BEHAVIORAL RESPONSES OF THE MELON FLY, *ZEUGODACUS CURCUBITAE* (CONQUILLETT, 1849) (DIPTERA: TEPHRITIDAE) TO HOST PLANT VOLATILE ORGANIC COMPOUNDS

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Behavioral Responses of the Melon Fly, *Zeugodacus curcubitae* (Conquillett, 1849) (Diptera: Tephritidae) to Host Plant Volatile Organic Compounds

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DECLARATION

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

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DEDICATION

I would dedicate this thesis to my entire family and friends for the support they gave me throughout the study.

I also dedicate this thesis to my late parents Irene Waithera Kariuki and Dominic Njuguna Wamae. May their souls rest in peace.

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ACRONYMS AND ABBREVIATIONS

ANOVA	Analysis of Variance
ARCU	Animal Rearing and Containment Unit
BCED	Behavioral and Chemical Ecology Department
BC	Biological Control
CC	Capillary Column
FID	Flame Ionization Detector
GC-EAD	Gas Chromatography-Electroantennographic Detection
GC-MS	Gas Chromatography-Mass Spectrometry
ICIPE	International Centre of Insect Physiology and Ecology
IPM	Integrated Pest Management
MM	Moneymaker
MSD	Mass Selective Detector
RT	Retention Time
SIT	Sterile Insect Technique
VOCs	Volatile Organic Compounds

ABSTRACT

The Melon fly, *Zeugodacus cucurbitae* (Coquillett, 1849) (Diptera: Tephritidae), is a major pest of Cucurbitaceae but relevant field observations suggest that Solanaceous plants such as tomato have also become a major host of the pest. Solanaceous plants are highly susceptible to *Z. curcubitae* damage which may range from 30-100% globally depending on the season. Management of this pest in the past has focused mainly on the application of synthetic chemical insecticides which have resulted in negative effects on the environment and non-target beneficial organisms. Non-chemical control options such as fruit bagging are also employed but are labor intensive and/or expensive to small scale farmers. The aim of this study therefore was to compare attraction levels of *Z. cucurbitae* toward tomato varieties and compare volatile organic compounds (VOCs) produced by most attractive tomato variety with those of the main host, cucumber in order to understand the chemical basis of host shift in the pest. Behavioral responses of sexually mature and immature male and female *Z. curcubitae* to VOCs from three tomato varieties *viz.* Moneymaker (MM), Anna F1and Cal-J were investigated using a dual choice olfactometer. Experimental insects (immature and mature male and female *Z. curcubitae*) and plants (vegetative and flowering Moneymaker, Anna F1, and Cal-J tomato varieties and Ashley Cucumber) were used. Volatiles were collected from the potted plants using super-Q, eluted using nitrogen gas under ice, and subsequently identified based on their mass spectral data and authentic standards using a Gas Chromatography- Mass spectrometer (GC-MS) with helium as a carrier gas. Antennal responses of immature and mature male and female flies to host plant VOCs were evaluated using Gas Chromatography-Electroantennographic Detection (GC-EAD). Results from olfactometer assays showed that both sexes of immature and mature *Z. curcubitae* were attracted to all varieties of tomato with Cal J being the most attractive in pairwise comparisons. The results further showed that there was no significant difference in attraction of *Z. cucurbitae* to tomato (Cal J) and cucumber. The results for GC-MS analysis showed similarities among VOCs released by the three tomato varieties and cucumber (variety Ashley). About 11 electro physiologically active compounds from the three tomato varieties were revealed in the GC-EAD results. A comparison of cucumber and Cal J tomato variety revealed seven active compounds which were among the shared VOCs. The results suggest that there exists host plant variety discrimination in attraction hence odor perception is the key for selection of most suitable host plant variety. Results further showed qualitative and quantitative differences among VOCs released by Anna F1, Cal-J and MM tomato varieties in vegetative and flowering stages of growth. This suggests that Cal J tomato variety can be highly susceptible to *Z. cucurbitae* infestation than the other two varieties in monoculture farming of the three tomato varieties. In conclusion, shared volatiles between tomato plant (Solanaceous) and Ashley cucumber plants (Cucurbitaceous) have made tomato plant become major hosts of *Z. cucurbitae*. A similarity of EAD active compounds among tomato and cucumber plant profiles may explain the preference of *Z. cucurbitae* to tomato plants.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Plant volatiles are of strong ecological importance shaping behavioral responses in insects (Schoonhoven *et al.,* 2005). They provide important cues to insect species for locating food sources, finding suitable oviposition sites, Facilitate mate finding and also modify mate selection strategy. In addition, many studies have revealed that polyphagous fruit flies orient to different plants by using odors that are shared by the hosts (Seyoum *et al.,* 2014).

The melon fly, *Zeugodacus cucurbitae* (Coquillett, 1849) is an economically important pest of horticultural crops in Africa, attacking a wide range of fruits and vegetables, and causing losses of 30% to 100%, depending upon the season. Its polyphagous nature is demonstrated by its ability to complete its life cycle on several host plants belonging to different families that reflect the presence of a particular attractants (Weems *et al.,* 2015) Ovipositing females of the *Z. cucurbitae* attack host plants and lay up to 300 eggs in flowers, stems and leaf stalks, with developing larvae feeding and causing damage to plant tissues (Lanjar *et al.,* 2013). The damaged tissues serve as entry points for opportunistic microorganism infection leading to additional damage (Sulaeha *et al.,* 2017). Significant efforts have been made in the past to control the *Z. cucurbitae* and other damaging fruit flies using integrated management approaches. Examples of these approaches include fruit bagging, field sanitation, host plant resistance, use of soft insecticides and traps baited with protein and semiochemical lures that target males (Klungness *et al.,* 2005; Prokopy, 2004) . Semiochemical baits have been attempted to target females for example, a previous study showed that freshly sliced host fruit odors play an important role in attracting females in cage experiments (Miller *et al.,* 2004). These experiments demonstrated that odors released by the cucurbitaceous fruits

cucumber, zucchini, bitter melon, kabocha, cantaloupe and ivy gourd attracted the *Z. cucurbitae*, with cucumber and cantaloupe fruit odors being more attractive than tomato fruit odors to females. This study also showed that female attraction was stage-dependent, with protein-fed females more responsive than protein-deprived females to fruit odor. However, in this study, the volatiles mediating attraction were not identified. Another study on the melon fruit fly focused on fresh and aged puréed cucumber fruit odors and identified several candidate attractive blends comprising of the compounds (*E,Z*)-2,6-nonadienal, (*E*)-2-nonenal, (*Z*)-6-nonenal, nonanal, (*Z*)-6-nonen-1-ol, 1-nonanol, (*E*)-2-octenal, hexanal, 1-hexanol, acetic acid and 1-octen-3-ol. In an outdoor rotating olfactometer, McPhail traps baited with a 9-component blend derived from these compounds attracted predominantly females(Siderhurst, M. S.; Jang, 2010). A more recent study using a blend comprising the seven compounds (*Z*)-6-nonenal, (*Z*)-6-nonen-1-ol, 1-octen-3-ol, (*E,Z*)-2,6-nonadienal, (*E*)-2-nonenal, hexanal, and 1-hexanol loaded in PVC plugs or glass capillaries was found to be effective in trapping the *Z. cucurbitae* (Jang *et al.*, 2017). Surprisingly, the role of host plant foliar and floral volatiles in attracting females of the *Z. cucurbitae* has not been reported.

Although it's preferred hosts are both cultivated and wild cucurbitaceous plants, in this decade, however, the *Z. cucurbitae* has emerged as one of the most devastating pests of the solanaceous tomato plant, *Solanum lycopersicum* Mill in eastern Africa (Weems *et al.*, 2015). It is well known that biological and environmental factors drive the host range expansion in insect species, transforming some species to become major pests of less preferred hosts (Tallamy., 1999) In this context, the plant chemistry due to genetic manipulation and biotic and abiotic stressors, could alter both the quality and quantity of host plant volatiles, as well as olfactory responses of pests and parasitoids associated with the host plant (Becerra., 1997; Berenbaum., 1990). Also, the presence and extent of cultivation of congeneric plants in the landscape can also contribute to enhancing the pest status of an insect (Cock *et al.*, 2013). Given this scenario, it is therefore important to understand the chemical basis of the interaction between the *Z. cucurbitae*; cucumber and tomato host plants. Knowledge of this interaction will likely contribute to the

development of additional kairomonal lures for use in surveillance of both sexes of *Z. cucurbitae* during their early stages of establishment (Fombong *et al.*, 2016).

Although it has been postulated that plant odors are responsible for *Z. cucurbitae* interactions with cucumber (Siderhurst and Jang., 2010) and tomato (Pinero *et al.*, 2006), limited attempts have been made to identify the specific active plant volatiles attractive to *Z. cucurbitae*. Detailed understanding of the chemical ecology of the pest in question before applying that knowledge to pest management is important (Morrison *et al.*, 2018). In the current study, we investigated the olfactory basis of the interaction between *Z. cucurbitae* and tomato plants, and compared this interaction with that involving its preferred natural host plant cucumber. Specifically, we used electrophysiological, chemical and behavioral analyses to identify the chemicals mediating the interactions

1.2 Statement of the problem

Tomato production in Kenya has increased considerably in the recent past with greenhouse production being adopted for both export and local consumption. But recently in Kenya, *Z. cucurbitae* have been observed to be highly attracted to tomatoes even in the presence of their major host species like cucumber for oviposition hence posing threat to its production and utilization. This has caused considerable damage of economic importance to this crop during its early stages of growth particularly to small scale farmers who rely on agriculture for their livelihood (Dhillon *et al*., 2005).

Farmers have relied on chemical pesticides to control pests of tomatoes which have resulted in environmental damage, pest resurgence, and development of resistance to pesticides, and lethal effects on non-target beneficial organisms (Bokonon-Ganta *et al*., 2007). Using chemical pesticides such as Dipterex 80 SP and Imidacloprid for the control of *Z. curcubitae* in tomatoes is increasingly inaccessible to farmers especially in developing countries like Kenya due to the high cost and unavailability to the farmers. Other pest management strategies for example fruit bagging, augmentation of bio-

control agents, collection and destruction of infested produce are used but are labour intensive and also expensive to small scale farmers. However, use of semiochemicals as attractants in traps is not only effective and environmental friendly, but also highly specific hence have no effect on non-target beneficial organisms (Klungness *et al*., 2005). Identification of volatile organic compounds attractive to *Z. cucurbitae* will provide valuable information on development of effective semiochemicals attractants in the management of *Z. cucurbitae*.

1.3 Justification of the study

Vitamin deficiency and malnutrition related problems in urban and rural populations have led to an increased sensitization on the need to incorporate fruits and vegetables in the diet resulting to an increase in demand and supply of cultivated fruits and vegetables (Worldbank, 2013). Tomato being one of the most important sources of production and export in Kenya is threatened by *Z. curcubitae* infestation despite the presence of cucurbitaceous plants such as cucumber which is the natural host of the pest. *Z. curcubitae* causes damage of up to 100% if not checked, hence contributing to low supply and high costs of the fruits (Mkiga and Mwatawala., 2015). Vegetables and fruits producers rely heavily on the use of chemical insecticides in pest management. However, the continued and overuse of insecticides is associated with some deleterious effects that includes environmental pollution, development of resistance to insecticides, negative effect on *Z. curcubitae* natural enemies, and more importantly, increase of chemical insecticides residual levels in fruits. Therefore, alternative effective management practices have been developed for incorporation into integrated pest management of *Z. curcubitae* while in the process alleviating the problems posed by chemical pesticides. These management practices includes, mass trapping using plant host VOCs attractants to *Z. curcubitae,* use of entomopathogenic microorganisms, mass release of sterilized males using either the sterile insect technique, or lufenuron as a chemosterilant, biological control with parasitoids (Rendon *et al.,* 2006), and nematodes (Todd *et al.,* 2017) . Use of VOCs management option could be more accessible to

farmers compared to chemical pesticides and other management practices. In addition, this pest control strategy has no adverse effect to environment and or humans; it is highly selective and could also reduce the problem of pest resurgence since it is unlikely to meet pest resistance. Therefore, the aim of this study was to investigate the role played by volatile organic compound in attraction of *Z. cucurbitae* to tomato host plant varieties as the basis for its effective management.

1.4 Hypothesis

1. Host plant volatile organic compounds of tomato and cucumber do not elicit olfactory behavioral responses to *Z. cucurbitae*

2. There are no differences in the chemical composition of different tomato varieties and cucumber

3. Tomato and cucumber volatile organic compounds do not elicit antennal responses to *Z. curcubitae*

1.5 General objective

The general objective of this study was to investigate behavioral responses of the melon fly, *Zeugodacus cucurbitae* (Diptera: Tephritidae) to host plant volatile organic compounds of tomato and cucumber.

1.6 Specific objectives

1. To investigate the olfactory behavioral responses of *Z. curcubitae* to different tomato varieties and Cucumber.

2. To identify volatile organic compounds produced by different tomato varieties and Cucumber.

3. To identify volatile organic compounds in different tomato and cucumber varieties that elicits antennal responses to *Z. cucurbitae*

CHAPTER TWO

REVIEW OF LITERATURE

2.1Tomato, Solanum Lycopersicon

The tomato *Lycopersicon esculentum* (Mill) is a berry of the nightshade *Solanum lycopersicum* commonly known as tomato plant belonging to Solanaceae family. The species originated in western South America (Adam *et al.*, 2018) and is the second most important vegetable in economic importance and consumption in the world, second only to potatoes (Ibitoye *et al.*, 2009). It was introduced to Kenya in 1933 by early missionaries (Atherton and Rudich, 1986).

Tomato plant is fairly adaptable and grows well in warm conditions. It requires optimum temperatures of 20-25°C during the day and 15-17°C degrees at night, moisture of about 600mm well distributed throughout the growing season and well drained soils, light loam with high organic matter content and pH of 5-7.5 (Obeng-Ofori *et al.*, 2007).

Tomato is rich in vitamins A and C and is gaining importance since it contains lycopene which is a food component known to reduce the incidences of prostate cancer, heart and age related diseases (AVRDC., 2003). It is one of the most important local market and widely consumed vegetable in Kenya (Muendo and Tschirley, 2004). It is also an important cash crop for small-scale growers with potential for increasing incomes in rural areas, improving standards of living and creating employment opportunities (Ssejjemba, 2008). However, Tomato production in Kenya is threatened by fruit flies particularly *Z. cucurbitae* that brings considerable damage.

Tomato plant produces volatiles and fragrances that play an important role in host recognition and attraction of insects from short and long ranges for oviposition (Linn *et*

al., 2003). These complex volatile compounds which are an outcome of the plant metabolism play a role in the co-evolution between plants and insects.

The role of specific and general host fruit odors has been widely investigated in pest-tomato fruits interaction Stepwise bioassays of whitefly-tomato interaction revealed a clear preference of the white flies to tomato (money maker) plant VOCs and the role of monoterpenes (ρ-cymene, α-terpinene and α-phellandrene) were positively identified as repellant compounds in tomato-white fly interaction (Bleeker *et al.,* 2009). Piñero *et al.,* (2006) found that cucumber (*Cucumis sativus* L) odor was more attractive to female *B. cucurbitae* than odors of the papaya (*Carica papaya* L), zucchini (*Cucurbita pepo* L var. medullosa Alef) and tomato (*Lycopasicon esculentum* Mill). But in this study, VOCs emited by these plants were not identified. Solomon *et al.,* (2005) revealed that 2-butanol and 1, 4-butanediamine in Roma and Grosse lisse varieties of tomato respectively were responsible for the high oviposition preference by *Bactrocera tryoni* Froggatt. Despite all these studies that sows *Z. cucurbitae* being attracted to the host plants, little is known about the role played by tomato plant odor in attraction of *Z. cucurbitae* to tomato plant and odor discrimination among tomato plant varieties in vegetative and flowering stages of growth. This hypothesizes that tomato plant produces volatile organic compounds that are attractive to *Z. curcubitae* which will be addressed using behavioral assays, chemical analysis and antennal responses. In this study we (a) investigated the role of olfaction in location of tomato and cucumber host plant varieties by immature and mature male and female *Z. cucurbitae* (b) Investigated the *Z. cucurbitae* odor discrimination of three tomato plant varieties namely; Anna F1, Cal-J and moneymaker and Ashley cucumber. (c) Identified the volatile organic compounds of the odor of the three varieties of tomatoes and Ashley cucumber and (d) Identified the odor components the elicited the antennal responses.

The identified VOCs will increase our knowledge of *Z. curcubitae* -tomato interactions and have the potential to be used as attractants thus increasing monitoring and/or trap and kill efficiency (Webster *et al.,* 2010). In the long run, this will also add important

information to plant breeders for the use of natural tomato attractant production which could be manipulated in such a way that it alters the *Z. curcubitae* behavior and dramatically decrease the plant attractiveness.

2.1.1. Tomato production in Kenya

Tomato is a popular crop in Kenya whose fruits are used in salads, cooked as vegetables, processed in to tomato paste, Sause and puree (MOA, 2009). The total production in Kenya between the year 2015 and 2019 is shown on Table 2:1 below

Table 0.1: Tomato production in Kenya for the period of 2015-2019

Year	Area (Ha)	Production (Mt)
2015	19027	402513
2016	21921	410033
2017	14595	283000
2018	15856	308467
2019	17491	356104

Tomato is a commonly used vegetable crop and is cultivated throughout the year to increase income for small scale farmers in rural areas, improve living standards and source of employment (Ssejjemba, 2008). The crop is mainly cultivated in the open fields but in the recent past, adoption of greenhouse technology is on the increase (Wachira *et al*., 2014).Tomato production in the 2012 was 397,00 MT valued at 12.8 billion shillings (HCDA, 2013) . The major tomato producing counties in Kenya

includes; Kirinyaga (13.7%), Kajiado (9.1%) and Taita Taveta (6.9%) (HCDA., 2013). Mainly, The Determinate varieties are cultivate in the open fields while Indeterminate ones in greenhouses (Odame, 2009).

2.1.2 Tomato Pests

Tomato plants are subject to infestation by wide range of pests such as sucking insects that include white fly (*Bemisia tabaci*), cotton aphid (*Aphis gossypii*), Red spider mites (*Tetranychus evansi*), thrips (*Ceratothripoides brunneus*), and the tomato russet mite (*Aculops lycopersicum*) among others. It is also attacked by the African mole cricket (*Gryllotalpa Africana*) which cuts newly transplanted seedling while the African bollworm (*Helicoverpa armigera*) attacks the ripped and pre-ripped fruits and exposing them to fungi. Leaf miner (*Liriomyza trifolii*) attacks tomato leaves causing various losses (Bonsu, 2002). Fruit flies attacks tomato plant and fruits hence one of the most threatening family of pests. They have been reported to cause considerably high tomato yield losses and are spreading to areas where they were not previously found (Boopathi *et al.,* 2017)

2.2 Volatile organic compounds

2.2.1 Host plant volatiles

Plant volatile organic compounds are products of diverse metabolic pathways but many are derived from the isoprenoid or terpenoids pathways (Sacchettini and Poulter, 1997; Degenhardt *et al.,* 2009). In many insect-plant interactions, plant derived odors have been shown to facilitate many behavioral and physiological responses that include, location of food sources, oviposition site, mates as well as nesting sites (Linn *et al.,* 2003; Bruce & Pickett, 2011). Therefore, insects herbivores olfactory cues plays a very important role in insect orientation towards and acceptance of specific hosts plants within a plant community (Bruce and Pickett, 2011).

The headspace of undamaged plants varies with genotype, phonological stage, and environmental conditions. Insect use the volatile signals that correlate with these

variations to distinguish the most suitable hosts (Bengtsson *et al.,* 2001). It is well known that biological and environmental factors drive the host range expansion in insect species, transforming some species to become major pests of less preferred hosts.(Tallamy, 1999) In this context, the plant chemistry due to genetic manipulation and biotic and abiotic stressors, could alter both the quality and quantity of host plant volatiles, as well as olfactory responses of pests and parasitoids associated with the host plant (Becerra, 1997; Berenbaum, 1990).

Brevault and Quilici (2010) reported that plant infestation by insect pests is facilitated by vegetative and flower odor that acts as short and long range host recognition cues. Even though Solanaceous plants like tomato produce a suite of terpenes that likely serve as defense agents against herbivores (Kennedy, 2003; Bleeker *et al.,* 2009; Kang *et al.,* 2010), certain plant varieties have been reported to show significant attractant towards *Z. curcubitae*. Individual host variety of plant emits its own scent that may act as Kairomone in attracting female *Z. cucurbitae* to lay eggs. White *et al.*, (2000) found that some plant species contain volatile and fragrant compounds that include 4-ally-1, 2-dimethoxybenzene (methyl eugenol), 4-(4-acetoxyphenyl)-2-butanone (Cue Lure) or closely related compounds that attract insects from short and long ranges.

2.2.2 Tomato host plant volatile organic compounds

The major compounds emitted by tomato plant *Solanum lycopersicum* (L) are terpenes and terpenoids in both undamaged and damaged leaves (Buttery and Ling, 1993). The volatiles in tomato leaves have been investigated by Carrasco *et al.,* 2015 who identified α-pinene, β-pinene, α-terpinene, γ-terpinene, β-phellandrene, α-terpinolene, α-thujene, *p*-cymene, β-caryophyllene, limonene, and α-humulene. This list was later supplemented with 2-carene, β-myrcene, α-phellandrene, hexanal, *cis*-3-hexenal, *trans*-2-hexenal, hexanol, *cis*-3-hexenol, several oxygenated terpenes and some aromatic compounds (Buttery *et al.,* 1987; Buttery and Ling, 1993; Ishida *et al.,* 1993). In damaged leaves, Ishida *et al.,* (1993) found the concentrations of C_6 volatiles of at least 10 fold being higher compared to undamaged leaves.

More than 30 volatile components were identified from fresh tomato head space (Buttery *et al.*, 1987). The volatiles associated with tomato flavor are derived from Linoleic acid (hexanal) and linolenic acid *(cis*-3-hexenal, *cis*-3-hexenol, and *trans*-2-hexenal). Other important volatile compounds in tomato fruit include phenyl acetaldehyde, 2-phenylethanol, methyl silicylate, and apocarotenoids (for example β-ionone, geranylacetone, and 6-methyl-5-hepten-2-one).

2.3 The *Zeugodacus cucurbitae* (Coquillett)

2.3.1 Scientific Classification of *Z. cucurbitae*

The *Z. cucurbitae* belongs to the domain: Eukaryota, Kingdom: Animalia, Phylum: Arthropoda, Class: Insecta, Order: Diptera, Section: Schizophora, Family: Tephritidae, Genus: *Zeugodacus*, Species: *Zeugodacus cucurbitae* (Coquillett, 1849). *Z. cucurbitae* was originally named as *Bactrocera cucurbitae* but later renamed as *Zeugodacus cucurbitae*. Other synonyms includes, *Chaetodacus cucurbitae*, *Dacus cucurbitae* and *Strumeta cucurbitae*

2.3.2 Body coloration

The *Zeugodacus cucurbitae* are slightly larger than houseflies. They measure 1/3 to 1/2 inch long with a wingspan of 1/2 to 3/5 inch. The head and eyes are dark brown. Their bodies are yellowish brown with a yellow spot above the base of the first pair of legs. A yellow stripe, with curved lines on either side, is present down the center of the back. The tip of the body furthermost from the head is yellow. Wings are patterned with a thick brown band extending along the leading edge, ending in a larger brown spot at the tip. Another thin band extends from the wing base just inside the trailing edge of each wing. A brown spot occurs near the wing margin. Abdomens are reddish yellow with darker bands on the second and third abdominal segments. Legs are yellowish. They have a similar appearance to the oriental fruit fly except for the patterned wing. Eggs hatch within 24-72 hours in to white larvae. Pupa is dull white to yellowish brown.

