*
142	18.54	Dauca-5,8-diene	156.8±11.8	*	*	*
143	18.56	*γ*-Gurjunene	*	*	90.4±2.4	*
144	18.69	Butylated hydroxytoluene	*	54.65± 4.7	*	*
145	18.56	Premnaspirodiene	*	*	97.3±1.4	*
146	18.65	*β*-Bisabolene	628.5±63.6	*	*	68.8±5.4
147	18.82	Cadina-1(10),4-diene	154.59± 22.0	*	90.4±2.1	*
148	19.28	*allo*-Aromadendrene epoxide	152.1±43.5	*	*	*
149	19.30	Octahydro-1,4,9,9-tetramethyl-1H-3a,7-methanoazulene	*	*	88.6±2.6	*
150	19.46	(*E*)-α-Damascone	*	*	*	95.8±14.8
151	19.51	Z-Sesquilavandulol	*	*	89.9±2.0	*
152	19.65	1-[2-(Isobutyryloxy)-1-methylethyl]-2,2-dimethylpropyl-2-methylpropanoate	*	*	105.5± 1.6	*
153	19.67	Caryophyllene oxide	730.8±250.0	63.1±5.6	101.9±6.7	131.5±30.1
154	19.65	(*Z*,*E*)-2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-,	*	*	90.0±1.8	*
155	19.84	Dehydro-aromadendrane	189.7±23.6	150.4±38.9	*	*
156	19.65	Humulene epoxide II	343.3±42.8	66.9±8.9	93.3±5.0	*
157	19.92	1,5,5,8-Tetramethyl-12-oxabicyclo[9.1.0]dodeca-3,7-diene	*	*	93.3± 5.0	*
158	20.02	Germacrene D-4-ol	*	*	87.0±5.6	*
159	20.15	1,1,7-Trimethyl-4-methylenedecahydro-1H-cyclopropa[e]azulen-7-ol /spathulenol	88.2 ±12.6	*	*	*
160	20.27	Caryophylla-4(12),8(13)-dien-5-alpha-ol	105.4 ±15.0	*	*	*
161	20.64	14-Hydroxy-(*Z*)-caryophyllene	132.6 ±17.7	*	*	*
162	20.82	Amorpha-4,9-dien-2-ol	156.7±45.4	*	*	*

Figure 4.9 The calibration curves for the linear equations used in quantification of compounds identified from gas–chromatography–linked mass spectrometry (GC–MS).
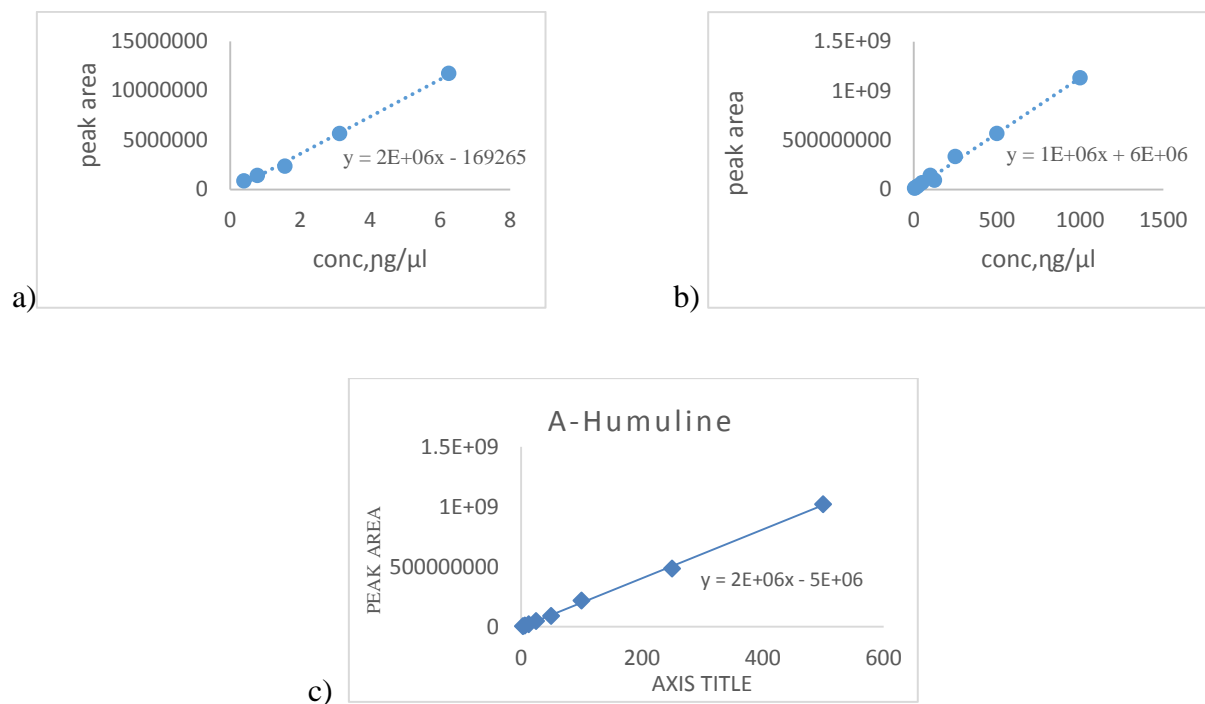

a)

$y = 2E+06x - 169265$

b)

$y = 1E+06x + 6E+06$

A-Humuline

$y = 2E+06x - 5E+06$

c)

Table 4.10 Number of *T. absoluta* migrating to either side of the olfactometer when blends were used.

Treatment	SS	NSS	SS-NSS	SS+NSS	PI	std error
control vs Blend A	28	10	-18	38	-47.3684	0.24
control vs Blend B	33	8	-25	41	-60.9756	0.40
control vs Blend C	34	4	-30	38	-78.9474	0.37
Cultivated tomato vs Blend B	40	2	-38	42	-90.4762	0.5
Wild tomato vs Blend B	31	4	-27	35	-77.1429	0.71

APPENDIX II: LETTER FOR NACOSTI RESEARCH GRANT

NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY AND INNOVATION

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Ref No.
NACOSTI/RCD/ST&I/7TH CALL/MSe/037

Date:
25th April 2016

Rapheal Njurai Miano
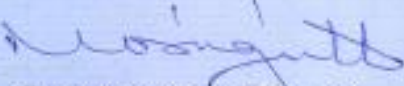Kenyatta University
P.O. Box 43844
NAIROBI

RE: SCIENCE, TECHNOLOGY AND INNOVATION RESEARCH GRANT (MSe/MA)

I'm pleased to inform you that National Commission for Science, Technology and Innovation (NACOSTI) has awarded you a research grant for your **MSe/MA research proposal**.

The NACOSTI has approved an amount of Kenya shillings *One Hundred and Seventy Thousands only* (Kshs 170,000) towards your project titled *"Behavioural responses of Tuta absoluta towards wild and cultivated tomato and characterization of the mediating semiochemical blends"* Your awarded grant will be disbursed in one instalment.

Find the enclosed *Research Grant Contract Form (NACOSTI/ST&I/CONTRACT/FORM 1C)* that should be duly completed. In the contract form, provide clearly itemized yearly budget in the format provided and attach grant acceptance letter if you take up the offer.

Your duly signed contract form and acceptance letter should be sent back to reach us not later than 6th May 2016 for our further actions.

DR. MOSES K. RUGUTT, PhD, HSC.
DIRECTOR GENERAL

cc: Vice Chancellor,
      Kenyatta University

National Commission for Science, Technology and Innovation is ISO 9001:2008 Certified