Adult dorsum of the thorax is reddish yellow with light yellow markings and yellowish head with black spots (Weems *et al.*, 2015).

2.3.3 Morphology

Legless larvae which are cylindrical, elongated and with narrow anterior end. The larva of the *Z. cucurbitae* is particularly distinctive in having a dark sclerotized horizontal line below the spiracular region on the caudal end, with a curved ridge on each side of it. Larva grows to a length of 7.5-11.8mm inside the host fruit. The pupa is 5-6 mm long, elliptical. The adult is 6-8 mm in length with a distinct wing pattern, long third antennal segment (Weems *et al.*, 2015)

2.3.4 Distribution and ecology of *Z. curcubitae*

The *Zeugodacus cucurbitae* (Coquillett) is believed to have originated in India sub-continent and is widely distributed in temperate, tropical, and sub-tropical regions of the world (Dhillon *et al.*,., 2005) (Plate 2:1 below).

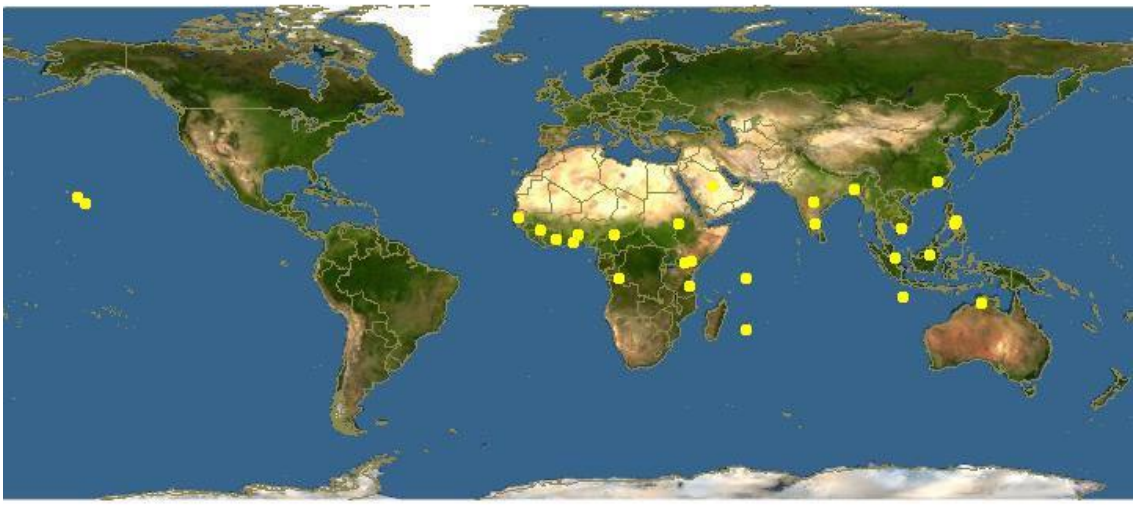


Plate 0.1: Distribution of the *Z. cucurbitae* in the World shown by the yellow marks.

It is mainly found in tropical Asia and the South Pacific islands as well as Mauritius, Re-Union, Africa, and Hawaii (De Meyer *et al.*, 2015).The invasion potential of *Z. curcubitae* is determined by development of international trade in fruits and vegetables, its likelihood to be transported and carried away from one place to another in infested farm produce, by its ability to adapt in the new environment and ability to reach its hosts to reproduce hence it has been reported as a pest in China, Bangladesh, Pakistan, Philippines, Nepal, New Guinea, Mariana, Hawaii islands and most parts of South East Asia. The *Z. curcubitae* has been in Africa and Kenya in particular for years without a clear date of introduction (White, 2006). Having originated from Asia, the invasion of alien pest species (*Z. cucurbitae*) can cause extensive, economic, and ecological damage with unpredictable negative effects on native population of crops (Ekesi, 2010)It has also been reported in, Tanzania, Egypt and Kenya among other countries (Weems and Hepner, 2001).

Temperature plays a very special role in regulating the oviposition behavior of the fruit fly adults which indicates a positive correlation of prevailing temperature with the number of ovipositing females hence increasing the fruit damage. However, humidity variation and rainfall have a non significant impact on fruit damage (Ahmad *et al.*, 2006). The optimal temperature for the development of *Z. curcubitae* is 25-28°C. Hence, exposure to warm weather results to its population build up. In addition, seasonal rise of *Z. curcubitae* population concides with the air temperature, availability and fruiting period of the hosts plants. During the severe winter months, adults of *Z. curcubitae* hide together under dried leaves of bushes and trees while in hot and dry seasons, the flies take shelter under humid and shady places and feed on honey dew of aphids infesting the fruits (Dhillon *et al.*, 2005)

2.3.5 Life cycle of *Z. cucurbitae*

The *Z. curcubitae* undergoes a complete metamorphosis thus egg, larva, pupa and adult which requires 14-17 days under favorable environmental conditions. However, the developmental periods may be extended considerably up to 28 days by cool weather.

Mating in majority of Tephritid fruit flies occurs at dusk. In general, the life cycle follows a pattern of adult mating, usually in the foliage of plants surrounding or near the host but not necessarily on the host (Raghu *et al.*, 2002). *Z. curcubitae* actively breeds when temperature falls below 32.2°C and the relative humidity ranges between 60-70%. A single mating ensures the production of fertile eggs for life, but more frequent mating appears to be required to sustain maximum fertility (Parmet, 1999).

Oviposition occurs about 10 days after emergency and continues at intervals. Females have slender pointed ovipositors used to lay up to 300 slender and white eggs under the skin of the host stems, flowers, leaves and fruit in natural conditions. Olfactory and visual cues are involved in the location of a suitable host by gravid females seeking for oviposition. Females then explore it thoroughly before selecting the actual site for oviposition. They then deposit the eggs 2-5 millimeters deep inside the host in bunches of 1-40 using their long ovipositor. The oviposition period varies from 39-95 days. A single female may lay as many as 1000 eggs (Weems and Hepner, 2001).

Eggs hatch within 24-72 hours in to larvae. The larva feed on the host and undergoes three larval instars which take 6-11 days. At the end of the third larval instar, larvae tunnel through host then emerges and drops down to the ground. It then burrows in the soil and forms a pupa. The pupation usually takes place on the ground inside the upper layer of soil (Mkiga and Mwatawala, 2015). During warm weather the pupal stage lasts 9-11 days and develops to adult which then emerges from the soil. Adult emergency occur around morning and are controlled by light and temperature. There are 8-10 generations in a year (Weems and Hepner, 2001).

2.3.6 Host plants of *Z. curcubitae*

Insect pests use plant volatiles to locate their hosts (Bruce *et al.*, 2005). *Zeugodacus curcubitae* is highly polyphagous hence has been reported to be attracted to bitter gourd (*Momordica charantia*), Musk melon (*Cucumis melo*), Snap melon (*Cucurbita melo var. momordica*), Snake gourd (*Trichosanthes anguina*), pumpkin (*Cucurbita maxima* Duchesne), and tomato (*Lycopersicon esculentum* Mill) (Weems and Hepner, 2001). It

has also been observed feeding on the flowers of Chinese bananas (*Ensete lasiocarpum*), juice exuding from sweet corn (*Zea mays var saccharata*), and sunflower (*Helianthus annuss*) among others.

Zeugodacus cucurbitae is a major pest of beans, bitter lemon, winter melon, cucumbers, eggplant, green beans, hyotan, luffa, peppers, squashes, togan, water melon, and zucchini. Among these hosts, eggplant and tomato are considered as occasional hosts. However, the two might be considered as equivalent hosts like other hosts of the family Cucurbitaceae (Humayra *et al.*, 2010).

2.3.7 Economic importance of *Z. curcubitae*

Tephritid fruit flies are recognized worldwide as the most important threat to the horticultural industry. Cucurbits and Solanaceaes are infested by several insect pests which are considered to be significant obstacle for its economic development. Among them, *Zeugodacus curcubitae* (Coquillett) is the major pest responsible for considerable damage (Dhillon *et al.*, 2005).

The *Z. curcubitae* is a polyphagous fruit fly infesting up to 125 plant species most of them belonging to Cucurbitaceae and Solanaceae with loses ranging from 30-100% worldwide having been reported depending on the season with dry season recording the most damage (Dhillon *et al.*, 2005). *Zeugodacus curcubitae* females cause direct losses to fruits through oviposition under the skin of fruits and succulent stems hence making them unfit for human consumption. Females have very high egg laying potential, superior mobility and dispersive power, and polyphagy hence a single female can destroy large number of fruits in her life time (Weems *et al.*, 2015). At times, the eggs are laid in the corolla of the flower and the larva feeds on the flowers hence affecting the reproduction of the host plant and consequent production of fruits resulting in the reduction of fruit yield (Lanjar *et al.*, 2013). The fruits attacked in early stages of their development fail to develop and drop or rot on the plant due to the action of saprophytic organisms like fungi and bacteria (Gleason and Edmunds., 2006). If the infested fruits

do not rotten, they deform due to larval feeding galleries and become unfit for human consumption (Nasiruddin *et al.*, 2013).

A few larvae have been observed to feed on the stems hence damaging the plant transport tissues thus xylem and phloem. This interferes with the translocation of synthesized food materials, transportation of water and mineral salts in the host plant resulting to poor growth that culminates to economic loss. Exporting farmers incur additional losses if their agricultural produce is rejected by European markets from countries where *Z. curcubitae* management practices are undertaken as quarantine measure to control its spread. According to the governments in these countries, in the event of infection, the economic damage caused by invasive insect pests is immense partly due to lack of their natural enemies to an extent of endangering local agricultural production (Enkerlin & Mumford., 1997). It is therefore necessary to device means to reduce damage of this pest without adverse effect on the agro-ecosystem.

2.3.8 Management of *Z. cucurbitae*

Significant efforts have been made in the past to control the *Z. cucurbitae* and other damaging fruit flies using integrated management approaches. Examples of these approaches include fruit bagging, field sanitation, host plant resistance, use of soft insecticides and traps baited with protein and semiochemical lures that target males (Klungness *et al.*, 2005; Prokopy., 2004). The management of *Z. curcubitae* has been difficult because of its internal feeding behavior, high population growth due to short life cycle, extremely broad host ranges including many non-economically important plants, the increase of abandoned orchards, and the effects of global warming. Several environmentally sound control strategies have been developed in *Z. curcubitae* management. Chemical control of *Z. curcubitae* is relatively ineffective because of the development of resistance and concealed feeding behavior in its larval stage. The damage is much more pronounced especially to the three highly producing varieties of tomato (money maker, Cal-J and Anna F1) during there early stages of growth. These

three varieties are widely grown in Kenya and hence the subject of investigation to this study.

Chemical control in *Z. curcubitae* is inappropriate due to possible changes of insecticidal residual toxicity in fruits and vegetables. Hence most of the efforts in *Z. curcubitae* management have focused on mature adult through the use of attractant volatile organic compounds in traps (McQuate and Vargas, 2007).

2.3.9 Local area integrated pest management

The management of *Z. curcubitae* is utilized with an aim to suppress its population rather than eradicating it (Jang *et al.,* 2017). Under this management, a number of methods are used thus cultural, biological, use of plant resistance, use of traps, legal approaches and use of pesticides That suppresses pest population levels below those causing economic injury (Flint, 2012).

Gleason and Edmunds., (2006) suggested using chemical, cultural, biological or legal approaches are effective in *Z. cucurbitae* management but the component of these methods are not always feasible and the growers do not use (Akhtaruzzaman, 1999). The current trend in crop production are towards reducing the use of pesticides by appling multiple control tactics (Raini *et al.,* 2005). The approaches for the control of pests in Kenya inlude biological, chemical cultural and physical methods (Waiganjo *et al.,* 2006)

2.3.10 Chemical control of *Z. cucurbitae*

Control of *Z. curcubitae* is dependent upon the insecticides application of various nature notably among these are dipterex, imidacloprid, triazophos, and neem products. In most countries where *Z. cucurbitae* is present, farmers frequently spray broad-spectrum insecticides to control the pest. Fumigation with methyl bromide has been widely used as a regulatory control to kill flies and allow movement of produce from within quarantine areas to locations outside the quarantine boundaries. Direct foliar spray of insecticides fails to control this pest as the larva develops inside the fruit.

Chemical control of *Z. curcubitae* is often successful but can be hazardous and toxic to human beings and environment (Bokonon-Ganta *et al.*, 2007). It has been estimated that the world wide damage caused by pesticides reaches $100 billion annually due to high toxicity, non-biodegradable properties of insecticides, and the residues in fruits, soils, water sources and crops that affect public health (Akhtar *et al.*, 2009). Insects may also develop resistance to pesticides due to continuous application.

In order to reduce the excessive use of pesticides in tomato fields, environmentally sound control strategies have been developed that includes cultural control measures (crop rotation, selective removal and destruction of infested plant material) (Korycinska and Moran, 2009) the use of natural enemies (Parasitoids, predators, entomopathogens, and nematodes) (Todd *et al.*, 2017) and resistant varieties of tomatoes (Gil, 2015)

2.3.11 Physical and Cultural control of *Z. cucurbitae*

Field sanitation thus picking of infested fruits, bagging of fruits and early harvesting among others are very effective control measures of this pest (Akhtaruzzaman, 1999). Covering of fruits by polythene bags is an effective control method of fruit fly as it has been tested in teasels gourd where the fruit fly incidence occurred in bagging of fruits (4.2%) while the highest (39.38%) was recorded in the fruits of control plots (Anonymous, 1988). But the fruit bagging is labour intensive and raises the cost of production. Sanitation within the field must be observed which involves the removal of fruits as they ripen and if they fall to the ground, they should be buried not only to kill any larvae in them but also to prevent further infestation and consequent survival of the pest. Early harvesting of uninfected tomatoes reduces the rate of infestation. Monoculture agro ecosystem with low diversity may be more susceptible to pests outbreaks hence reliance on diverse planting, provide a range of natural enemies that are supported by these plants, and associated crop management strategies can in some places help maintain pest populations below economic thresh holds (Altieri and Nicholls, 2004).

In the management of *Z. curcubitae*, the level of pest infestation is monitored to establish the pest status hence aid in evaluation and use of the best strategies. It is important for the farmer to be familiar with its life cycle and other hosts plants and determine when these plants are fruiting. If possible, crop rotation should be practiced so that crops do not fruit when other hosts are fruiting.

2.3.12 Biological control of *Z. cucurbitae*

Biological control involves the use of living organism to suppress the population density of a specific organism, making it less abundant or less damaging (Eilenberg *et al.*, 2001). It is increasingly viewed as a safe and economical means of fruit fly control that includes use of predators, parasitoids, nematodes, and entomopathogens (viruses, bacteria, and fungi) (Van Driesche and Bellows, 1996). Preditors such as chicken, guinnea hens and wild birds have been seen digging through the infested fruits for larvae. Parasites can lay their eggs in the egg, larva, or pupa of a developing *Z. curcubitae*. During the location finding process, numerous studies have shown that female parasitoids respond to various stimuli from the plant, the host population, the host itself or their interractions: those stimuli are mainly volatile semiochemicals, though visual and/or mechanical cues are also used (Quilici and Rousse, 2012). The egg parasitoid *Fopius arisanus* and the larval parasitoid *Psyttalia fletcheri* (Silvestri) are fruit fly parasitoids introduced in Hawawii to parasitize *Z. curcubitae* in which they did not harm any other species (Bautista *et al.*, 2004). Use of nematodes such as Mexican strain nematode *Steinernema carpocapsae* (weiser) is an important method in *Z. curcubitae* management (Urbaneja *et al.*, 2012). Entomopathogenic fungi such as *Metarhizium anisopliae* and *Beauveria bassiana* have been used in fruit fly suppression in Kenya.

2.3.13 Sterile insect technique

The sterile insect technique (SIT) as a method of pest control using area-wide inundative release of sterile insects to reduce fertility of a field population of the same species. The technique involves releasing large number of sterile males to a population in order to increase chances of their mating with wild females. The technique is highly

expensive hence not widely used. In Africa for example, South Africa has limited application of Sterile male technique mainly at Natal province where it is used in suppresion and eradication of *Ceratitis capitata* and *Ceratitis rosa*. In Japan, the SIT was employed to eradicate *Z. curcubitae* in Okinawa and all of Japan's south-western islands (Ito *et al.,* 2003). However, this method relies on the ability to rear millions of flies for release, is species specific and requires huge investiments.

2.3.14 Use of resistant varieties of tomato

Host plan resistant to *Z. cucurbitae* is an important component in integrated pest management programs. Cultivation of host plants resistant to insect attack reduces the economic loss for example; many growers have found that small tomato varieties can be harvested with less infestation than large varieties (Yang *et al.,* 1994). Varieties with thicker of tougher skins prevent the *Z. cucurbitae* from being able to oviposit and infest the fruit. Resistant varieties can be developed by transferring resistant genes in the cultivated genotypes from wild relatives resistant to *Z. cucurbitae* through wide hybridization (Dhillon *et al.,* 2005)

2.3.15 Legal approaches of controlling *Z. cucurbitae*

The import and export of infested plants material from one area or country to other non-infested places is the major model of the spread of Tephritids (Dhillon *et al.,* 2005). Phytosanitary quarantines are imposed on wide varieties of plants and plant products as a means to deter introductions of *Z. cucurbitae*. The insect receives a lethal treatment inducing very high mortality while the plant tissue is minimally affected. Hot treatment at 40ºC for 24 hours reduce the estimated surviving population by 99.5-100% (Yang *et al.,* 1994). Quarantine implementation is associated with undesirable effects including restriction of commodity availability, increased costs, and decreased commodity quality.

2.3.16 Semiochemical control of *Z. cucurbitae*

Behavioral control involves attraction of flies to chemical lure and phago-stimulatory food attractant. The attraction is enhanced by use of traps with specific visual cues (yellow, green and red) (Pinero *et al.,* 2006). Yellow spheres or sticky panels are also

used to monitor and reduce population of fruit flies in tomato field which should be checked regularly. Mass trapping with protein baits for male and female *Z. cucurbitae* or with chemical attractant are used in the management of *Z. cucurbitae* (Barry *et al.*, 2006). Protein bait acts as a food attractant and its effectiveness relies on the fact that immature adult flies need a protein source to become sexually mature. Nu-lure® (a yeast extract) and Staley's Fly Bait® (a corn extract) hydrolyzed proteins are therefore effective attractants in traps for monitoring and mass trapping of *Z. curcubitae* (Piñero *et al.*, 2006b). Parapheromone lures are highly volatile and longer lasting than protein baits. They include cue-lure and trimedlure that attracts male flies which has been used for monitoring and mass trapping of *Z. curcubitae* in bitter gourd. Traps baited with cue-lure are used in detection programs world-wide (Gonzalez and Troncoso, 2007). Earlier research suggested that a chemically similar compound (raspberry ketone formate) is more attractive than cue-lure and thus might improve surveillance efforts (Sulaeha *et al.*, 2017).

Use of protein- bait-insecticide mixture on to nearby non-crop plants for example protein hydrolysate compound such as Nu-lure® or Staley's® bait can be combined with an insecticide. Historically, protein bait sprays and the highly attractive male kairomone lures methyl eugenol (4-allyl-1,2-dimethoxybenzene-carboxylate) and cue-lure 4-(ρ-acetoxyphenyl)-2-butanone have been used in conjunction with organophosphate insecticides in area wide fruit fly campaigns (Vargas *et al.*, 2014).

Although, several management options such as hydrolyzed protein spray, para-pheromone trap, spraying of ailanthus and cashew leaf extracts, neem products, bagging of fruits, field sanitation, food baits, and spray of chemical insecticides have been in use for the management of *Z. curcubitae*, some of them either fail to control the pest and /or are uneconomical and hazardous to non-target organism and the environment (Iyaniwura, 1991).

CHAPTER THREE

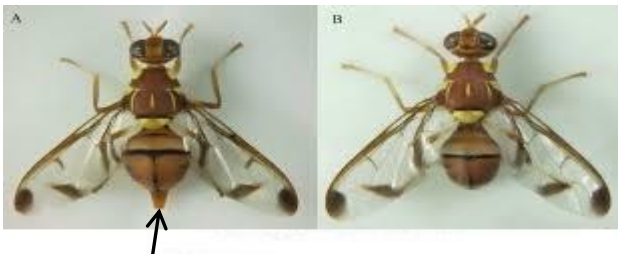
MATERIALS AND METHODS

3.1 Study site

The project was carried out at the International Centre of Insect Physiology and Ecology (*icipe*) located at Duduville Campus, Nairobi. This Campus is at S01°17'; E36°49'. The altitude is 1661m above sea level and receives an average rainfall amount of 950mm per year with two main rain seasons; the short rains between October and December and the long rains between March and June. The temperature ranges between 16°C and 28°C. Bioassays, collection and analysis of volatiles were done at this campus in the laboratories of Behavioral and Chemical Ecology Unit (BCEU).

3.2 Experimental design

Ten mature female *Z. curcubitae* and males were randomly selected from a cage with an already reproducing population of both sexes (16-20 days old). They were distinguished since females have long ovipositor at posterior part of the abdomen as shown on Plate 3.1A below while males have not as shown on Plate 3.1 B below. The same was repeated for the immature female and immature male adults (2-5 days old; before they had reached sexual maturity age of 8-10 days).



Ovipositor

Plate 0.1: Female (A) and Male (B)

One group at a time was assayed against each variety of tomato plant (Anna F1, Cal-J and Moneymaker) and a variety of cucumber at vegetative and flowering stages of growth against blank control.

Similar experiments were repeated when the three varieties of tomatoes were compared thus two different plants at a time (without control). The most attractive tomato variety (Cal-J) was compared with 'Ashley' cucumber (The preferred host of *Z. cucurbitae*). Flies that were found at within 25 cm of both ends of the olfactometer (Figure 3:1) at the end of ten minutes were regarded to have made a positive response to either control or test odors. The number of *Z. cucurbitae* that were found at the middle region (25 cm from either sides of release hole) at the end of the 10 min were regarded as non-respondents and therefore not included in the data analysis.

At the end of each experiment, the used *Z. cucurbitae* were discarded and a new batch of 10 flies selected for the subsequent experiment. Between experiments, air was passed through the chamber for one minute (without potted plant) to remove any volatile residues. After testing 10 batches of *Z. cucurbitae*, the olfactometer was rinsed with acetone (Sigma-Aldrich, Germany). The experiments were replicated five times with different potted plants used in each replication.

3.3 Sampling design

Sampling of ten immature and mature male and female *Z. cucurbitae* for each bioassay and electroantennographic detention experiments were done using randomized sampling design. Systematic sampling technique was used to obtain a total of five individuals from each variety of tomato and cucumber plant populations for bioassay experiments, and the other group of five for collection of volatiles in each stage of the plant growth (vegetative and flowering stages) to be used in GC/MC and GC/EAG analysis.

3.4 Rearing of *Z. cucurbitae*

The parent colony of *Z. curcubitae* was obtained from a colony maintained at the Animal Rearing and Containment Unit (ARCU) of the International Centre of Insect Physiology and Ecology (*icipe*), *Duduville* campus, Nairobi Kenya. ARCU colony was established from wild *Z. cucurbitae* collected from infested tomato fruits at Chala (03° 15.371S, 037° 44.604 E, elevation 924m) and Mbomeni (03° 26.301 S, 037° 40.835 E, Elevation 736 m) divisions inTaita-Taveta County, Kenya in January 2014.

Zeugodacus cucurbitae rearing was carried out as previously described (Kachigamba *et al.*, 2012) with a slight modification in which oven bags were used to enclose the test plants instead of glass chambers. Ten ripe tomatoes fruits bought from the local farmers to serve as egg-laying substrates were placed in plastic containers for 10 days to ripen and to ensure they were free of insect larvae. Tomato fruits free of larvae were then washed with distilled water, dried with cotton cloth, and then placed in a clean Petri-dish (8 cm diameter; 1 cm height) and exposed to 80 (sex ratio 1:1) mature adult *Z. cucurbitae* (16-20 days old) in a rearing clear ventilated Perspex cage (35 cm × 30 cm × 30 cm) for 24 h to oviposit as indicated on Plate 3:1 A and B below.

Plate 0.2: *Zeugodacus cucurbitae* ovipositing on tomato substrate (A) and infested tomato (B)

The tomato fruits with eggs were then transferred into a clean sterile plastic container (20 cm long × 14 cm wide × 8 cm high) with a lid fitted with 0.5 mm diameter pore size netting material in the middle to facilitate aeration. The larvae were then allowed to

develop up to the third instar stage before being transferred into sterilized-sieved-sand for pupation as indicated on Plate 3:2 A and B respectively below.

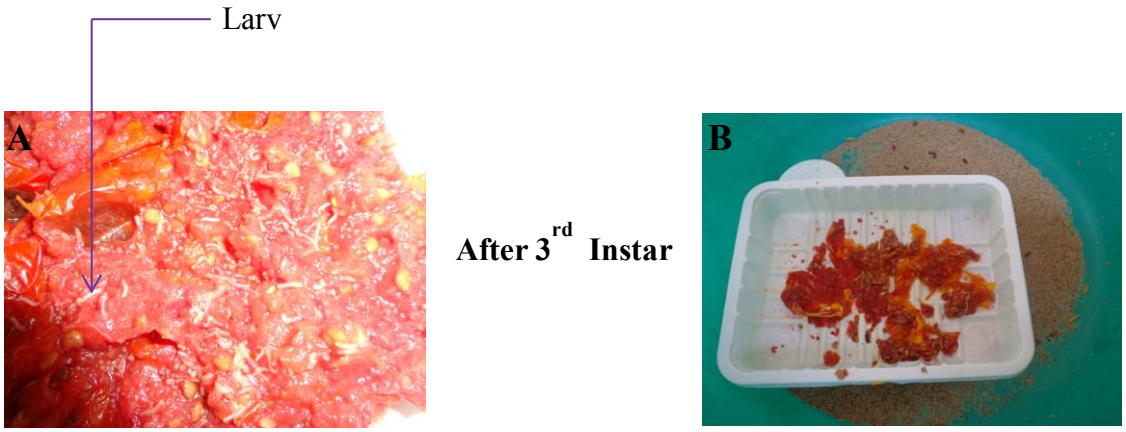


Plate 0.3: *Zeugodacus cucurbitae* larvae feeding (A) and pupation (B)

Pupae were separated from the sand through a 1 mm mesh size sieve (plate 3:3) after which they were then transferred into a holding cage until eclosion. Adults that emerged were then reared in a clear ventilated Perspex cage (35 × 30 × 30 cm) in a room maintained at 27 ± 2°C, 65± 5% RH and 12:12 h L:D) as indicated on Plate 3:3 A and Plate 3:3 B below.

Plate 0.4: Pupa in a petri dish (A) and *Z. cucurbitae* in a rearing cage (B)

They were fed on artificial diet (2:1 volumetric mixture of dry sugar and enzymatic yeast hydrolysate ultrapure; United State Biochemical, Cleveland, OH, USA) and

watered in 1cm height Petri dishes filled with pumice granules to prevent drowning. For bioassays, 3-5 day old flies were used for the immature stages and 16-20 day old for mature ones.

3.5 Tomato and Cucumber plants

The three tomato varieties used included indeterminate (Varieties that grows to a fixed mature size) Anna F and Cal-J and determinate (Varieties that continues to extend in length throughout the growing season) money maker while the cucumber variety was Ashley. They were selected based on their susceptibility to damage by *Z. cucurbitae* and widely grown in Kenya. Seeds were purchased from Simlaw Seeds Company Limited, Kenya and established separately in seedling trays (Chamak Polymers Pvt. Ltd, India) containing autoclaved fine sand for delicate seedlings and sieved farmyard manure mixed in the ratio of 2:1 and moistened with water (Plate 3:4A). The trays were kept in screen house at 26 ± 2°C temperature and 12hrs light and 12 dark (L12:D12) lighting regime to facilitate seed germination and growth. Light watering in the morning and evening each day followed and continued up to the last week in nursery (5-6 weeks) which was then slightly withheld to harden the seedlings as indicated on Plate 3:4B below.

The 5-6 weeks old seedlings of tomato and cucumber were then transplanted into a five litre planting pots (with drainage holes) filled with mixture of volcanic soil, sand and manure in the ratio of 3:2:1 (fertile draining soil) as indicated on Plate: 3:4 C below.

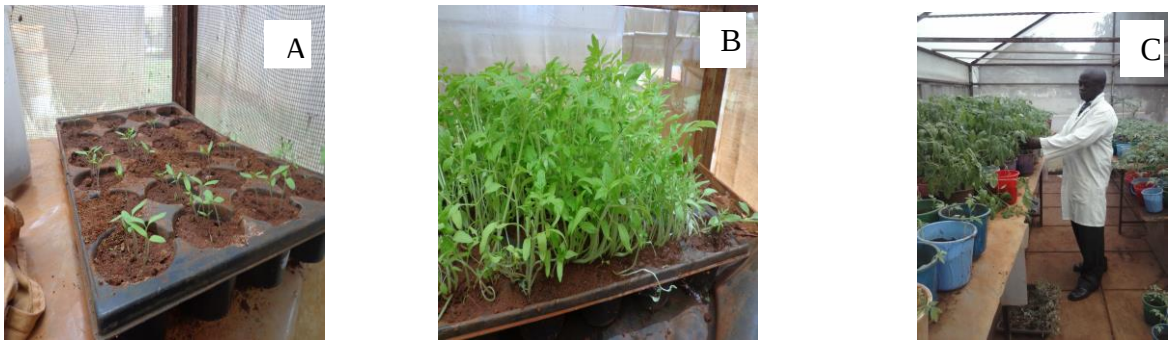


Plate 0.5: Early tomato growth on seedling tray (A), late tomato growth on a seedling tray (B) and tomato growing in pots (C).

Each seedling was staked during transplanting using stakes of approximately 12 cm in length to minimize damage of the root system at later stage. The plants were then maintained in a screen house at 26 ± 2 °C, 55 ± 5 % RH. Pests and weeds were controlled through sanitation and hand picking and the plants watered in the morning and in the evening daily. Vegetative and flowering stages of growth were used for the experiments.

3.6 Olfactometer assays for objective one

The procedure for olfactometer behavioral assays was carried out according to the protocol used by Nyasembe *et al.,* (2012) as indicated on figure 3:1 below but with a slight modification i.e. use of oven bags to enclose the test plant instead of glass chamber. Systematically selected test plants from each tomato plant variety and Ashley cucumber were transferred to the laboratory 12 hours prior to conducting bioassays to allow the plants to acclimatize. The selection of mature and immature *Z. cucurbitae* was as described in section 3.1 above.

The olfactometer was a glass chamber (30 cm × 31 cm ×100 cm) which was marked to divide it in to four equal parts (1^{st} or 4^{th} - control region, 2^{nd} and 3^{rd} - non respondent region and 1^{st} or 4^{th} - tomato odor region) such that the boundary between the second and

the third parts lied at the Centre of the release hole. One of the sides had one hole for releasing *Z. cucurbitae* in to the chamber and at the top had two holes for recovering the flies at the end of the experiment. Both ends of the chamber were connected to a square pyramidal aluminium funnel each connected by Teflon tubing to oven bags (in case of volatiles from the plants). Vegetative plants, flowering plants, were used as sources of volatiles. To avoid mixing of volatiles in the arena, it was fitted with a 14 cm × 14 cm vacuum fan (Nikko Company, Japan) at the top of the mid-section of the chamber that sucked the air plus odor out of the system at 700 ml/min. In addition, the laboratory was fitted with extraction hood that sucked the air plus the odor out allowing more fresh air to come in. Two 40W bulbs were placed above the olfactometer to uniformly illuminate the test arena as indicated on Figure 3:1 below.

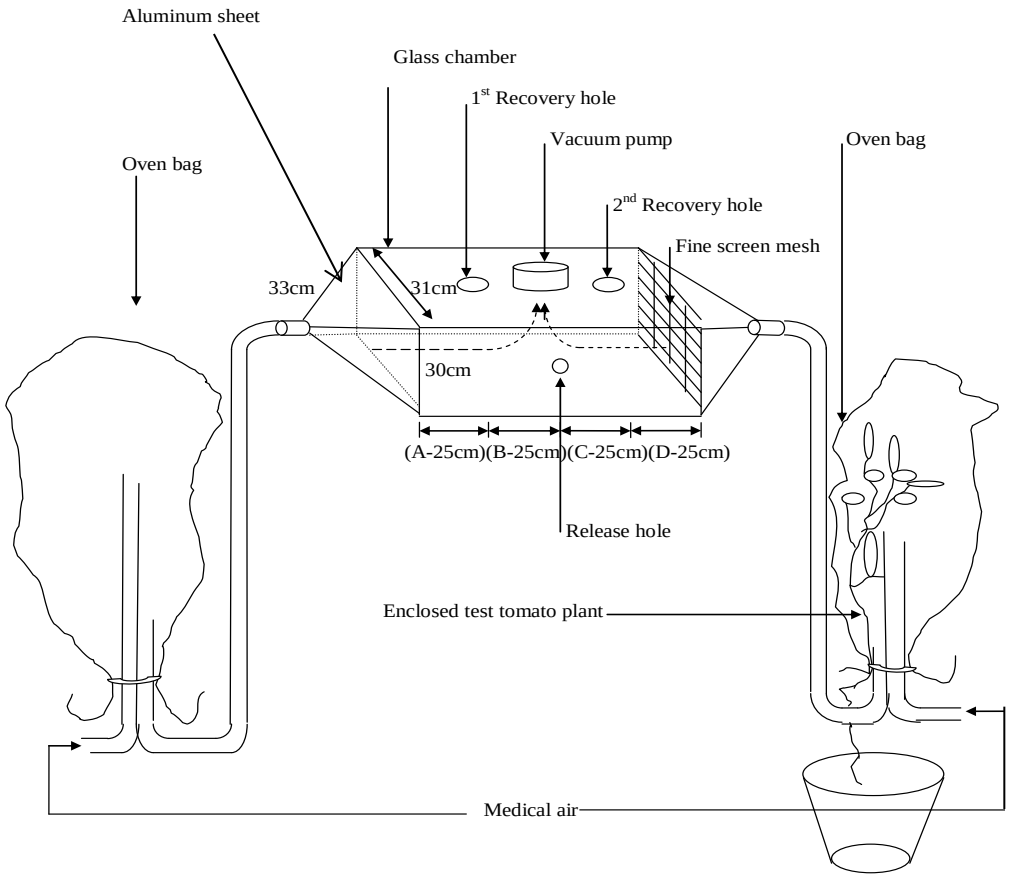


Figure 0.1: Schematic drawing of a dual choice Olfactometer (not drawn to scale) (Nyasembe *et al.*, 2012).

The assays were conducted in a laboratory under controled conditions of temperature, 27 °C and relative humidity of 70 % at 1000-1500 hrs. Compressed medical air (BOC gases, Kenya) was humidified by passing it through distilled water and then split in to two equal channels. One channel was passed through an oven bag enclosing a potted tomato plant and then in to one arm of the olfactometer at a flow rate of 350 ml/min via Teflon® tubes. The other channel was passed through a blank oven bag (control) or over another oven bag with a different potted plant in pair wise comparison assays. For each growth stage of the three tomato varieties and Ashley cucumber (vegetative and flowering), a group of 10 of each mature and immature males and females *Z. cucurbitae* was tested first against a control (clean empty oven bag) and then against another tomato

variety for pair wise comparisons among the three selected tomato varieties. Similar comparison experiments were repeated where the most attractive tomato variety (Cal-J) and the known preferred host plant of *Z. cucurbitae* Ashley cucumber were compared using the groups of 10 mature male and female *Z. cucurbitae*. The positions of the test units and control in the olfactometer arms were interchanged between two consecutive runs to prevent any positional biasness.

The *Zeugodacus cucurbitae* that occupied each of the two regions of the olfactometer (control and odor regions) at the end of ten minutes were regarded to have responded to them hence counted. The number of *Z. cucurbitae* that occupied non-respondent region (did not make a choice) at the end of 10 minutes was not included in the data analysis. At the end of each experiment, the used *Zeugodacus cucurbitae* were discarded and a new batch of ten selected for the following experiment. In-between experiments, air was passed through the olfactometer arena for 5 min without the treatments to remove any volatile residues and then cleaned with an acetone cotton swab and flushed with air again. The experiments were replicated five times on different days in a randomized complete block design.

3.7 Objective 2

3.7.1 Collection and elution of volatiles

Volatiles from the test plants were collected and eluted to be used for GC MS identification of VOCs (objective 2) and for GC EAD antennal responses (objective 3). The selected two months old (for vegetative stage) and about three months (for flowering stage)test plants were taken to the laboratory 12 h prior to start of the experiments in order for the plants to acclimatize. Volatiles released from the intact aerial parts of Anna F1, Cal-J, Moneymaker and Ashley cucumber during the vegetative and flowering stages were collected according to the protocol used by Nyasembe *et al.*, (2012), but with few modifications as indicated on Plate 3:6A below. Transparent oven bags (450 mm x 400 mm, Classic Consumer Products, Inc, Englewood, NJ, USA) were

pre-conditioned at 98°C for 12 hours. In each stage of growth, each of the four intact treatment plants was enclosed in a pre-cleaned oven bag as shown on Plate 3:5A below. A fifth oven bag with no plant (control) was included in the set-up. Each oven bag was supplied with a stream of purified and humidified air at a flow rate of 350 ml/min at room temperature. The mixture of air and volatiles in each oven bag was then sucked in to adsorbent Super-Q traps (30 mg, Analytical Research System, Gainesville, Florida, USA) for volatile collection and then out through Teflon® tubes by a vacuum pump (Vacuum Brand, MZ 2C, Wertheim, Germany). Volatiles were collected from each selected plant for six hours (from 0900 h to 1500 h). Preliminary experiments and previous studies showed that 6hrs was sufficient time to trap volatiles from intact plant head space. In each stage of plant growth, each variety was replicated five times using a different plant in each replicate. Volatiles trapped by each Super-Q filter were eluted using 100 µl dichloromethane (Analytical grade, Sigma Aldrich, St, Louis, MO, USA) under a stream of pure Nitrogen gas in to 2 ml vial. The vial was immersed in an ice bucket to prevent the loss of volatiles during elution as indicated on plate 3:5B below and then stored in a freezer (New Brunswick Scientific Freezer, U725-86G, eppendorf company, Hamburg, Germany) at -80°C until used for GC/MS and GC/EAD analysis.

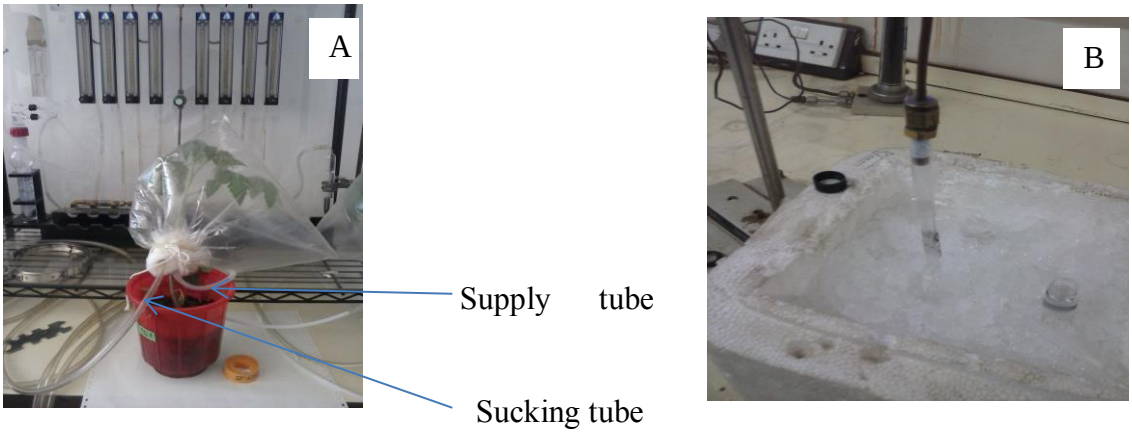


Plate 0.6: Collection (A) and Elution (B) of Plant Volatile Organic Compounds

3.7.2 Coupled Gas Chromatography-Mass spectrometer (GC-MS) analysis

Eluted volatiles were identified using an Agilent technologies series A 7890 gas chromatography (GC) coupled to a 5975C inert XL EI/CI mass spectrometer (MS), equipped with an HP-5MS column (30m in length× 250 µm internal diameter × 0.25 µm film thickness, Agilent, Palo Alto, CA). For each tomato head space volatile collected and eluted, 1µl of it was injected in to the GC/MS in splitless mode at an injector temperature of 270 °C. The GC was programmed as follows: Oven temperature held at 35 °C for 3 min, then increased at the rate of 10 °C/min to 280 °C and maintained at this temperature for 10 min for a total of 50 min. Helium was used as a carrier gas at a flow rate of 1.2 ml/min. Mass spectra were obtained using electron impact mode at 70 eV. Recording was done by a computer connected to GC and MS as shown on Plate 3:6 below.

Identification of compounds was done according to their retention time and comparison of the sample's mass spectra data with mass library data; NIST05a library (NIST 2005a), Adams2 library (Adams 1995) and chemecol library.

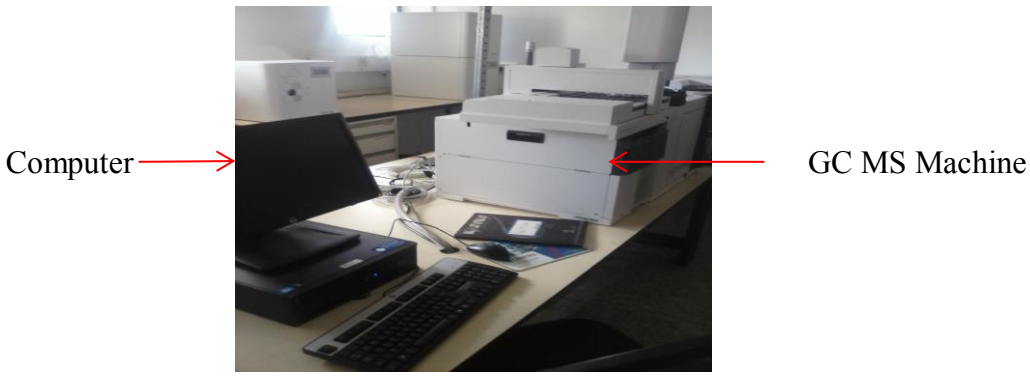


Plate 0.7: Gas Chromatography Mass Spectrometer connected to a Computer

3.7.3 Quantification of profile components

Quantification of VOCs from three tomato varieties and Ashley cucumber in vegetative and flowering stages of growth was done by use of external standards of identified monoterpene (β-phellandrene) and sesquiterpene (*α*-cedrene) compounds (Sigma®

Chemicals Co, St Louis, MO, USA). The two compounds were selected for use as external standards since they were common and abundant in all the GC MS profiles of vegetative and flowering stages of tomato and cucumber varieties in addition to elicit antennal responses in GC EAD experiments. 2,000 ng/µL stock solutions of each external standard were prepared and then serially diluted to give a range of concentrations from 0.005 to 1200 ng/µL. The preparation and running of known concentrations of external standards were done where the highest peak area generated by each standard was slightly higher than the highest peak area within retention time range of sample profile. The same was produced of slightly lower external standard peak area than the sample components within the specified retention time ranges. The GC conditions for quantitative analyses including injection operation of the standards, capillary column dimensions and oven temperature were the same as those for GC/MS.

The peak area of each component between retention time (RT) 1 and 15 minutes were compared with peak areas from the equation of the line ($y = 1E+06x + 61.536$, $R^2 = 0.9998$) generated by external standard monoterpene (β-phellandrene) of known concentrations. The same was repeated for components between 16-20 minutes RT where equation of the line ($y = 3E+06x + 7.7698$, $R^2 = 0.9998$) generated by external standard sesquiterpene (α-cedrene) was used. (Monoterpenes separated from GC column between 1-15 minutes RT while sesquiterpenes within 16-20 minutes RT)

3.8 Objective 3

3.8.1 Collection and elution of volatiles

Volatiles were collected and eluted as per objective 2

3.8.2 Coupled GC-Electroantennographic Detention (GC-EAD) analysis

The GC/EAD analysis was done to detect the physiologically active components of the four plant odors. It was done using GC coupled with Flame Ionization Detector (FID) and Electroantennographic detector (EAD) with nitrogen as a carrier gas.

Preparation of *Z. cucurbitae* antenna was done as previously described (Kugel M., 1977). Antenna from *Z. cucurbitae* was pulled off the head capsule then the scape and pedicel cut off and the flagellum inserted in to a glass micropipette containing Ringers solution. Humidified air was delivered at 1 ml/min over the mounted antenna. The microelectrodes were connected via antennal holder to a universal AC/DC amplifier in DC mode. VOCs were analyzed in a splitless mode at an injector temperature of 250°C and a split valve delay of 1 min. The oven temperature started at 35°C for 5 min and then increased to 280°C at 10°C/min and maintained at this temperature for 5 min. Column effluent was split in to 1:1 of which one part flowed to FID and the other part to the stimulus delivery tube that drained it over the antenna which was connected to EAD. The simultaneous detection by FID and EAD were recorded using a computer as shown on Plate 3:7 below.

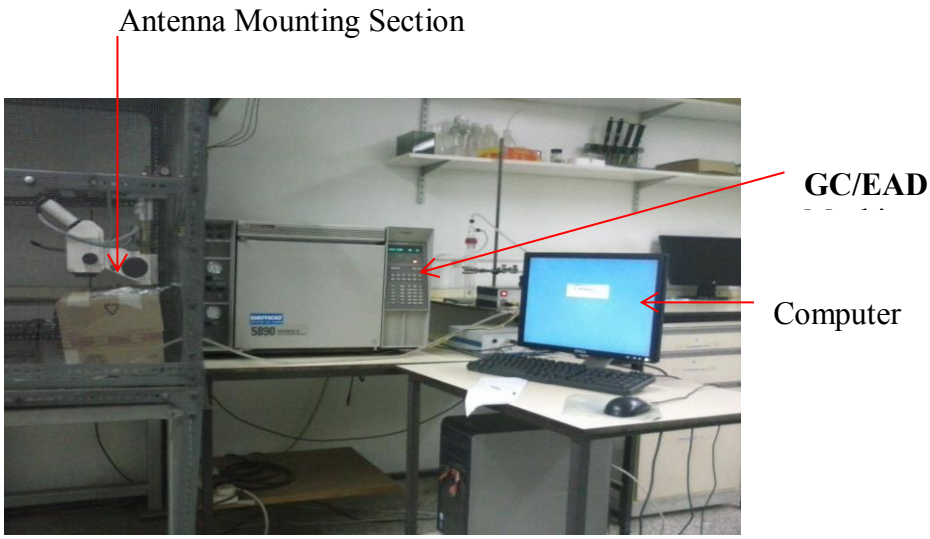


Plate 0.8: Gas Chromatography electroantennographic detector

VOCs from each variety of tomatoes were analyzed with fresh antennae of each of the four groups of the *Z. cucurbitae* (Mature and Immature males and females). While VOCs from Ashley cucumber were analyzed with antennae of mature male and female

Z. cucurbitae only. Each test was replicated three times. Identification of EAD-active components was carried out by GC/MS using the same oven conditions as described above 3.7.1.

3.9 Data analysis

The number of *Z. cucurbitae* in each arm of the olfactometer at the end of 10 min observation period was recorded and the data converted to a percentage based on the number of respondents, then used as a measure of response as previously described (Nyasembe *et al.,* 2012) from the formula

$$PR= [(SS-NSS)/ (SS+NSS)] \times 100$$

Where PR represent the percentage response, SS is the number of *Z. cucurbitae* responding to the test plant odors and NSS the number of *Z. cucurbitae* responding to the control odors (Carlsson *et al.,* 1999). The percentage response was to be zero if count numbers of *Z. cucurbitae* on the test plant and control were the same and 100 if all the flies preffered one side of olfactometer. Positive preference index shows most of the *Z. cucurbitae* responding to the test odors while the negative shows most of the them responding to the control. Percentage responses were subjected to a sample Chi-square ($\chi^{2)}$ test to examine if mature and immature males and female responses differed from zero. All statistical analysis were done at an α level of 0.05 using R software (R Core Team., 2014).

The differences in chemical composition of the samples from all of the three tomato varieties in vegetative and flowering stages of growth were analysed using principal component analysis (PCA) in which their concentrations in ng/plant/hour were subjected to logarithmic transformation. Scaling was focused on correlation among varieties where each variety score was divided by its standard deviation.

CHAPTER FOUR

RESULTS

4.1 Objective 1

4.1.1 Olfactometer assays results

In the vegetative stage, immature females were significantly more attracted to the three host tomato varieties (Anna F1: PR =62%, χ^2 =15.7209, DF=1, p<0.001; Cal-J: PR = 80%, χ^2 =24.025 DF=1, p<0.001; Moneymaker: PR =68.5%, χ^2 =5.0256, DF=1, p<0.05) than to the control as shown on Figure 4:1 **A** below. For the paired assays, there was no significant difference in attraction of immature females to Anna F1 (PR =52.40%, χ^2 =0, DF=1), Cal-J (PR =52%, χ^2 =1.561, DF=1) and moneymaker (PR =51%, χ^2 =1.481, DF=1) as indicated on Figure 4:1 **B** below.

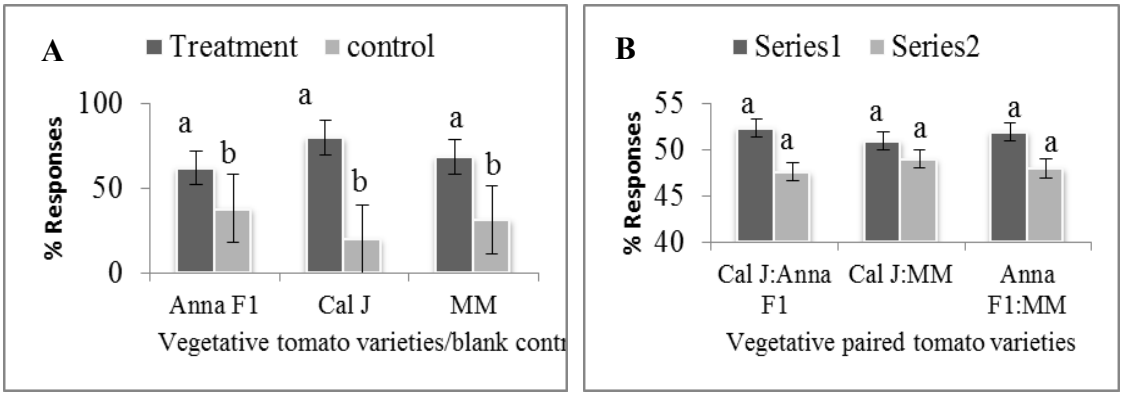


Figure 0.1: Responses of immature females to vegetative tomato varieties vs control (A) and pairwise comparison (B)

Immature females were more attracted to the three tomato varieties during their flowering stage (AnnaF1: PR =61.1%, χ^2 =12.25, DF=1, p<0.001; Cal-J: PR =70%, χ^2 =14.025, DF=1, p<0.01; Moneymaker: PR =62.9%, χ^2 =5.6, DF=1, p<0.05) than to control as shown on Figure 4:2 **A** below. Paired assays showed immature females being

significantly more attracted to Anna F1 (PR =65.1%, χ^2 =3.8919, DF=1, p<0.05) than to Moneymaker (PR =34.9%, χ^2 =1.8919, DF=1). However, there was no significant difference in attraction to AnnaF1 (PR =45.7%, χ^2 =2.3824, DF=1,) versus Cal-J and Cal-J (PR =50.6%, χ^2 =1.7297, DF=1) versus moneymaker recorded (PR =49.4%, χ^2 =1.6373, DF=1) as shown on Figure 4:2 **B** below.

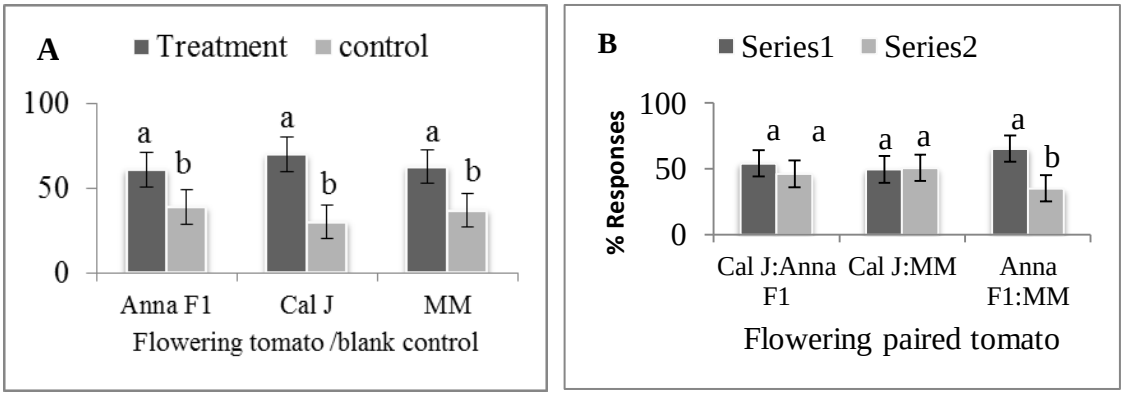


Figure 0.2: Responses of immature females to flowering tomato varieties vs control (A) and pairwise comparison (B)

Immature males were significantly more attracted to the three host tomato varieties than to the control in the vegetative stage of growth (Anna F1 PR =81.8%, χ^2= 20.4848, DF= 1, P<0.001

; Cal-J PR = 86.3%, χ^2=7.9024, DF=1 p<0.01 Moneymaker PR=82.9%, χ^2=22.4, DF=1, p<0.001) as shown on Figure 4:3 **A** below. In paired assays of vegetative stage, immatur e males were significantly more attracted to Cal J (PR =67.5%, χ^2= 3.7812, DF=1, p<0.0 1) variety than Anna F1 (PR =32.5%, χ^2= 1.4731, DF=1, p<0.01) and Moneymaker (PR =28%, χ^2= 1.2816, DF=1). In addition, there was no significant difference in attraction o f immature males to Anna F1 (PR =48%, χ^2= 1.4793, DF=1) and Money maker (PR =52 %, χ^2= 1.5016, DF=1) as shown on Figure 4:3 **B** below.

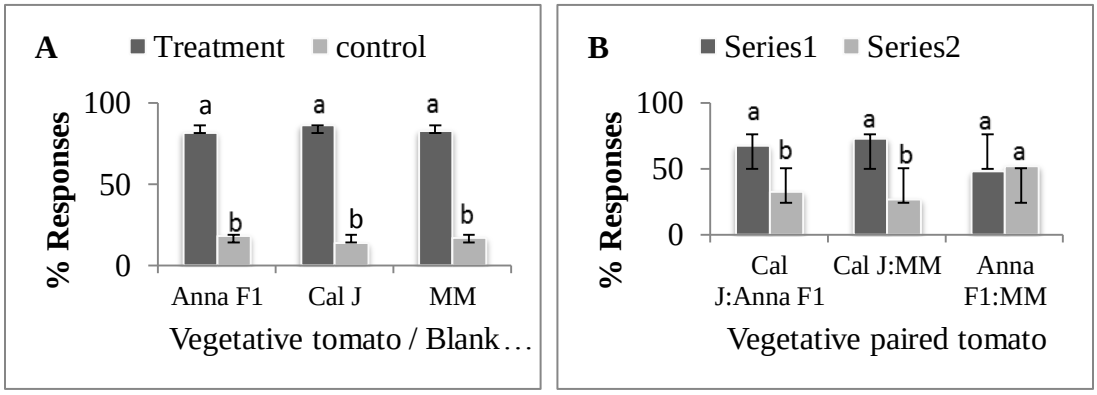


Figure 0.3: Responses of immature males to vegetative tomato varieties vs control (A) and pairwise comparison (B)

In flowering stage, Immature males were significantly more attracted to the three host to mato varieties than to the control (Anna F1 PR =68.8%, χ^2= 12.3333, DF=1, p<0.01; Cal -J PR =71% χ^2=14.0488, DF=1, p<0.001and Moneymaker PR =58.9%, χ^2=4.6944, DF= 1, p<0.05) shown on Figure 4:4 **A** below. In addition, they were significantly more attrac ted to Cal J (PR =76.8%, χ^2= 4.4474, DF=1, p<0.001) than both Anna F1 (PR =23.2%, χ^2= 2.2875, DF=1) and Moneymaker (PR =33%, χ^2= 3.5928, DF=1) tomato varieties in p air-wise comparisons as shown on Figure 4:4 **B** below.

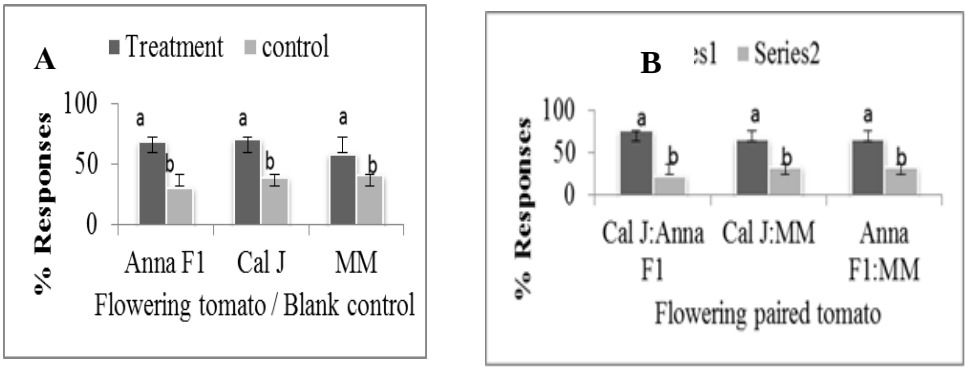


Figure 0.4: Responses of immature males to flowering tomato varieties vs control (A) and pairwise comparison (B)

Mature females were significantly more attracted to both vegetative Anna F1 (PR =78.4%, χ^2 =21.1892, DF=1, p<0.001), Cal-J (PR =87.5%, χ^2 =9.41, DF=1, p<0.01) and

moneymaker (PR =78%, χ^2 =7.45, DF=1, p<0.01) than to the control as shown on Figure 4:5 **A** below.

Paired assays indicated no significant differences in attraction of mature females to both Anna F1 (PR =45%, χ^2 =0, DF=1, p<0.01) and Cal-J (PR =55%, χ^2 =11.025, DF=1) tomato varieties. However, they were significantly more attracted to Anna F1 (PR =64%, χ^2 = 11.8321, DF=1, p<0.001) and Cal-J (PR =72%, χ^2 = 12.4654, DF=1, p<0.001) than to moneymaker varieties (PR =28%, χ^2 = 1.7149, DF=1) as indicated on Figure 4:5 **B** below.

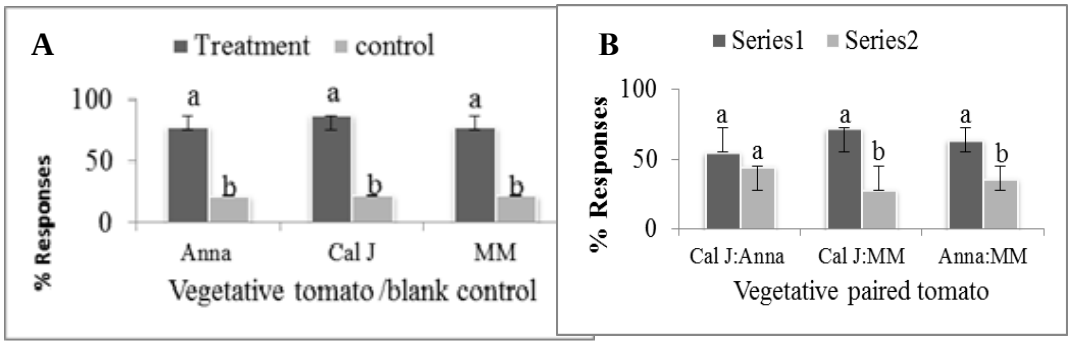


Figure 0.5: Responses of mature females to vegetative tomato varieties vs control (A) and pairwise comparison (B)

Mature females were significantly more attracted to the flowering stage of the three tomato varieties (Anna F1: PR =72.2%, χ^2 =17.3611, DF=1, p<0.001; Cal-J: PR = 79.7%, χ^2 =14.3636, DF=1, p<0.001 and Moneymaker: PR =62.6%, χ^2 =9.5, DF=1, p<0.001) than to the control as indicated on Figure 4.6 **A** below. Paired assays indicated mature females being significantly more attracted to Cal J (PR =74%, χ^2 =9.0256, DF=1, p<0.001) than to Anna F1 (PR =26%, χ^2 =1.0429, DF=1) and moneymaker (PR =32%, χ^2 =2.8571tomato varieties. However, mature females attraction to Anna F1 (PR =55%, χ^2 =3.3971, DF=1, p<0.01) and moneymaker (PR =45%, χ^2 =2.6388, DF=1, p<0.01) in pair-wise comparison had no significant differences as indicated on Figure 4.6 **B** below.

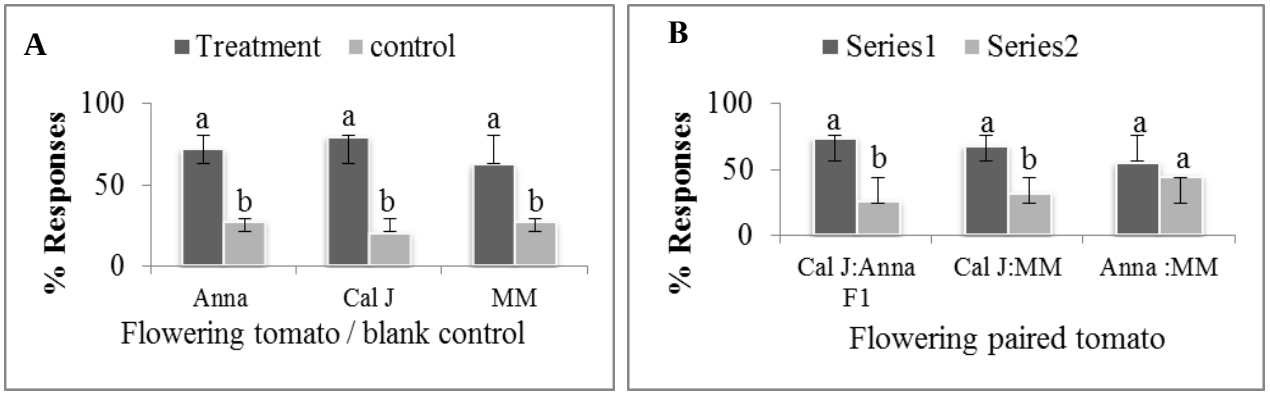


Figure 0.6: Responses of mature females to flowering tomato varieties vs control (A) and pairwise comparison (B)

Olfactometer bioassays of mature male *Z. curcubitae* and vegetative tomatoes varieties indicated the insects being significantly more attracted to Anna F1 (PR =84.2%, χ^2 = 15.2895, DF=1, p<0.001) and Cal-J (PR =76.5%, χ^2 = 5.14, DF=1 p<0.01) than to control. Similarly, they were significantly more attracted to moneymaker variety (PR =58.2%, χ^2= 2.5641, DF=1) than to the control as indicated on Figure 4:7 **A** below. Data for the paired assays in vegetative stage showed no significant difference in attraction of mature males to Anna F1 (PR =49%, χ^2 =1.641, DF=1), Cal-J (PR =51%, χ^2 =2.5641, DF=1) and Moneymaker (PR =46%, χ^2 =1.4417, DF=1) tomato varieties as indicated on Figure 4:7 **B** below.

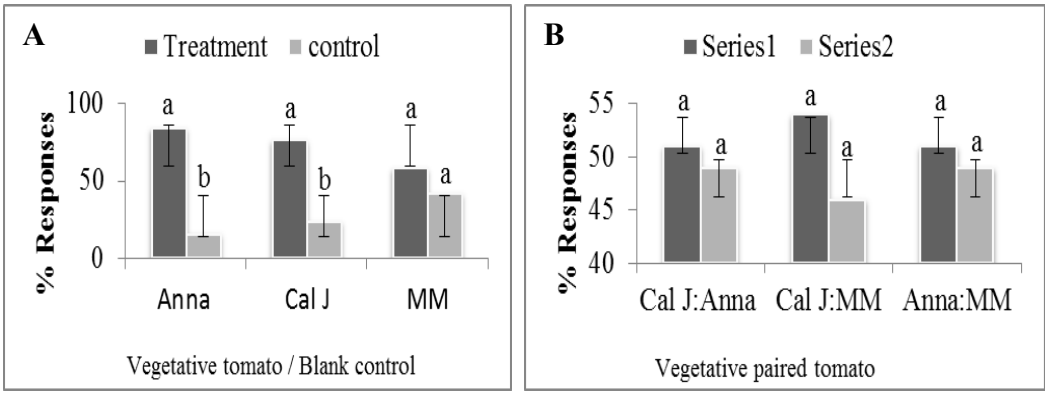


Figure 0.7: Responses of mature males to vegetative tomato varieties vs control (A) and pairwise comparison (B)

In the flowering stage, mature males were significantly more attracted to Anna F1 (PR =69.2%, χ^2 =17.3333, DF=1, p<0.001), Cal J (PR =68.6%, χ^2 =7.3143, DF=1, p<0.01), and Moneymaker (PR =63.6%. χ^2 = 6.5641, DF=1, p<0.05) tomato varieties than to the control as indicated on Figure 4:8 **A** below. In the flowering stage, the paired assays indicated that the males were significantly more attracted to Cal J (PR =78.9%, χ^2 =4.6944, DF=1, p<0.05) than to Anna F1 (PR =21.1%, χ^2 =1.0378, DF=1) and moneymaker (PR =19%, χ^2 =1.0248, DF=1, p<0.05) tomato varieties. However, there was no significant difference in attraction of mature males to Anna F1 (PR =45.7%) and Moneymaker tomato varieties (PR =54.3%) as indicated on Figure 4:8 **B** below.

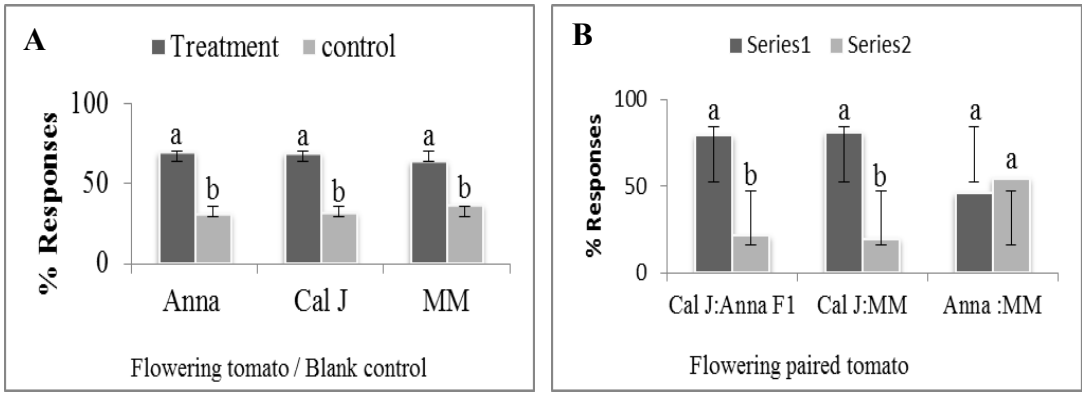


Figure 0.8: Responses of mature males to vegetative tomato varieties vs control (A) and pairwise comparison (B).

Both mature female (PR=81.8%; χ^2 = 8.26; P < 0.001) and mature male (PR=76.7%; χ^2= 4.34; P < 0.001) *Z. cucurbitae* were significantly more attracted to the odor of the flowering cucumber plant than the control as shown on Figure 4.9 **A** below. Similarly, mature females (PR=87.5%; χ^2 = 9.41; P < 0.01) and mature male (PR=76.5%; χ^2 = 5.14; P < 0.01) *Z. cucurbitae* were significantly more attracted to the odors of the flowering Cal J tomato plant than of the control as indicated on Figure 4.9 **B** below. In paired assays, there was no significant difference in attraction of both female (PR=52%; χ^2 = 0.02; P = 0.64) and male (PR=51%; χ^2 = 0.01; P = 0.76) *Z. cucurbitae* to Ashley cucumber and Cal J tomato odors as shown on Figure 4.9 **C** below.

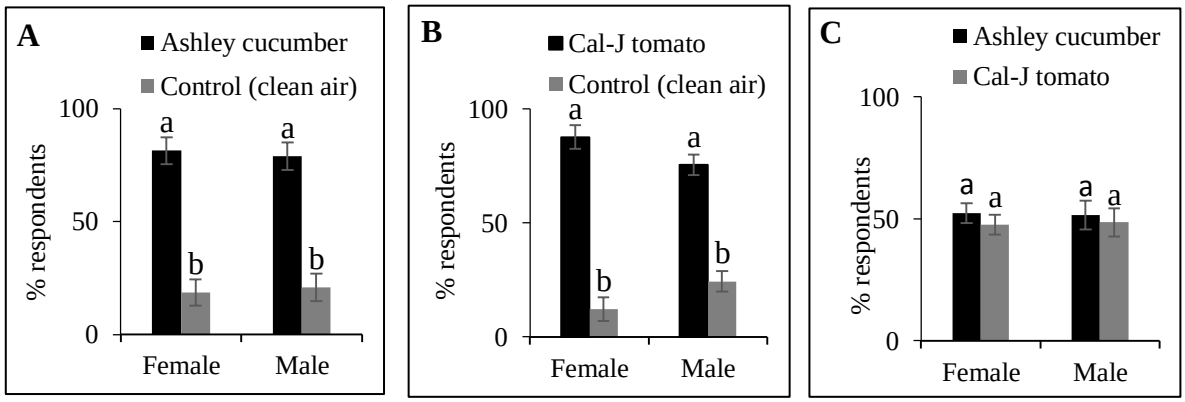


Figure 0.9: Responses of Males and Females *Z. cucurbitae* to odor of Ashley Cucumber vs control (A); Cal J Tomato vs control (B) and pairwise comparison(C)

4.2 Objective 2

4.2.1 Gas Chromatography Mass Spectrometer Results

Analyses of headspace volatile organic compounds released from Anna F1, Cal-J and Moneymaker tomato varieties during their vegetative and flowering stages of growth revealed qualitative differences in composition (Table 4:1). In the vegetative stage, analysis identified 25 VOCs in Moneymaker tomato variety that included Hexanal (**1**), 4-methyl-2-Hexanol (**2**), 3-methyl-2-Hexanol (**8**), 3-methyl-2-Butenal (**13**), α-Pinene (**15)**, o-Cymene (**17**), (E)-Isolimonene (**19**), Myrcene (**21**), δ-2-Carene (**22**), α-Phellandrene (**23**), α-Terpinene (**25**), p-cymene (**26**), β-Phellandrene (**27**), (E)-β-Ocimene (**29**), Sabinene (**30**), γ-Terpinene (**3**1), Terpinolene (**33**), n-Nonanal (**34**), iso-Sylvestrene (**36**), n-Decanal (**41**), δ-Elemene (**4**6), 10-Octadecenal (**50**), α-Cedrene (**54**), (E)-Caryophyllene (**55**) and α-Humulene (**60**) as indicated on Figure 4:10 below

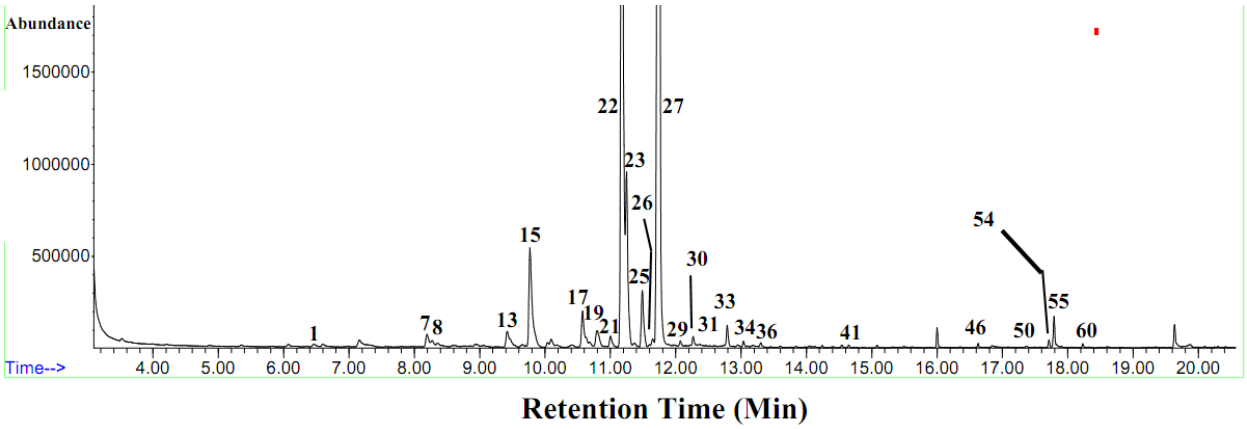


Figure 0.10: GC MS profile of vegetative money maker tomato variety

The GC MS analysis of vegetative Anna F1 identified 28 VOCs that included, 4-methyl-2-Hexanol **(1),** 5-methyl-2-Hexanol **(6),** 4-methyl-2-Hexanol **(7),** Heptanal **(11),** α-Pinene **(15),** 3,3-Dimethyl-2-pentanol **(16),** o-Cymene **(17),** β-Pinene **(18),** (E)-Isolimonene **(19),** 6-methyl-5-Hepten-2-one **(20),** Myrcene **(21),** δ-2-Carene **(22),** α-Phellandrene **(23),** δ-3-Carene **(24),** p-cymene **(26),** β-Phellandrene **(27),** (E)-β-Ocimene **(29),** γ-Terpinene **(31),** Terpinolene **(33),** n-Nonanal **(34),** iso-Sylvestrene **(36),**

n-Decanal **(41),** 6-Undecanone **(45),** δ-Elemene **(46),** β-Elemene **(51),** α-Cedrene **(54),** (E)-Caryophyllene **(55)** and α-Humulene **(60)** as shown on Figure 4:11 below.

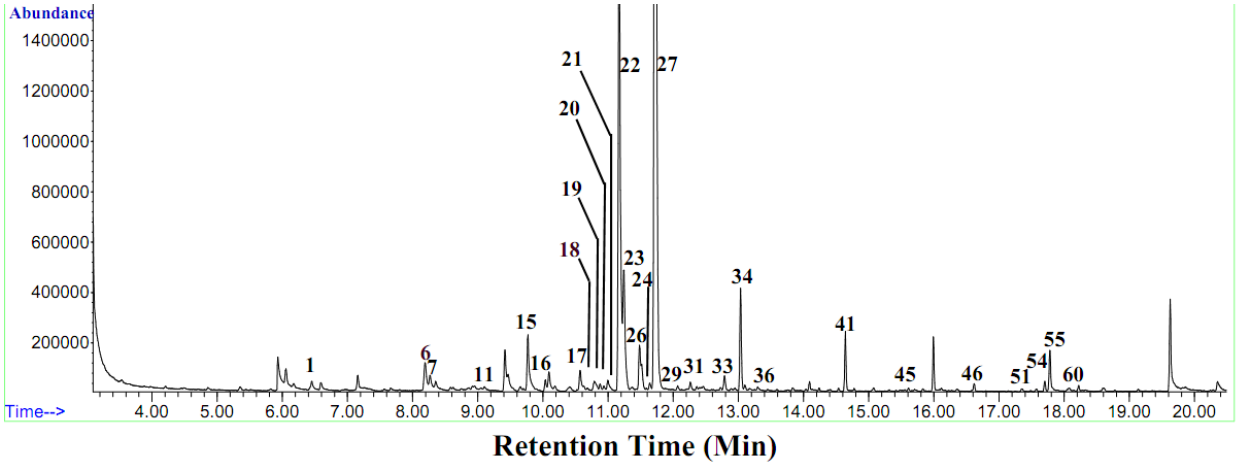


Figure 0.11: GC MS profile of vegetative Anna F1 tomato variety

In the GC MS analysis of vegetative Cal J tomato profile, 34 different VOCs were identified that included: Hexanal **(1)**, 4-methyl-2-Hexanone **(2)**, Ethylbenzene **(5)**, 5-methyl-2-Hexanol **(6)**, 4-methyl-2-Hexanol **(7)**, 3-methyl-2-Hexanol **(8)**, α-Pinene **(15)**, *o*-Cymene **(17)**, (E)-Isolimonene **(19)**, Myrcene **(21)**, δ-2-Carene **(22)**, α-Phellandrene **(23)**, α-Terpinene **(25)**, β-Phellandrene **(27)**, butyl-Benzene **(28)** , (E)-β-Ocimene **(29)**, γ-Terpinene **(31)**, m-Cymene **(32)**, Terpinolene **(33)**, n-Nonanal **(34)**, 1,3,8-p-Menthatriene **(35)**, iso-Sylvestrene **(36)**, allo-Ocimene **(37)**, Methyl salicylate **(40)**, n-Decanal **(41)**, (Z)-2-Dodecene**, (42)**, δ-Elemene **(46)**, α-Copaene **(48)**, 1-Hexadecene **(49)**, β-Elemene **(51)**, (E)-Caryophyllene **(55)**, γ-elemene **(56)**, Zonarene **(57)**, α-Humulene **(60)** and α-Selinene **(68)** as indicated on Figure 4:12 below.

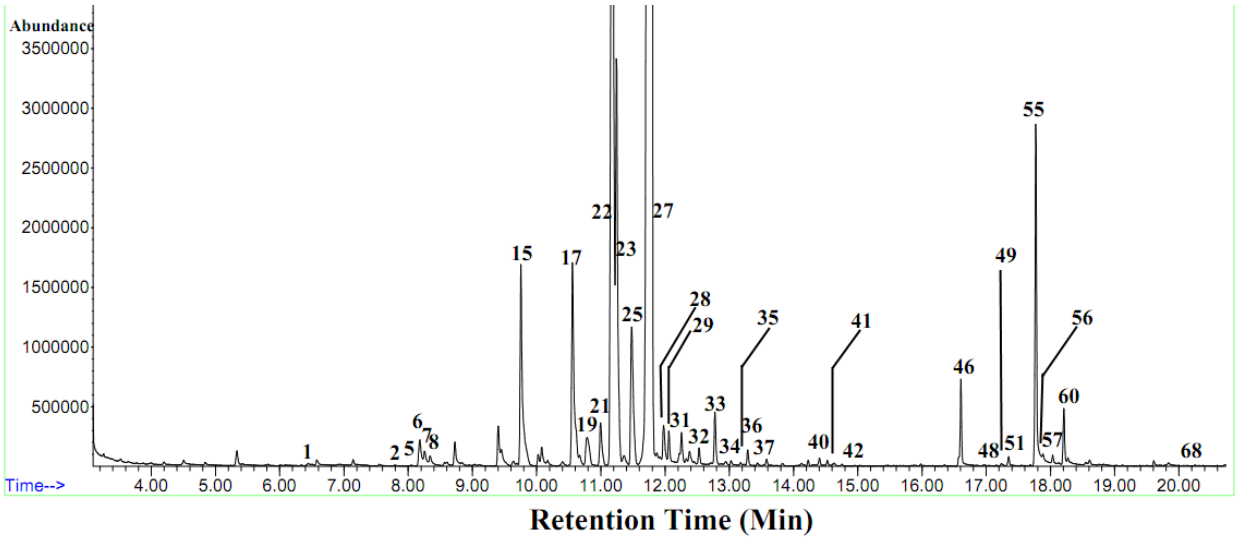


Figure 0.12: GC MS profile of vegetative Cal J tomato variety

In flowering stage, the GC MS analysis identified 37 VOCs from moneymaker tomato variety that included: 4-methyl-2-Hexanol **(1)**, Ethylbenzene **(5)**, 4-methyl-2-Hexanol **(7)**, 4-Heptanone **(9)**, Propyl butanoate **(10)**, α-Pinene **(15)**, o-Cymene **(17)**, β-Pinene **(18)**, (E)-Isolimonene **(19)**, Myrcene **(21)**, δ-2-Carene **(22)**, α-Phellandrene **(23)**, α-Terpinene **(25)**, p-cymene **(26)**, β-Phellandrene **(27)**, (E)-β-Ocimene **(29)**, γ-Terpinene **(31)**, Terpinolene **(33)**, n-Nonanal **(34)**, iso-Sylvestrene **(36)**, allo-Ocimene **(37)**, Camphor **(38)**, 1-Decene **(39)**, Methyl salicylate **(40)**, n-Decanal **(41)**, 6-Undecanone **(45)**, δ-Elemene **(46)**, Eugenol **(47)**, β-Elemene **(51)**, Methyl eugenol **(52)**, α-Cedrene **(54)**, (E)-Caryophyllene **(55)**, 6,10-dimethyl-5,9-Undecadien-2-ol **(59)**, α-Humulene **(60),** γ-Cadinene **(62)**, α-Gurjunene **(63)**, and Spathulenol **(69)** as shown on Figure 4:13 below**.**

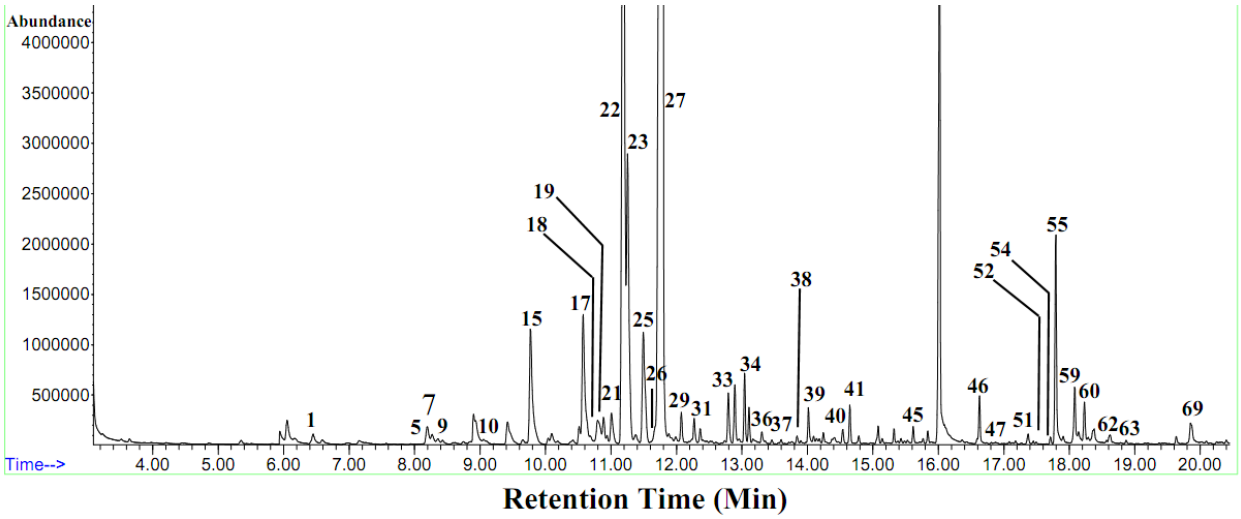


Figure 0.13: GC MS profile of flowering money maker tomato variety.

There were 26 different VOCs identified from GC MS analysis of flowering Anna F1 tomato variety. The VOCs were: 4-methyl-2-Hexanol **(1)**, 4-methyl-2-Hexanone **(2)**, 5-methyl-2-Hexanol **(6)**, α-Thujene **(14)**, α-Pinene **(15)**, o-Cymene **(17)**, (E)-Isolimonene **(19)**, δ-2-Carene **(22)**, α-Phellandrene **(23)**, α-Terpinene **(25)**, β-Phellandrene **(27)**, (E)-β-Ocimene **(29)**, γ-Terpinene **(31)**, m-Cymene **(32)**, Terpinolene **(33)**, n-Nonanal **(34)**, 1,3,8-p-Menthatriene **(35)**, iso-Sylvestrene **(36)**, allo-Ocimene **(37)**, n-Decanal **(41)**, Piperitone **(44)**, δ-Elemene **(46)**, α-Cedrene **(54)**, (E)-Caryophyllene **(55)**, Zonarene **(57)**, andα-Humulene **(60)** as indicated on Figure 4:14 below.

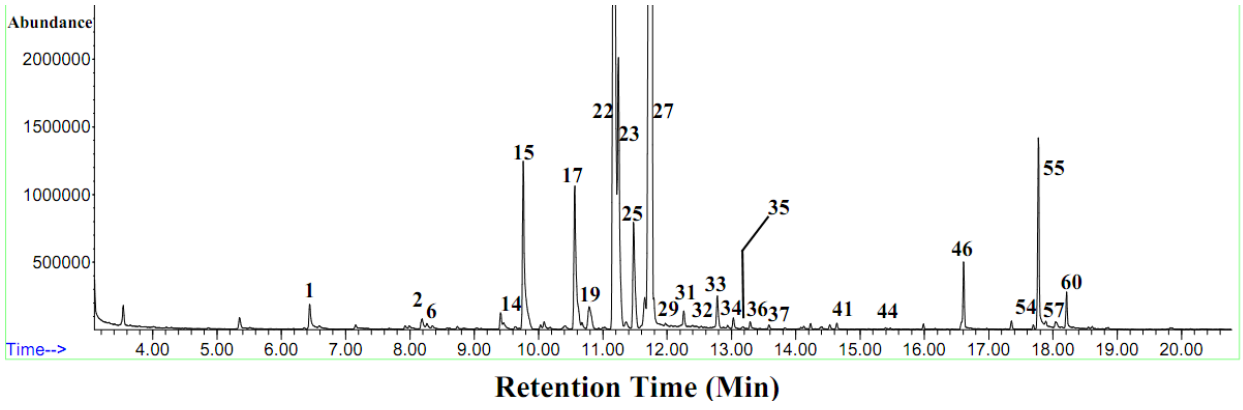


Figure 0.14: GC MS profile of flowering Anna F1 tomato variety

Lastly, 45 VOCs in flowering stage of Cal-J tomato variety were identified. They included: 4-methyl-2-Hexanol **(1)**, (E)-2-Hexenal **(3)**, (Z-)3-Hexenol **(4)**, 5-methyl-2-Hexanol **(6)**, 4-methyl-2-Hexanol **(7)**, 3-methyl-2-Hexanol **(8)**, 2E,4E-Hexadienal **(12)**, α-Thujene **(14)**, α-Pinene **(15)**, o-Cymene **(17)**, β-Pinene **(18)**, (E)-Isolimonene **(19)**, Myrcene **(21)**, δ-2-Carene **(22)**, α-Phellandrene **(23)**, δ-3-Carene **(24)**, α-Terpinene **(25)**, β-Phellandrene **(27)**, butyl-Benzene **(28)**, (E)-β-Ocimene **(29)**, γ-Terpinene **(31)**, m-Cymene **(32)**, Terpinolene **(33)**, n-Nonanal **(34)**, 1,3,8-p-Menthatriene **(35)**, iso-Sylvestrene **(36)**, allo-Ocimene **(37)**, Methyl salicylate **(40)**, n-Decanal **(41)**, Umbellulone **(43)**, δ-Elemene **(46)**, α-Copaene **(48)**, β-Elemene **(51)**, β-Longipinene **(53)**, α-Cedrene **(54)**, (E)-Caryophyllene **(55)**, γ-elemene **(56)**, 6,9-Guaiadiene **(58)**, α-Humulene **(60)**, Germacrene D **(61)**, α-Gurjunene **(63)**, δ-Amorphene **(64)**, Germacrene B **(65)**, Caryophyllene oxide **(66)** and Spathulenol **(69)** as indicated on Figure 4:15 below.

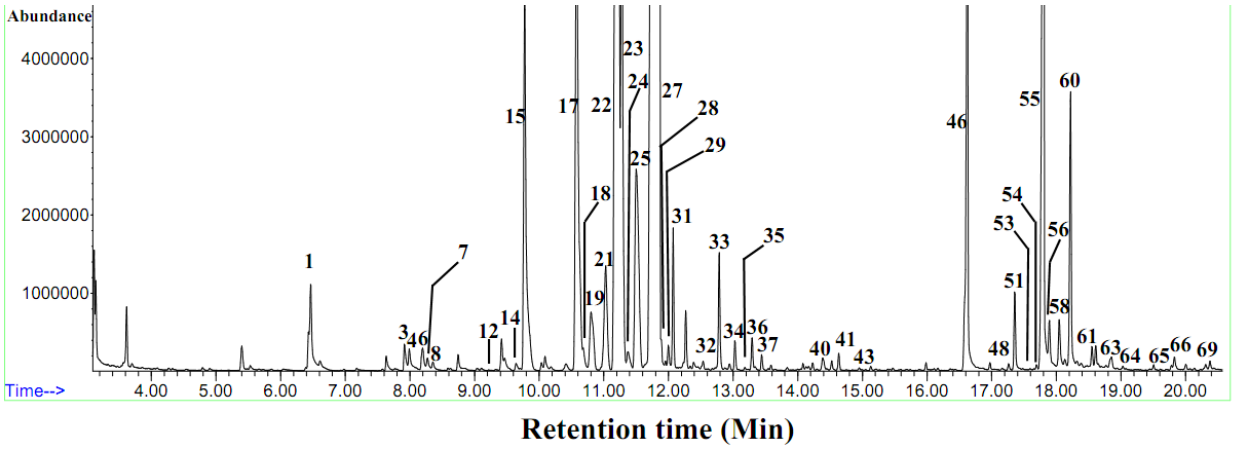


Figure 0.15: GC MS profile of flowering Cal J tomato variety

In the comparison of GC/MS analyses of preferred Cucurbitaceous Ashley cucumber and Solanaceous Cal J tomato, I identified 21 and 34 components respectively mainly dominated by terpenes as indicated on Figure 4:16 below.

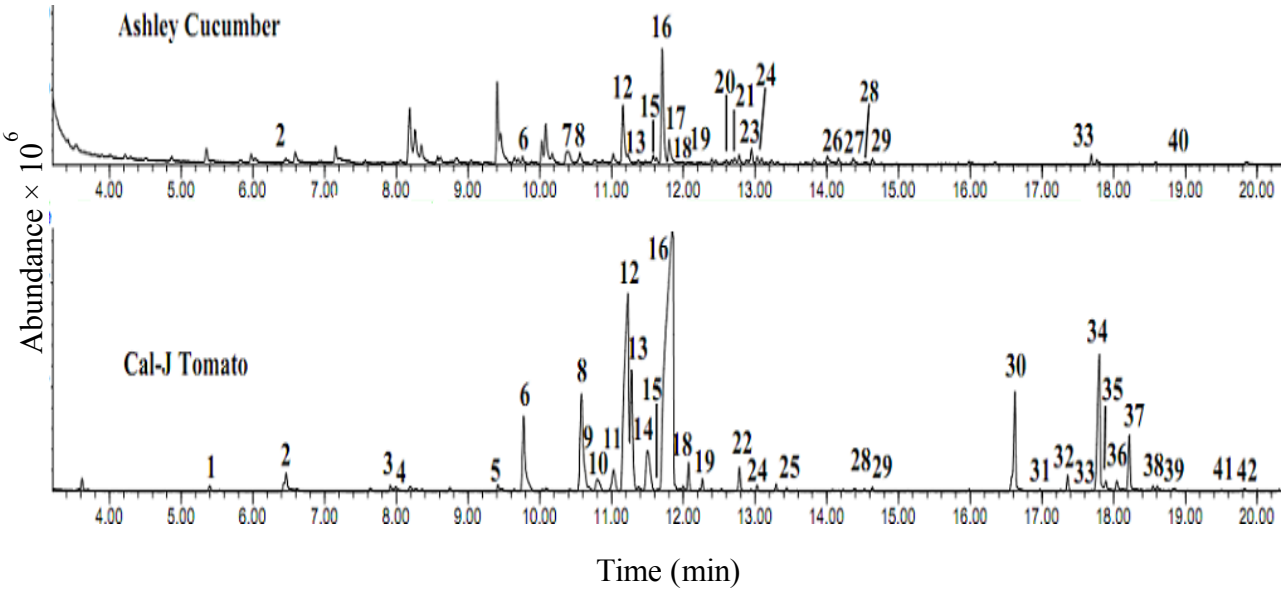


Figure 0.16: GC/MS analysis of Ashley cucumber and Cal J tomato odors

The headspace of vegetative Moneymaker tomato plants contained the least number of VOCs (twenty five). In total, sixty nine volatile organic compounds were identified in the vegetative and flowering stages of the three tomato varieties. These included terpenes (monoterpenes and sesquiterpenes), alcohols, aldehydes, esters, ketones among others. Out of all identified volatile organic compounds, fifteen were common in each stage of the three tomato varieties in varying abundance. These included hexanal, 4-methyl-2 hexanol, α-pinene, o-cymene, α-phellandrene, β-phellandrene, γ-Terpinene, Terpinolene, n-Nonanal, iso-Sylvestrene, n-decanal, δ-elemene, α-cedrene, (E)-caryophyllene, and α-humulene. β-phellandrene was the major component in each of the six profiles of which in flowering Anna F1, it stood out to be the highest overall. The highest number of eleven components including (E)-2-hexenal, (Z)-3-hexenol, propyl

butanoate, 2E,4E-Hexadienal, Umbellulone, 6,9-Guaiadiene, Germacrene D, δ-Amorphene, Germacrene B, Caryophyllene oxide, and Spathulenol were specifically found in significant amounts in the headspace of flowering Cal-J variety. Odor from vegetative moneymaker variety had the least number of compounds that were specifically present in the headspace of each stage of a variety

Thirteen compounds including hexanal, *α*-pinene, *o*-cymene, *δ*-2-Carene, *α*-phellandrene, *p*-cymene, *β*-phellandrene, (*E*)-*β*-ocimene, *γ*-terpinene, *n*-nonanal, methyl salicylate, *n*-decanal and *α*-cedrene were identified as common to the odors of both plants. Additionally, (*E*)-2-hexenal and (*Z*)-3-hexenol, toluene, (*E*,*E*)-2,4-hexadienal, (*E*)-Isolimonene, *β*-pinene, myrcene, *α*-terpinene, terpinolene, allo-ocimene, *δ*-elemene, *α*-Copaene, *β*-elemene, (*E*)-caryophyllene, *γ*-elemene, 6,9-guaiadiene, *α*-humulene, germacrene D and B, *α*-gurjunene and caryophyllene oxide were identified as specific to Cal J tomato odors. Cucumber-specific odors were identified as benzaldehyde, benzyl alcohol, *m*-cymene, (*E*)-linalool oxide, linalool, 2, 3-octanediol and naphthalene as indicated on Table 4:1 below.

4.2.2 Quantification

Gas Chromatography Mass Spectrometer analyses of headspace volatile organic compounds released from Anna F1, Cal-J and Moneymaker tomato varieties during their vegetative and flowering stages of growth revealed quantitative differences in composition. Generally, more compounds were detected and at relatively greater amounts in the headspace of flowering Cal-J plants than in the headspace of its vegetative stage or both stages of the other two tomato plant varieties. The α-Phellandrene and β-Phellandrene were highest in amount relative to the other VOCs produced by the three tomato varieties. Vegetative Anna F1 produce the least amount compared to other stages of tomato varieties but on the other hand its flowering stage produced the highest amount of both α-Phellandrene and β-Phellandrene which were common in all tomato varieties headspace as indicated on Table 4:1 below.

Table 0.1: Gas Chromatography Mass Spectrometer Identification and quantification of headspace volatiles of the moneymaker, Cal J and anna F1 tomato varieties

Peak Number	RT (MIN)	Name of Compounds	Vegetative MM in ng/plant/h ± SEM	Vegetative Cal-J in ng/plant/h ± SEM	Vegetative Anna F1 in ng/plant/h ± SEM	Flowering MM in ng/plant/h ± SEM	Flowering Cal-J in ng/plant/h ± SEM	Flowering Anna F1 in ng/plant/h ± SEM
1	6.4	Hexanal	3.32±1.47	2.84±0.73	5.32±0.43	8.83±1.81	40.46±2.97	13.54±4.60
2	7.8	4-methyl-2-Hexanone	—	0.23±0.07	—	—	—	—
3	7.9	(E)-2-Hexenal	—	—	—	—	12.61±4.03	—
4	8.00	(Z-)3-Hexenol	—	—	—	—	10.69±0.64	—
5	8.05	Ethylbenzene	—	0.38±0.12	—	0.31±0.08	—	—
6	8.18	5-methyl-2-Hexano	—	14.79±1.76	12.23±1.14	—	0.95±0.54	4.41±0.20

		l						
7	8.19	4-methyl-2-Hexanol,	6.00±0.76	8.65±0.92	7.06±0.70	9.66±2.64	5.71±0.50	7.69±0.07
8	8.20	3-methyl-2-Hexanol	10.21±1.42	—	—	—	4.72±0.18	
9	8.43	4-Heptanone	—	—	—	3.26±0.29	—	—
10	9.06	Propyl butanoate	—	—	—	5.22±2.43	—	—
11	9.11	Heptane	—	—	2.52±0.29	0.00	—	—
12	9.33	2E,4E-Hexadienal	—	—	—	—	0.72±0.03	—
13	9.40	3-methyl-2-Butenal	14.77±2.08	15.89±1.63	—	—	—	—
14	9.64	α-Thujene	—	—	—	—	2.92±0.15	2.93±0.15

15	9.76	α-Pinene	33.05±17.60	66.01±15.59	15.29±6.83	64.37±17.64	149.47±10.69	181.01±43.18
16	10.04	3,3-Dimethyl-2-pentanol	—	—	3.34±0.36	—	—	—
17	10.56	*o*-Cymene	17.20±8.17	73.42±14.04	5.58±2.22	58.63±12.17	230.39±15.28	208.28±69.89
18	10.68	β-Pinene	—	—	0.90±0.25	4.05±0.83	8.09±0.40	–
19	10.79	(E)-Isolimonene	6.51±3.43	14.39±3.47	4.01±1.79	17.98±4.69	36.05±1.86	40.85±11.30
20	10.94	6-methyl-5-Hepten-2-one	—	—	1.81±0.14	—	—	—
21	11.00	Myrcene	4.39±1.30	11.15±4.74	3.69±1.64	13.42±2.11	51.68±3.60	—
22	11.17	δ-2-Carene	172.68±90.91	–	107.80±50.38	367.44±105.08	—	875.00±179.63
23	11.25	α-Phellandrene	46.78±21.46	116.48±74.28	33.90±13.64	119.00±28.33	205.65±9.59	275.23±61.62
24	11.37	δ-3-Carene	—	—	1.73±0.55	—	11.37±0.26	—

25	11.49	α-Terpinene	16.07±7.82	29.52±13.56	—	53.84±13.32	13.05±7.12	123.88±32.58
26	11.64	p-cymene	2.90±0.98	—	2.18±0.66	—	—	—
27	11.74	β-Phellandrene	417.59±201.71	981.77±157.62	321.69±152.26	936.94±263.76	1478.70±70.17	1840.26±354.83
28	11.92	butyl-Benzene	—	2.08±0.82	—	—	3.05±0.19	—
29	12.06	(E)-β-Ocimene	2.49±1.21	8.47±3.81	1.50±0.71	10.04±2.31	32.65±2.14	8.77±4.18
30	12.23	Sabinene	2.07±1.44	—	—	—	—	—
31	12.26	γ-Terpinene	3.50±1.54	9.20±2.18	2.48±1.06	10.14±2.59	18.82±1.01	17.39±6.79
32	12.53	*m*-Cymene	—	4.30±1.60	—	—	4.15±0.41	3.70±0.74
33	12.78	Terpinolene	5.61±2.91	13.64±3.21	3.70±1.76	14.28±3.07	33.73±1.28	32.81±8.07
34	13.03	n-Nonanal	9.81±6.54	2.14±0.07	24.45±2.21	21.08±0.89	7.98±0.19	5.86±1.40
35	13.18	1,3,8-*p*-Mentha	—	1.21±0.19	—	—	5.88±2.49	3.00±0.94

		triene						
36	13.29	iso-Sylvestrene	1.82±0.71	3.90±1.00	1.74±0.87	7.98±3.52	8.89±0.44	8.30±1.91
37	13.44	allo-Ocimene	—	0.89±0.25	—	1.38±0.29	4.41±0.16	1.46±0.44
38	13.76	Camphor	—	—	—	1.31±0.12	—	—
39	14.10	1-Decene	—	—	—	2.66±0.05	—	—
40	14.53	Methyl salicylate	—	2.68±0.27	—	4.16±0.67	3.35±0.12	—
41	14.63	n-Decanal	5.19±5.05	1.33±0.33	13.73±1.30	11.38±0.44	4.92±0.16	2.83±0.29
42	14.75	(Z)-2-Dodecene,	—	0.53±0.15	—	—	—	—
43	14.95	Umbellulone	—	—	—	—	2.20±0.65	—
44	15.43	Piperitone	—	—	—	—	—	0.44±0.13
45	15.61	6-Undecanone	—	—	1.05±0.10	4.60±0.62	—	—
46	16.61	δ-Elemen	0.43±0.19	8.05±2.97	0.61±0.29	3.71±0.76	51.18±3.74	13.29±7.58

		e						
47	16.87	Eugenol	—	—	—	0.43±0.09	—	—
48	17.16	α-Copaene	—	0.13±0.01	—	—	0.42±0.03	—
49	17.24	1-Hexadecene	—	0.31±0.06	—	—	—	—
50	17.26	10-Octadecenal	0.11±0.05	—	—	—	—	—
51	17.35	β-Elemene	–	1.17±0.02	0.31±0.08	0.96±0.16	7.05±0.49	—
52	17.45	Methyl eugenol	—	—	—	0.29±0.06	—	—
53	17.59	β-Longipinnate	—	—	—	—	0.32±0.01	—
54	17.69	α-Cedrene	0.49±0.24	0.58±0.08	0.60±0.29	0.62±0.12	0.62±0.03	0.98±0.16
55	17.77	(E)-Caryophyllene	2.03±1.05	40.74±4.06	2.82±1.42	15.59±2.93	84.78±5.94	36.44±14.51
56	17.89	γ-elemene	—	3.58±0.42	—	—	6.94±0.39	—

57	18.04	Zonarene	—	1.95±0.19	—	—	1.49±0.10	2.59±0.89
58	18.05	6,9-Guaiadiene	—	—	—	—	6.14±0.44	—
59	18.09	6,10-dimethyl-5,9-Undecadien-2-ol,	—	—	—	5.20±0.97	—	—
60	18.21	α-Humulene	0.40±0.05	8.09±1.36	0.44±0.20	3.50±0.65	26.03±1.88	7.83±3.78
61	18.55	Germacrene D	—	—	—	—	2.54±0.18	—
62	18.57	γ-Cadinene	—	—	—	0.37±0.07		—
63	18.85	α-Gurjunene	—	—	—	0.43±0.07	2.49±0.21	—
64	19.03	δ-Amorphene	—	—	—	—	0.66±0.04	—
65	19.51	Germacrene B	—	—	—	—	0.63±0.05	—
66	19.83	Caryophyllene	—	—	—	—	1.80±0.15	—

		oxide						
67	20.09	Cedrol	—	—	—	0.47±0.11	—	—
68	20.26	α-Selinene	—	0.25±0.12	—	—	—	—
69	20.32	Spathulenol	—	—	—	—	0.89±0.07	—

Quantification of VOCs of Ashley cucumber and Cal J showed differences in the amount released from the two plants. Of the shared components, the Cal J tomato plant emitted relatively greater amounts of *α*-phellandrene and *β*-phellandrene, approximately 26- and 2.5-fold more of the two components than in cucumber plant odor respectively. Conversely, δ-2-carene, nonanal and α-cedrene were approximately 20-, 6- and 100-fold more abundant in cucumber odor than in Cal J tomato odor, respectively as indicated on Table 4:2 below.

Table 0.2: Gas Chromatography Mass Spectrometer Identification and Quantification of vegetative cucumber and Cal-J tomato variety headspace volatiles

Peak No	RT	Compound	Cucumber in ng/plant/h ± SEM	Cal-J in ng/plant/h ± SEM
1	5.4	Toluene	Trace	1.94±0.63
2	6.4	Hexanal	Trace	3.15±2.97
3	7.9	(*E*)-2-Hexenal		5.03±4.03
4	8.0	(*Z*)-3-Hexenol		1.07±0.64
6	9.8	α-Pinene	8.6 ± 5.0	12.09±10.69
7	10.4	Benzaldehyde	17.34± 2.0	

8	10.6	*o*-Cymene	15.2 ± 8.8	20.52±15.28
9	10.7	(E)-Isolimonene		0.96±0.40
10	10.8	β-Pinene		4.07±1.86
11	11.0	Myrcene		4.32±3.60
13	11.2	δ-2-Carene	49.8 ± 28.7	2.21±0.26
12	11.2	α-Phellandrene	Trace	26.00±9.59
14	11.5	α-Terpinene		13.05±7.12
15	11.6	*p*-Cymene	10.6 ± 6.1	Trace
16	11.7	β-Phellandrene	83.1 ± 48.0	199.64±70.17
17	11.8	Benzyl alcohol	17.68± 2.7	
18	12.1	(*E*)-β-Ocimene		2.98±2.14
19	12.3	γ-Terpinene	Trace	2.78±1.01
20	12.7	*m*-Cymene	6.1±1.3	
21	12.7	(*E*)-Linalool oxide	11.1 ± 6.4	
22	12.	Terpinolene		4.81±1.28

	8			
23	13.0	Linalool	14.6 ± 8.4	
24	13.0	n-Nonanal	8.8 ± 5.1	1.36±0.19
25	13.4	*allo*-Ocimene		0.62±0.16
26	14.0	2,3-Octanediol	10.4 ± 6.0	
27	14.4	Naphthalene	6.63± 1.8	
28	14.5	Methyl salicylate		0.46±0.12
29	14.6	n-Decanal	Trace	0.71±0.16
30	16.6	δ-Elemene		4.00±3.74
31	16.9	α-Copaene		0.04±0.03
32	17.4	β-Elemene		0.60±0.49
33	17.7	α-Cedrene	8.3 ± 4.8	0.08±0.03
34	17.8	(*E*)-Caryophyllene		7.04±5.94
35	17.9	γ-elemene		0.84±0.39
36	18.1	6,9-Guaiadiene		0.13±0.44

37	18.2	α-Humulene		2.08±1.88
38	18.6	Germacrene D		0.20±0.18
39	18.9	α-Gurjunene		0.20±0.21
40	18.9	Butylated hydroxytoluene	Trace	
41	19.5	Germacrene B		0.07±0.05
42	19.8	Caryophyllene oxide		0.16±0.15

4.3 Objective 3

4.3.1 Gas chromatography Electroantennographic detection results

Gas chromatography Electroantennographic detection analysis of the three varieties of tomato plant odors isolated 34 EAD-active components using antennae of both sexes of immature and mature *Z. cucurbitae* of which 11 were consistently detected in at least two out of the three runs.

In general, antennae of the mature female *Z. cucurbitae* appeared to be more sensitive in detecting the plant odors of the three varieties of tomato than those of mature males. Cal J tomato volatiles produced the highest number of antennal responses among the four groups of *Z. cucurbitae* s as indicated on Figure 4:17 below.

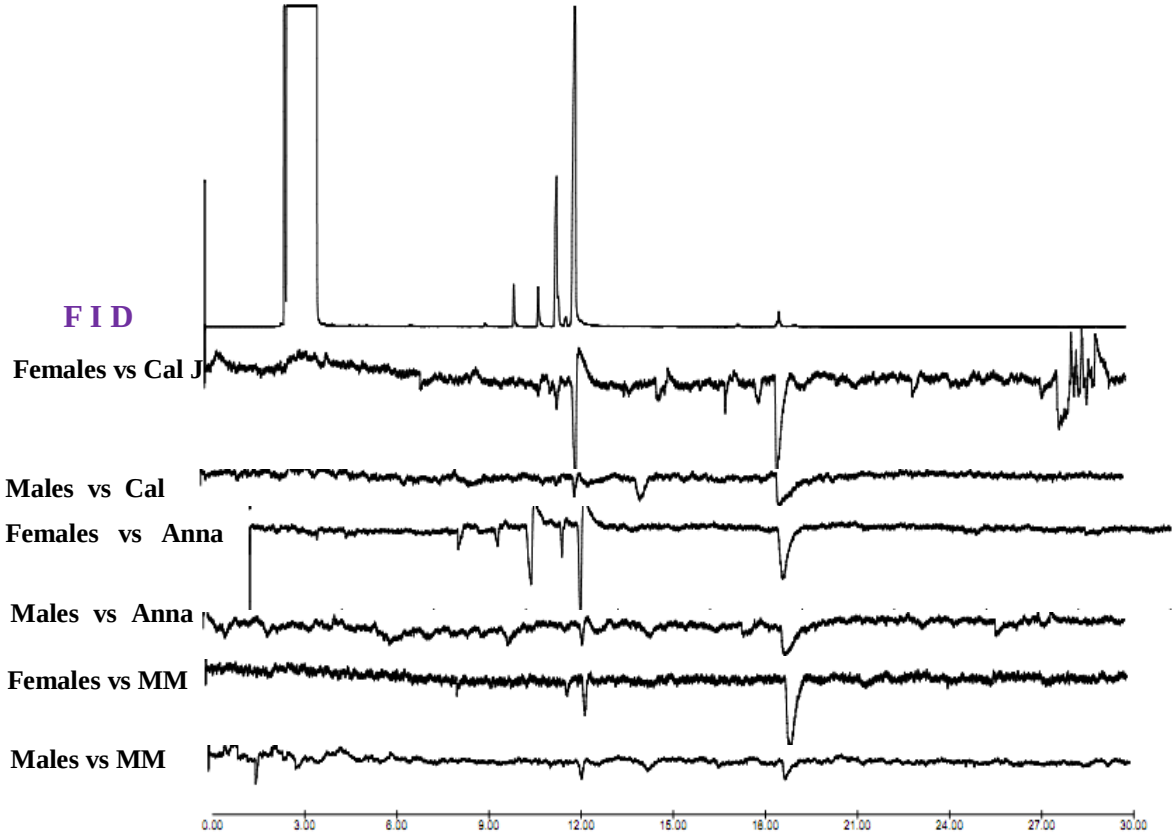


Figure 0.17: EAG responses of selected mature *Z. cucurbitae*

Gas chromatography Electroantennographic detection analysis of Solanaceous Cal J tomato and cucurbitaceous Ashley cucumber plant odor isolated 10 EAD-active components using antennae of both sexes of the mature *Z. cucurbitae* (Figure 3) of which 7 were consistently detected in at least two out of the three runs. These 7 components were among the 13 shared components as indicated on Figure 4:18 below.

The identities of the seven EAD-active components were confirmed by comparison of GC/EAD and GC/MS retention times and fragmentation patterns with those of authentic standards of *o*-cymene, *p*-cymene, *α*-phellandrene, *β*-phellandrene, *β*-ocimene, methyl salicylate, and *α*-cedrene.

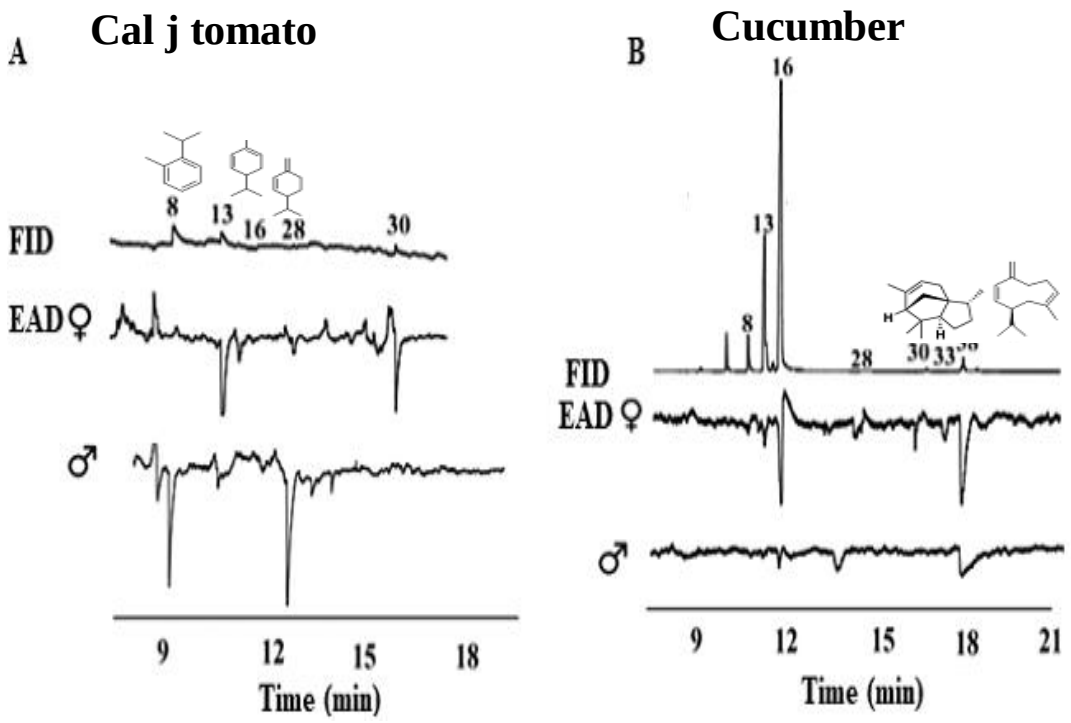


Figure 0.18: EAG responses of mature male and female *Z. cucurbitae* to Cal J (A) and Cucumber (B) odors

In general, antennae of the female *Z. cucurbitae* appeared to be more sensitive in detecting the plant odors than those of males, while immature females were more responsive to plant odors than immature males. Immature males showed the least responses. The α-Phellandrene and β-Phellandrene volatile organic compounds being common in both vegetative and flowering stages of tomato varieties produced the highest number of responses among the four groups of *Z. cucurbitae* as indicated on Table 4:3 below.

Table 0.3: EAG responses of mature and immature male and female *Z. cucurbitae* to Anna F1, Cal J and Moneymaker tomato volatile organic compounds

	Vegetative stage			**Flowering stage**		
EAG active Compound Name	Anna F1	Cal-J	Money maker	Anna F1	Cal-J	Money maker
Toluene					IF	
Hexanal	MF, MM					
5-methyl-2 hexanol	MF				IF	
4-Methyl-2-hexanol	MF, MM	MF, MM,	MF, MM	MF, IF		IM
4-heptanone						IM
Heptane	IF					
α-Thujene				MM		
α-Pinene	IF	IM	MF	MF		MF,IF
3,3-Dimethyl-2-pentanol	MM					
o- Cymene	MF	MF, IM		MF	MF, IF, IM	IF
β-pinene						MM
(E)-Isolimonene	MM,IF		MM	IF		
Myrcene		IF,IM				
δ-2-Carene	IF					
α-Phellandrene	MM	MF,IF, IM	MM	MF, IF, MM	MF,MM,IF, IM	MF,MM,IF
δ-3-Carene	MF					
α-Terpinene			MF			

p-Cymene					IF,IM	
β-Phellandrene	MF,M M, IF	MF, MM, IF, IM	MF, MM	MF, MM, IF	MF, MM, IF	MF, MM
β-Ocimene		IF			IM	
γ-Terpinene	MF	MM				IF
Terpinolene			MM			
Tridecane					IM	
1-Decene						MM
Methyl salicylate		MM			MF,MM	IF,IM
n-Decanal		MM		MM		IF
δ-Elemene	MF, IF			IF	MF,IM	
β-elemene		MF		MM		
α-Cedrene					MF,IF	
(E)-caryophyllene	IF	IM				
γ-elemene					IM	
α-Humulene	MM	MF,MM,IF,IM				MF,MM
Germacrene D				MF, MM	MF,MM,IF, IM	IF
γ-Cadinene						IM

MF represents mature female antennae, MM represents mature male antennae, IF represent immature female antennae while IM represents immature male antennae.

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDAIONS

5.1 Discussions

The study carried out provides results of the evaluation of odors from the three host tomato varieties at different stages of growth to both mature and immature male and female *Z. cucurbitae* in dual choice olfactometer. Both mature and immature males and females *Z. curcubitae* portrayed positive responses to odors from vegetative and flowering stages of Anna F1, Cal-J and Moneymaker varieties of tomato. The data further showed the existence of crude odor-based tomato variety discrimination by both immature and mature male and female *Z. cucurbitae* adults. Little attention had been paid to the contribution of olfactory cues in observed host plant variety selection. Indeed the fact that vegetative and flowering Cal J was more attractive than Anna F1 and moneymaker indicates that odor perception is a key in to selection of most suitable oviposition, mating and feeding sites by *Z. cucurbitae*. The results of this study suggest attractiveness of mature females *Z. curcubitae* to all the three tomato varieties in vegetative and flowering stages of their growth compared to control. This is similar to previous reports of female based attraction of oriental fruit fly to volatiles from leaves of several host plants (Chen and Dong, 2000). The data further showed the existence of odor based variety discrimination in vegetative and flowering stages by mature females. Since ovipositing females will choose the plants that are most likely to sustain offspring development than hosts less likely to do so (Solomon *et al.,* 2005), group of compounds detected by mature female *Z. curcubitae* may signal the availability of enough resources for the survival of the larval instars of the insect up to the time of pupation (Kimbokota *et al.,* 2013) hence facilitating location of their host. In addition, the detection of these compounds may be an indication of gravid flies' preference of the soft texture of vegetative and flowering stages of tomato stems and leaves that can easily be penetrated by *Z. curcubitae* ovipositor during oviposition and fully grown larvae ready for pupation

in the soil. The results on mature males' attraction to the three varieties of tomato in both stages of growths concur with earlier reports that Tephritid fruit flies have evolved a wide range of mating systems and host plants odors play an important role in shaping male sexual behavior and mating success in many ways (Aluja *et al.,* 2000). Mating enhancing chemicals mainly elicit strong attraction for males who have been frequently reported to feed on the source of the odor. According to (Landolt and Phillips, 1997), many phytophagus insects aggregate at the primary feeding and oviposition sites preferred by females. In some species, exposure of males to particular plant compounds of hosts species confers a mating advantage over individuals denied access to such substances (Shelly, 2006). Males of oriental fruit fly *Bactrocera dorsalis* are strongly attracted to methyl eugenol which is ingested and used as a precursor in the synthesis of sex pheromone (Tan and Nishida, 1996). Attraction of *Z. curcubitae* to the three tomato varieties may be indicative of the presence of certain group of volatile organic compounds that are biosynthesized and secreted during the tomato host initial stages of growth and detected by antennal olfactory receptors of both immature and mature males and females *Z. cucurbitae*. The findings on attraction responses of immature males and females *Z. cucurbitae* to odor of the three varieties of tomato in vegetative and flowering stages are similar to earlier reports that sexually immature males and females *Bactrocera cucurbitae*, *Bactrocera dorsalis*, and *Ceratitis capitata* are attracted to bacteria volatiles growing in soya meal. Besides host finding, feeding, and oviposition, plant chemicals may also influence developmental rates and the progress of maturation (Kouloussis and Katsoyannos, 2006).

GC/MS analysis of tomato volatile organic compounds revealed chemical similarities in the head space of the Anna F1, Cal-J and MM varieties in vegetative and flowering stages of growth dominated by monoterpenes and sesquiterpenes. This may explain why the three groups of *Z. cucurbitae* were attracted to all tomato varieties in their vegetative and flowering stages of growth. The two groups of compounds are members of terpenes that form one of the dominant classes of volatile organic compounds released by plants (Dudareva *et al.,* 2004; Pichersky & Gershenzon, 2002). An evaluation of differences in

volatile composition of the three varieties of tomato in both vegetative and flowering stages has shed an insight in clarification of their role in odor based host plant discrimination that led to stimulation of response. Indeed, the fact that Cal-J was significantly more attractive than Anna F1 which was more attractive than moneymaker during the vegetative and flowering stages has been confirmed by the fact that quantitatively and qualitatively, the composition of volatile organic compounds emanating from the vegetative and flowering stages of the three varieties of tomato are different, and odor perception is a key to selection of the most suitable host plant variety by *Z. cucurbitae*.

The four groups of *Z. cucurbitae* showed high antennal responses to the odors of Cal J compared to the other two varieties which differed in quality and quantity of VOCs. My results lend to support of previous reports which indicated that for many insect pests, the quality and quantity of olfactory cues are very important and are used by the insect to orientate towards and accept a specific host plant odor within a plant patch (Bruce and Pickett, 2011). This is due to the fact that, plant volatiles form a vital part of the total phagostimulation flavor of the plant and potential nutrient content of the plant is a complimentary factor (Saxena and Okech, 1985). GCEAD comparison of Ashley cucumber and Cal J tomato variety revealed 7 active compounds which were among the shared VOCs in the two plant odors. The shared antennal active VOCs were higher in amount in Cal J odor profile than in Ashley cucumber, this might explain the host shift of *Z. cucurbitae* to Solanaceous plant (tomato).

The low attractiveness of vegetative and flowering moneymaker plant volatile organic compounds to both mature and immature males and females' *Z. cucurbitae* indicates that in a monoculture farming situation, moneymaker variety may be likely to be susceptible to lower rate of infestation than Cal-J and anna F1 hence lower damage. Likewise, the high attractiveness of Cal J in both early stages of growth to mature and immature adult *Z. cucurbitae* implies that in cultivating the three varieties, Cal J may be susceptible to

the highest rate of infestation. Shared volatiles between Solanaceous and cucurbitaceous plants have made Solanaceous plants become major hosts for *Z. cucurbitae.*

5.2 Conclusions

In conclusion, olfactory behavioral responses of *Z. curcubitae* showed the flies being attracted to different tomato varieties and Cucumber attributed to similarity of VOCs among the plants. In addition, high quality and quantity of VOCs in Cal J tomato variety may explain its high attractiveness to *Z. cucurbitae.*

The identified volatile organic compounds produced by different tomato varieties and Cucumber showed similarities and differences in the compositions and concentrations.

Similar VOCs identified from the three tomato varieties and cucumber that elicited antennal responses to *Z. cucurbitae* were among the shared volatiles in the three tomato varieties and cucumber hence explaining the attractiveness of Z. *cucurbitae.*

5.3 Recommendations

- Further laboratory bio-assays should be done using synthetic standards of identified antennal responsive components of tomato odor to identify the specific VOCs that are attractants to *Z. cucurbitae* with their most effective concentrations.
- More tests should be carried out using synthetic standards of VOCs that are highly attractive in bioassays with their determined concentration in the field using traps.
- In the management of *Z. cucurbitae* for which monitoring is a desirable tool, the design of an efficient odor-baited trap should be based on a careful determination of the quality and quantity of appropriate odor eliciting most positive responses.

- More research work to be carried out in order to determine the relationship between active odor components and available resources on the host plant for larval development.

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APPENDICES

Appendix I: Publication on "Cucumber and Tomato Volatiles: Influence on attraction in the Melon Fly Zeugodacus cucurbitae (Diptera: Tephritidae)"

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Cucumber and Tomato Volatiles: Influence on Attraction in the Melon Fly *Zeugodacus cucurbitae* (Diptera: Tephritidae)

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Supporting Information

ABSTRACT: The main hosts of the melon fly *Zeugodacus cucurbitate* are cultivated and wild cucurbitaceous plants. In eastern Africa, the melon fly is a major pest of the Solanaceae plant *Solanum lycopersicum* (tomato). We hypothesized that shared species-specific volatiles may play a role in host attraction. We tested this hypothesis by comparing the olfactory responses of the melon fly to *Cucumis sativus* (cucumber) (Cucurbitaceae) and tomato plant odors in behavioral and electrophysiological assays, followed by chemical analysis to identify the key compounds mediating the interactions. Our results identified 13 shared components between cucumber and tomato plant odors. A synthetic blend of seven of the shared components dominated by monoterpenes at concentrations mimicking the volatile bouquet of cucumber and tomato attracted both sexes of the melon fly. Our results suggest that the presence and quantity of specific compounds in host odors are the main predictors for host recognition in *Z. cucurbitate*.

KEYWORDS: *Zeugodacus cucurbitate, electrophysiology, melon fly, kairomone, Cucumis sativus, Solanum lycopersicum*

INTRODUCTION

Phytophagous insects exploit plant volatiles to locate their food sources, find suitable oviposition sites, and in some insects to find mates.[1,2] Many studies have shown that interactions between a phytophagous insect and its host plant are influenced by several factors including the quality and quantity of volatile organic compounds released by the plant, which in turn are determined by plant species, plant part, cultivar, and whether the plant is undamaged, mechanically, or herbivore-damaged.[1,2] For some polyphagous tephritid fruit flies such as the invasive species, *Bactrocera invadens*, it has been shown that shared host odors contribute to host plant finding.[3]

The melon fly, *Zeugodacus cucurbitae* (Coquillett), another tephritid fruit fly, has a wide host plant range. In Africa, it is an economically important pest of horticultural crops, attacking a wide range of fruits and vegetables, and causing losses of 30−100%, depending upon the season. Its polyphagous nature is demonstrated by its ability to complete its life cycle on several host plants belonging to different families that may reflect the presence of particular attractants in these plants.[4] However, its preferred hosts are both cultivated and wild cucurbitaceous plants.[4] Ovipositing females of the melon fly attack host plants and lay up to 300 eggs in flowers, stems, and leaf stalks, with resultant developing larvae feeding and causing damage to plant tissues.[5] Additionally, the damaged tissues serve as entry points for opportunistic microorganism infection leading to further damage.[6] Significant efforts have been made in the past to control the melon fly and other damaging fruit flies using integrated management approaches. Examples of these approaches include fruit bagging, field sanitation, host plant resistance, use of soft insecticides and traps baited with protein, and semiochemical lures that target males.[7,8]

Traps baited with host plant odors also have been used in attempts to target females. For example, a previous study showed that freshly sliced host fruit odors play an important role in attracting females in cage experiments.[9] These experiments demonstrated that odors released by the cucurbitaceous fruits cucumber, zucchini, bitter melon, kabocha, cantaloupe, and ivy gourd attracted the melon fly, with cucumber and cantaloupe fruit odors being more attractive than tomato fruit odors to females. This study also showed that female attraction was stage-dependent, with protein-fed females more responsive than protein-deprived females to fruit odors. However, in this study, the volatiles mediating attraction were not identified. Another study on the melon fruit fly focused on fresh and aged puréed cucumber fruit odors and identified several candidate attractive blends comprised of the compounds (*E*,*Z*)-2,6-nonadienal, (*E*)-2-nonenal, (*Z*)-6-nonenal, nonanal, (*Z*)-6-nonen-1-ol, 1-nonanol, (*E*)-2-octenal, hexanal, 1-hexanol, acetic acid, and 1-octen-3-ol. In an outdoor rotating olfactometer, McPhail traps baited with a nine-component blend derived from these compounds attracted predominantly females.[10] A more recent study using a blend comprising the seven compounds (*Z*)-6-nonenal, (*Z*)-6-nonen-1-ol, 1-octen-3-ol, (*E*,*Z*)-2,6-nonadienal, (*E*)-2-nonenal, hexanal, and 1-hexanol loaded in PVC plugs or glass capillaries

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was found to be effective in trapping the melon fly.[11] Surprisingly, the role of host plant foliar and floral volatiles in attracting females of the melon fly has not been reported.

In this decade, the melon fly has emerged as one of the most devastating pests of the solanaceous plant tomato, *Solanum lycopersicum* Mill, in eastern Africa.[4,12] It is well-known that biological and environmental factors drive the host range expansion in insect species, transforming some species to become major pests of less preferred hosts.[13] In this context, the plant chemistry due to genetic manipulation and biotic and abiotic stressors could alter both the quality and the quantity of host plant volatiles, as well as olfactory responses of pests and parasitoids associated with the host plant.[14,15] Additionally, the presence and extent of cultivation of congeneric plants in the landscape may also contribute to enhancing the pest status of an insect.[16] Given this scenario, it is therefore important to understand the chemical basis of the interaction between the melon fly and cucumber and tomato host plants. Knowledge of this interaction will likely contribute to the development of additional kairomone-based lures for use in surveillance of both sexes of the melon fly during their early stages of establishment.[17]

Although it has been postulated that plant odors are responsible for melon fly interactions with cucumber[6,10] and tomato,[18] limited attempts have been made to identify the specific plant volatiles attractive to melon flies. Detailed understanding of the chemical ecology of the pest in question before applying that knowledge to pest management is important.[19,20] In the current study, we investigated the olfactory basis of the interaction between the melon fly and tomato plants, and compared this interaction to that involving its preferred natural host plant, cucumber. Specifically, we used electrophysiological, chemical, and behavioral analyses to identify the chemicals mediating the interaction.

MATERIALS AND METHODS

Insects. Melon flies, *Zeugodacus cucurbitae*, were obtained from a first generation colony maintained on sugar-yeast feed at the Animal Rearing and Containment Unit (ARCU) of the International Centre of Insect Physiology and Ecology (*icipe*), Duduville Campus, Nairobi, Kenya. The ARCU colony was established from wild melon flies collected from infested tomato fruits at Chala (03°15.371′ S, 037°44.604′ E, elevation 924 m) and Mbomeni (03°26.301′ S, 037°40.835′ E, elevation 736 m) sub-counties in Taita-Taveta County, Kenya, in January 2014.

The melon fly was reared as previously described[21] with few modifications. Ten ripe tomato cultivar "Cal-J" fruits, to serve as egg-laying substrates, were bought from the local farmers and were placed in plastic containers for 10 d to ripen and to ensure they were free of insect larvae before use. Tomato fruits free of larvae were washed in distilled water, dried with cotton cloth, and then placed in a clean Petri-dish (8 cm diameter; 1 cm height) and exposed to 80 (sex ratio 1:1) mature adult melon flies (16−20 d old) in a rearing clear ventilated Perspex cage (35 cm × 30 cm × 30 cm) for 24 h to oviposit. The tomato fruits with eggs were then transferred into a clean sterile plastic container (20 cm long × 14 cm wide × 8 cm high) with a lid fitted with 0.5 mm diameter pore size netting material in the middle to facilitate aeration. The larvae were then allowed to develop up to the third instar stage before being transferred into sterilized-sieved-sand for pupation. Pupae were separated from the sand through a 1 mm mesh size sieve after which they were transferred into a holding cage until eclosion. Adults that emerged were then reared in a clear, ventilated, Perspex rearing cage (35 × 30 × 30 cm) in a room maintained at 27 ± 2 °C, 65 ± 5% RH, and 12:12 h L:D). They were fed an artificial diet (2:1 volumetric mixture) of dry sugar and enzymatic yeast hydrolysate ultrapure (United States Biochemical, Cleveland, OH) and watered in Petri dishes filled with pumice granules to prevent fly drowning.

Plants. Seeds of cucumber cultivar "Ashley" and tomato cultivar "Cal-J", which are commonly grown by small scale farmers and common hosts of the melon fly in Kenya, were purchased from Simlaw Seeds Co. Limited (Nairobi, Kenya). They were established separately in seedling trays obtained from Chamak Polymers Pvt. Ltd. (Ahmedbad, India), containing 2:1 sterilized fine sand and sieved farmyard manure mixture and moistened with water. The seedling trays were kept in a screen house (26 ± 2 °C, 55 ± 5% RH, 12:12 h L:D) and were watered twice daily until the seedlings were 5−6 weeks old. They were then transplanted into 5 L pots filled with 3:2:1 (v/v/v) volcanic red soil, sand, and manure mixture until they were 3 months old. This vegetative stage for both cucumber and tomato plants was used for all of the behavioral and chemistry experiments. They were transferred to the laboratory approximately 12 h prior to conducting bioassays to allow the plants to acclimatize.

Dual Choice Olfactometer Assays. Behavioral assays were carried as previously described[22] in an olfactometer (30 cm × 31 cm × 100 cm) with some modifications. Charcoal-purified and humidified air was split into two equal streams, with each passed into the arm of the olfactometer at a flow rate of 350 mL/min. An electrically powered vacuum fan placed at the top of the midsection pulled odors (including ones emitted by flies) out of the olfactometer at 700 mL/min. Because the melon fly is diurnal, the olfactometer was illuminated by placing two white 40 W fluorescent light bulbs producing 853 lx illuminations above it. The assays were conducted in a laboratory under controlled conditions of 27 °C and 70% RH at between 11:00 am and 3:00 pm, the time of day when the melon fly is active.[5,11] One arm of the olfactometer was permeated with odors from an intact plant whose vegetative parts were held in an oven-baked plastic bag (45 cm × 40 cm) (Classic Consumer Products, Inc., Englewood, NJ), while a similar bag without the plant served as the control. In pairwise comparisons, the oven bags held different potted plants.

To test responses of mated male and female melon flies (16−20 d old) to treatments, groups of 10 individuals were observed in separate assays using the following odor pairs: (a) cucumber plant against control (air); (b) tomato plant against control (air); and (c) cucumber plant against tomato plant. The positions of the test plant and control in the olfactometer arms were interchanged between runs to prevent any positional bias, and the arms were cleaned with acetone and oven-dried to remove residual odors.

In each assay, 10 melon flies were released at the center of the olfactometer, and this was replicated five times using different plants and melon flies on different days. The number of melon flies responding to the test and control odors was counted in each run after 10 min. Between experiments, air was passed through the olfactometer arena for 5 min, without the treatments, to remove any volatile residues and then cleaned with an acetone cotton swab and flushed with air again.

Collection of Volatiles. Volatiles released from the intact aerial parts of cucumber and tomato plants (3 months old) were collected as previously described[22,23] with a few modifications. Test plants were transferred to the laboratory 12 h to condition them prior to collecting volatiles. To collect volatiles, the test plants were enclosed in oven bags (45 cm × 40 cm) that had been precleaned in an oven at 100 °C for 12 h and thereafter allowed to cool. A stream of charcoal-purified and humidified air was pushed into the bag, and a vacuum line connected to a Super-Q trap (30 mg) (Analytical Research System, Gainesville, FL) pulled volatiles from the bag into the trap at 350 mL/min for 6 h. Volatiles were collected similarly from an oven bag with no plant (control). Each plant was sampled five times using a different plant in each sample. Volatiles were eluted using 100 μL of dichloromethane (Analytical grade, Sigma-Aldrich, St, Louis, MO) and stored at −80 °C prior to chemical analysis.

Analysis of Volatiles. Coupled gas chromatography−mass spectrometry (GC−MS) analysis was carried out on an HP7890A gas chromatograph coupled to an HP5975C inert XL EI/CI mass spectrometer (Agilent, Palo Alto, CA). The column used was a 30 m × 0.25 mm i.d., 0.25 μm Agilent HP-5 MS capillary column. An aliquot (1 μL) of extract of the volatiles was injected into the GC using splitless

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Table 1. Ratios of Individual Synthetic Shared EAD-Active Compounds in Cucumber and Tomato Odor Blends Tested in Bioassays[a]

	cucumber plant odor (ng/μL)			tomato plant odor (ng/μL)		
compounds	blend A	blend B	blend C	blend D	blend E	blend F
o-cymene	15	30	7.5	21	42	10.5
p-cymene	11	22	5.5	1	2	0.5
(*E*)-*β*-ocimene	3	6	1.5	3	6	1.5
α-phellandrene	1	2	0.5	26	52	13
β-phellandrene	83	166	41.5	200	400	100
methyl salicylate	1	2	0.5	1	2	0.5
α-cedrene	8	16	4	1	2	0.5
total	122	244	61	253	506	126.5

[a]Blends A and D represent the natural ratios for cucumber and tomato odors, respectively.

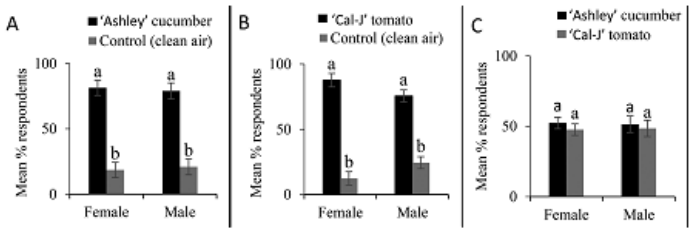

Figure 1. Behavioral responses of female and male *Z. cucurbitae* to odor of (A) cucumber plant and (B) tomato plant against control; and (C) pairwise comparison using odors of cucumber and tomato plants in the olfactometer. Pairs of bars with different letters indicate significantly different responses $P < 0.05$.

mode (270 °C; 6.83 psi), using helium as the carrier gas at a flow rate of 1.2 mL/min. The oven temperature was held at 35 °C for 3 min, then increased at the rate of 10 °C/min to 280 °C and maintained at this temperature for 10 min. Mass spectral data were obtained using electron impact mode at 70 eV. The detected compounds in the volatiles were tentatively identified by comparison of their retention times and mass spectral data with NIST07 library data and confirmed by comparison of their retention times and mass spectral fragmentation patterns with authentic samples where available. Retention times of *n*-alkane (C_8–C_{31}) standards were used to determine retention indices (RIs) of the identified compounds.

Electrophysiology. Coupled gas chromatography/electroantennographic detection (GC–EAD) analysis was carried out on a Hewlett-Packard 5890 Series II gas chromatograph (Agilent, Santa Clara, CA). The column used was a 30 m × 0.25 mm i.d., 0.25 μm, Agilent HP-5 MS capillary column, with nitrogen as the carrier gas at 1 mL/min. Injection was splitless at 250 °C with a split valve delay of 1 min. Oven temperature was held at 35 °C for 3 min, increased to 280 °C at 10 °C/min, and then held at this temperature for 10 min. Column effluent was split 1:1 with a fused silica outlet splitter (Alltech Associates Inc. Deerfield, IL) for simultaneous detection by electroantennographic detector (EAD) and flame ionization detector (FID). Silver wires in 1.5 mm internal diameter glass capillaries electrodes filled with Ringer solution served as reference and recording electrodes. The base of the excised head of 16–20 d old male or female of *Z. cucurbitae* was connected to the reference electrode and the tip of the antennae connected to a recording electrode. The electrodes were connected to an AC/DC amplifier in DC mode (Syntech, Kirchzarten, Germany). FID and EAD signals were detected through an INR-II probe (Syntech, Hilversum, The Netherlands) captured and processed with an IDAC-4 data acquisition controller, and data were analyzed using GC–EAD 2000 (Syntech, Hilversum, The Netherlands) software on a computer. An aliquot (3 μL) of volatile extract was analyzed with either fresh male or female antennae and was replicated three times. Identification of EAD-active components was carried out by GC–MS using the same oven conditions as described above.

For quantitation, stock solutions of the monoterpene *β*-phellandrene and the sesquiterpene *α*-cedrene (2000 ng/μL) were prepared and then serially diluted to give a range of concentrations from 0.005 to 1200 ng/μL. The GC conditions for quantitative analyses including injection operation of the standards, capillary column dimensions, and oven temperature were the same as those for GC–MS. Compound quantitation was done using calibration curves (comparison of peak areas and concentrations) generated for *β*-phellandrene and *α*-cedrene. Quantitated chemical composition of odors was expressed in ng/plant/h.

Chemicals. *o*-Cymene, *p*-cymene, *α*-phellandrene, methyl salicylate, and *α*-cedrene (>95% purity) were purchased from Sigma-Aldrich (St. Louis, MO), while *β*-phellandrene was donated by Prof. Phil Stevenson (University of Greenwich, UK) and *β*-ocimene was previously provided by coauthor P. Teal.

Bioassays with Synthetic Blends and Single Compounds. Behavioral responses of female and male *Z. cucurbitae* were tested using blends comprised of seven shared EAD-active compounds (*o*-cymene, *p*-cymene, *α*-phellandrene, *β*-phellandrene, *β*-ocimene, methyl salicylate, and *α*-cedrene) identified from both cucumber and tomato plant odors. Cucumber plant odor blends were tested at three concentrations and included blends A, B, and C. Blend A comprised the naturally occurring amounts of EAD-active components in the volatile extracts; blend B contained double the amounts in blend A; while blend C contained one-half the amounts in blend A (Table 1). Tomato plant odor blends at similar doses were blends D (naturally occurring ratio), E, and F (Table 1). Individual components in the blends were also tested at five concentrations: 0.32, 1.6, 8, 40, and 200 ng/μL. Each individual common component and blend were prepared in hexane and tested (100 μL of sample) against solvent (hexane) control (100 μL) separately. The treatments and controls were impregnated into 100 mg of Luna dental roll (Roeko, Langenau, Germany) and air-dried for 5 min at room temperature to allow the solvent to evaporate prior to bioassays. All of the tests were replicated five times with freshly impregnated dental rolls used for each replicate. The position of test and control odor sources was alternated after every replicate.

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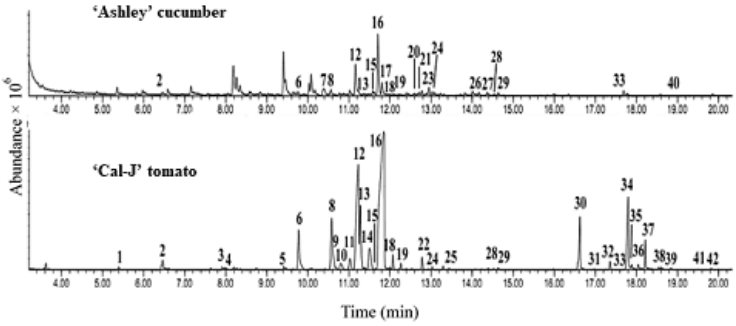

Figure 2. GC−MS profile of cucumber cultivar "Ashley" and tomato cultivar "Cal-J" plant odor. Numbers correspond to compounds listed in Table 2.

A group of 10 mated females (16−20 d old) was released in each of the five replicates and given a choice between treatment and control odors during a 10 min period. The numbers responding to the treatment and control were recorded for each group. Similar experiments were repeated for the 16−20 d old mated males.

Statistical Analyses. The number of melon flies in each arm of the olfactometer at the end of 10 min observation period was recorded and the data converted to a percentage based on the number of respondents, then used as a measure of response as previously described[22] from the formula PR = $[(SS - NSS)/(SS + NSS)] \times 100$ (where SS is the number of melon flies responding to test odors and NSS the number of melon flies responding to control odors). Where equal numbers of melon flies occur in each arm, the PR would be zero, and 100 if all melon flies preferred one side of the olfactometer. The number of melon flies that did not respond was not included in the statistical analysis. Female and male responses to odors of (a) cucumber plant versus control (air); (b) tomato plant versus control (air); (c) cucumber plant versus tomato plant; (d) blend A versus control (solvent); (e) blend B versus control (solvent); (f) blend C versus control (solvent); (g) blend D versus control (solvent); (h) blend E versus control (solvent); and (i) blend F versus control (solvent) were converted to percentage response (PR) and later subjected to a sample chi-square (χ^2) test to examine if both female and male responses differed from zero. All statistical analyses were done at an α level of 0.05 using R software.[19]

RESULTS

Responses of the Melon Fly to Host Plant Volatiles. Both female (PR = 81.8%; $\chi^2 = 8.26$; $P < 0.01$) and male (PR = 76.7%; $\chi^2 = 4.34$; $P < 0.01$) melon flies were significantly more attracted to the odor of the cucumber plant than of the control (Figure 1A). Similarly, female (PR = 87.5%; $\chi^2 = 9.41$; $P < 0.01$) and male (PR = 76.5%; $\chi^2 = 5.14$; $P < 0.01$) melon flies were significantly more attracted to the odors of the tomato plant than of the control (Figure 1B). In paired assays, there was no significant difference in attraction of both female (PR = 52%; $\chi^2 = 0.02$; $P = 0.64$) and male (PR = 51%; $\chi^2 = 0.01$; $P = 0.76$) melon flies to the odors released from cucumber and tomato plants (Figure 1C).

Analysis of Volatiles. GC−MS analyses identified 21 and 34 components in cucumber and tomato plant odors, respectively, dominated by terpenes (Figure 2 and Table 2). Thirteen compounds including hexanal, α-pinene, *o*-cymene, δ-2-carene, α-phellandrene, *p*-cymene, β-phellandrene, (*E*)-β-ocimene, γ-terpinene, *n*-nonanal, methyl salicylate, *n*-decanal, and α-cedrene were identified as common to the odors of both plants. Of the shared components, the tomato plant emitted relatively greater amounts of α-phellandrene and β-phellandrene, approximately 26- and 2.5-fold more of the two components than in cucumber plant odor, respectively (Table 2). Conversely, δ-2-carene, nonanal, and α-cedrene were approximately 20-, 6-, and 100-fold more abundant in cucumber odor than in tomato odor, respectively. Additionally, (*E*)-2-hexenal and (*Z*)-3-hexenol, toluene, (*E*,*E*)-2,4-hexadienal, (*E*)-isolimonene, β-pinene, myrcene, α-terpinene, terpinolene, *allo*-ocimene, δ-elemene, α-copaene, β-elemene, (*E*)-caryophyllene, γ-elemene, α-humulene, germacrene D and B, and caryophyllene oxide were identified as specific to tomato odors. Cucumber-specific odors were identified as benzaldehyde, benzyl alcohol, (*E*)-linalool oxide, linalool, and naphthalene (Table 2)

In GC−EAD analysis of the cucumber and tomato plant odors, antennae of both sexes of the melon fly detected 10 EAD-active components (Figure 3) of which seven were consistently detected in at least two out of the three runs. These seven components were among the 13 shared components. In general, antennae of the female melon flies appeared to be more sensitive in detecting the plant odors than those of males. The identities of the seven EAD-active components were confirmed by comparison of GC−EAD and GC−MS retention times and fragmentation patterns with those of authentic standards of *o*-cymene, *p*-cymene, α-phellandrene, β-phellandrene, β-ocimene, methyl salicylate, and α-cedrene (Figures 3 and 4).

Behavioral Responses to Synthetic Chemicals. Olfactometer assays showed that both sexes responded to the seven-component blend formulated to represent cucumber and tomato plant odors relative to control to varying levels (Figure 5). For the cucumber plant odor, females responded significantly to the seven-component blend A (PR = 75%, $\chi^2 = 0.93$, df = 1, $P < 0.01$) and blend B (PR = 70%, $\chi^2 = 16.84$, df = 1, $P < 0.01$) with nonsignificant response to blend C (PR = 55%, $\chi^2 = 0.07$, df = 1, $P = 1.00$). Males, on the other hand, responded significantly to blend A (PR = 80%, $\chi^2 = 14.66$, df = 1, $P = 0.01$) and blend B (PR = 80%, $\chi^2 = 2.61$, df = 1, $P < 0.01$) with nonsignificant response to blend C (PR = 77%, $\chi^2 = 0.95$, df = 1, $P = 0.44$) (Figure 5).

For the tomato odor synthetic representative, females responded significantly to the seven-component blend D (PR = 70.4%, $\chi^2 = 0.74$, df = 1, $P = 0.01$) and blend F (PR = 66.7%, $\chi^2 = 0.5$, df = 1, $P = 0.01$) with nonsignificant response to blend E (PR = 56.5%, $\chi^2 = 0.46$, df = 1, $P = 0.11$). In addition, males responded significantly to all blends: blend D (PR = 72%, $\chi^2 = 2.56$, df = 1, $P = 0.01$), blend E (PR = 70%, $\chi^2 = 2.43$, df = 1, $P =$

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Table 2. Volatiles Emitted by Cucumber Cultivar "Ashley" and Tomato Cultivar "Cal-J"

peak no.	retention time (min)	RI[a]	RI_L[b]	compound	cucumber cultivar "Ashley" (ng/h ± SEM)	tomato cultivar "Cal-J" (ng/h ± SEM)
1	5.36	748	762[D]	toluene[c]		1.9 ± 0.6
2	6.40	786	801[A]	hexanal[c]	trace	3.2 ± 3.0
3	7.90	844	852[B]	(*E*)-2-hexenal[c]		5.0 ± 4.0
4	8.00	848	836[C]	(*Z*)-3-hexenol[c]		1.0 ± 0.6
5	9.33	899	911[D]	(*E*,*E*)-2,4-hexadienal[c]		0.18 ± 0.0
6	9.76	918	932[A]	*α*-pinene[c]	8.6 ± 5.0	12.1 ± 10.7
7	10.36	945	953[D]	benzaldehyde[c]	17.3 ± 2.0	
8	10.59	956	1014[D]	*o*-cymene[c]	15.2 ± 8.8	20.5 ± 15.3
9	10.68	960	983[D]	(*E*)-isolimonene[d]		1.0 ± 0.4
10	10.79	965	980[A]	*β*-pinene[c]		4.1 ± 1.9
11	11.00	974	991[B]	myrcene[c]		4.3 ± 3.6
12	11.16	981	1001[A]	*δ*-2-carene[c]	49.8 ± 28.7	2.2 ± 0.3
13	11.23	985	1002[A]	*α*-phellandrene[c]	trace	26.0 ± 9.6
14	11.49	996	1014[A]	*α*-terpinene[c]		13.1 ± 7.1
15	11.58	1000	1020[A]	*p*-cymene[c]	10.6 ± 6.1	trace
16	11.74	1010	1031[A]	*β*-phellandrene[c]	83.1 ± 48.0	199.6 ± 70.2
17	11.81	1014	1031[B]	benzyl alcohol[c]	17.7 ± 2.7	
18	12.06	1029	1044[A]	(*E*)-*β*-ocimene[c]	trace	3.0 ± 2.1
19	12.26	1041	1054[A]	*γ*-terpinene[c]	trace	2.8 ± 1.0
20	12.67	1066		unknown 1	6.1 ± 1.3	
21	12.70	1068	1068[D]	(*E*)-linalool oxide[c]	11.1 ± 6.4	
22	12.78	1073	1086[A]	terpinolene[c]		4.8 ± 1.3
23	12.96	1084	1095[A]	linalool[c]	14.6 ± 8.4	
24	13.03	1088	1087[D]	nonanal[c]	8.8 ± 5.1	1.4 ± 0.2
25	13.44	1112	1119[D]	*allo*-ocimene[d]		0.6 ± 0.2
26	14.01	1146		unknown 2	10.4 ± 6.0	
27	14.36	1167	1178[D]	naphthalene[d]	6.6 ± 1.8	
28	14.53	1176	1199[A]	methyl salicylate[c]	trace	0.5 ± 0.1
29	14.63	1182	1203[D]	decanal[c]	trace	0.7 ± 0.2
30	16.61	1313	1335[A]	*δ*-elemene[d]		4.0 ± 3.7
31	16.90	1334	1378[B]	*α*-copaene[c]		0.1 ± 0.0
32	17.35	1366	1389[A]	*β*-elemene[d]		0.6 ± 0.5
33	17.69	1391	1413[B]	*α*-cedrene[c]	8.3 ± 4.8	1.1 ± 0.0
34	17.77	1396	1417[A]	(*E*)-caryophyllene[c]		7.0 ± 6.0
35	17.89	1405	1427[B]	*γ*-elemene[d]		0.8 ± 0.4
36	18.05	1418		unknown 3		0.1 ± 0.4
37	18.21	1430	1454[B]	*α*-humulene[c]		2.1 ± 1.9
38	18.55	1456	1480[D]	germacrene D[d]		0.2 ± 0.2
39	18.85	1479		unknown 4		0.2 ± 0.2
40	18.87	1481	1514[D]	butylated hydroxytoluene[c]	trace	
41	19.51	1532	1556[D]	germacrene B[d]		0.1 ± 0.1
42	19.83	1559	1589[B]	caryophyllene oxide[c]		0.2 ± 0.2

[a]Retention index relative to C8−C31 *n*-alkanes on an HP-5 MS column. [b]Retention index obtained from literature: (A),[48] (B),[49] (C),[50] (D).[51] [c]Compound whose identity was established on the basis of comparison of retention time and mass spectra data with authentic standard. [d]Compound identified tentatively based on library data only.

0.02), and blend F (PR = 78.6%, χ^2 = 0.36, df = 1, P = 0.01) (Figure 5D−F).

Similarly, both sexes of the melon fly showed significant sex- and concentration-dependent responses to the seven EAD-active compounds tested singly (Figure 6). In general, males were more responsive than females to the seven compounds to varying levels across all of the concentrations tested, with significant responses to methyl salicylate at three different concentrations: 0.32 ng/μL (PR = 65%, χ^2 = 15.36, df = 1, P = 0.01), 1.6 ng/μL (PR = 75%, χ^2 = 10.08, df = 1, P < 0.01), and 8 ng/μL (PR = 72.2%, χ^2 = 5.44, df = 1, P < 0.05) (Figure 6). Of the seven compounds, (*E*)-*β*-ocimene elicited the weakest response from both sexes of the melon fly, whereas *β*-phellandrene elicited significant responses from both sexes, with males at higher concentrations: 40 ng/μL (PR = 75%, χ^2 = 6.13, df = 1, P = 0.01) and 200 ng/μL (PR = 77.8%, χ^2 = 9, df = 1, P = 0.01) for males; and (PR = 71.4%, χ^2 = 6.10, df = 1, P = 0.01) and (PR = 81.3%, χ^2 = 10.13, df = 1, P < 0.01) for females, respectively. A significant response from both sexes was observed to a high concentration of *o*-cymene, intermediate concentrations of *p*-cymene, and at the lowest concentration of *α*-cedrene (Figure 6).

DISCUSSION

Polyphagous insects are known to use a variety of chemical blends to locate their hosts for feeding and oviposition. Most polyphagous insects are lepidopterans, coleopterans, hetero-

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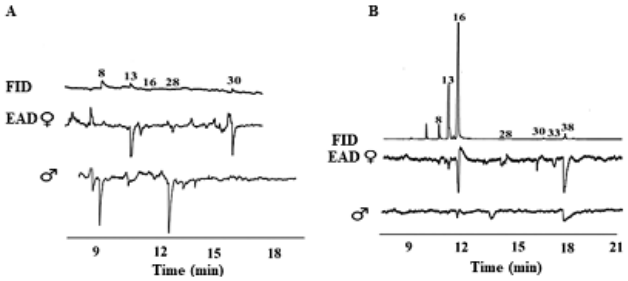

Figure 3. Representative GC–EAD profiles showing either female or male *Z. cucurbitae* antennal detection of specific components in (A) tomato and (B) cucumber plant odors: for example, *o*-cymene, **8**; α-phellandrene, **13**; β-phellandrene, **16**; methyl salicylate, **28**; δ-elemene, **30**; α-cedrene, **33**; and germacrene D, **38**. For each plant species, female and male detection of all seven components could only be established from more than one GC–EAD run. Numbers correspond to compounds listed in Table 2.

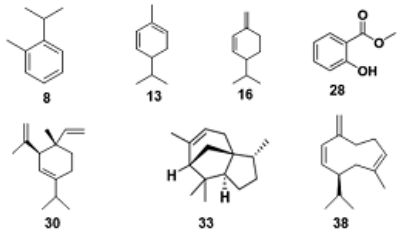

Figure 4. Structures of shared EAD-active components identified in tomato and cucumber plant odors.

pterans, and tephritids, to name a few, with a wide host distribution.[23−26] The melon fly *Z. cucurbitate*, a tephritid, fits into this group of insects. Our data indicate that both males and females of *Z. cucurbitate* responded to odors of cucumber and tomato plants relative to air controls, confirming the important role olfactory cues play in the polyphagous nature of host location process of the melon fly. Our results corroborate those previously reported for the polyphagous melon fly detecting and/or responding to a wider array of chemical blend than do oligophagous or monophagous fruit flies.[26−29] This result was expected because both sexes of *Z. cucurbitate* seek food and shelter, with plants not only providing this resource for insects in general, but also oviposition spots for females. In pairwise tests using odors of cucumber and tomato plants, both sexes of the melon fly were less discriminatory in their response, suggesting a possible overlap of the composition of the volatiles emitted by cucumber and tomato plants. It also suggests that although rearing *Z. cucurbitate* on fruits of the tomato cultivar "Cal J" could introduce learning behavior in the fruit fly, the effect did not appear significant in the presence of cucumber odors. Moreover, the composition of fruit odor may be different from that of foliar and floral odor. A previous study found that the melon fly showed a significant preference for freshly sliced cucumber odor over tomato odor.[10] Consistent with previous findings, it is not uncommon to find differences in insect responses to host odors, especially to different parts of the host

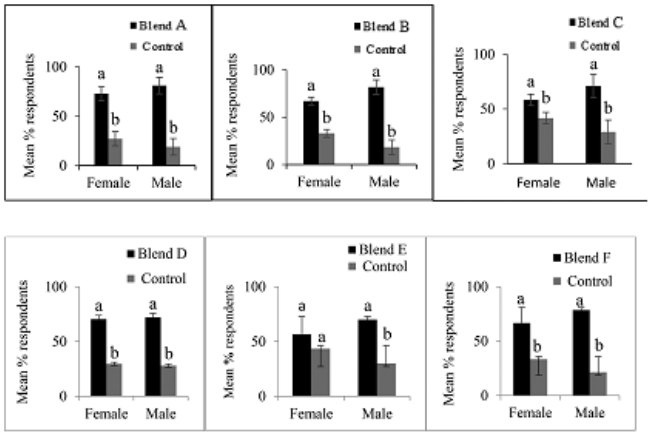

Figure 5. Responses of female and male *Z. cucurbitae* to seven-component (*o*-cymene, *p*-cymene, α-phellandrene, β-phellandrene, methyl salicylate, (*E*)-β-ocimene, and α-cedrene) blends against controls in the olfactometer. Blend A comprises naturally occurring amounts of EAD-active components in the volatile extract of cucumber plant; blend B contains double the amounts in blend A; while blend C contains one-half the amounts in blend A. Tomato odor blends at similar doses are blends D, E, and F at $P < 0.05$.

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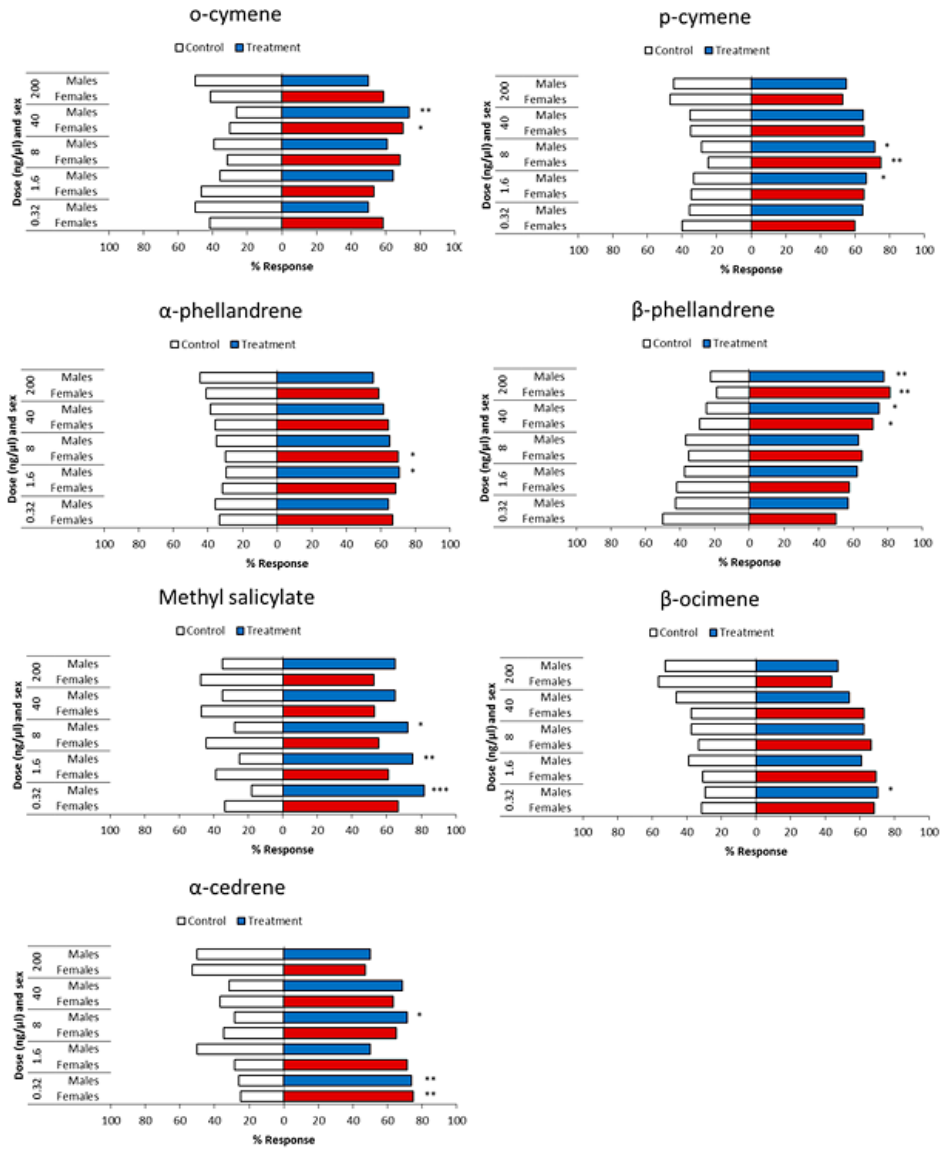

Figure 6. Responses of female (red) and male (blue) *Z. cucurbitae* to seven shared individual EAD-active components identified in cucumber and tomato plant odors tested singly at different concentrations at $P < 0.05$. "*" and "**" indicate statistically different responses at $P < 0.05$ and $P < 0.01$, respectively.

such as leaves, flowers, and fruits,[18,30] as well as to different cultivars[31,32] or developmental stages[33] of the same host plant. Insect differential responses to host odors are even more striking when comparing undamaged and damaged host plant odors and between odors released from different undamaged and damaged host plant species.[34] Thus, in a situation whereby different host plant species (undamaged or damaged) release volatiles that overlap in composition, as found in the present study for cucumber and tomato plants, this scenario can play a role in the host expansion of an insect pest such as the melon fly.

Chemical analysis showed distinct compositions for the odor profiles for cucumber and tomato plants. For example, tomato-specific odors were dominated by terpenes, whereas cucumber-specific odors comprised mainly a mixture of "green leaf volatiles", benzenoids, and terpenes. Interestingly, approximately 70% of the 13 shared compounds emitted by these two

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species of plants were dominated by terpenes. Surprisingly, in pairwise assays with intact cucumber and tomato plants, it appeared that these host-specific volatiles did not influence the response of the melon fly, even though in polyphagous insects, all volatile organic components of a host are not always essential for attraction.[3] However, our results suggest that the presence of key components, perhaps combined with or without specific background odors in host plants, determines the response of the melon fly. It is well-known that host plant odor perception and their central processing in insects could be influenced by either species-specific-, ratio-specific-, or whole-blend volatiles.[26−28,35−37]

The GC−EAD analysis of both cucumber and tomato plant volatiles followed by behavioral assays of identified compounds allowed us to determine which category of the three-odor perception described above influenced the melon fly attraction to host volatiles. GC−MS identified most of the EAD-active components as mainly terpenes: *o*-cymene, *p*-cymene, *α*-phellandrene, *β*-phellandrene, (*E*)-*β*-ocimene, and *α*-cedrene, Interestingly, our results show that these compounds are shared by the two hosts with the antennae of females detecting most of these odor components more strongly than their male counterparts. This differential sensitivity to odor components suggests that female antennal receptors are better tuned to detect host plant odors for various behavioral process such as feeding and oviposition, whereas males detect host plant odors mainly for feeding and mating purposes only, supporting previous findings on host plant odor detection in insects.[29,37] One major obstacle for investigating polyphagous herbivores is understanding how they recognize the odors of their many host plants.[32,38] Our seven-component blend derived from the shared components representing the natural volatile extract of either cucumber or tomato odor elicited significant behavioral responses from both sexes of the melon fly at varying concentrations. This is consistent with previous studies in which blends of volatile organic compounds have been found to elicit strong attraction from insect herbivores.[11,38] However, these results suggest that the melon fly's attraction was not influenced by the ratio-specific odor representing the different host plant species. Instead the presence and nature of key components in the odor blend appeared to be important in host recognition as shown in our tests with single compounds, which revealed significant sex- and concentration-dependent variability in the melon fly responses to these compounds. The fact that males responded to all seven compounds including the only benzenoid, methyl salicylate, suggests that males are less discriminatory in host plant selection. Perhaps this is because in most phytophagous insects, males tend to be more sensitive to female-produced odors, which are sex pheromones than to host plant odors.[39] It is also worth noting that both sexes responded significantly but variably to *p*-cymene, *o*-cymene, *α*-phellandrene, and *β*-phellandrene, especially the latter two monoterpenes, which are abundant in the odors of tomato plants. These results suggest that the presence of these two monoterpenes plus the background odor blend may serve as an olfactory signature for host location in the melon fly, which would require more experimentation. Also, the fact that the results of the present study showed a complete lack of an overlap in the composition of our blend and a previously reported kairomonal blend comprised of saturated and unsaturated aldehydes and alcohols identified from fresh and aged puréed cucumber fruit odors,[11] confirms our earlier suggestion of different host sources producing different volatiles.

The seven components *o*-cymene, *p*-cymene, *α*-phellandrene, *β*-phellandrene, methyl salicylate, (*E*)-*β*-ocimene, and *α*-cedrene constituting the behaviorally active blend for both sexes of the melon fly are known to play various roles in fruit flies species either individually or as part of a blend. For example, *p*-cymene and (*E*)-*β*-ocimene are components of the male-produced pheromones of *Anastrepha fraterculus* and *A. suspensa*.[40,41] Furthermore, female parasitic wasps *Psyttalia concolor* are attracted to (*E*)-*β*-ocimene,[42] while methyl salicylate serves as an attractant to the natural enemies of herbivores upon host plant infestation, parasitic microhymnoptera, and dance flies.[43] Of these compounds, the most reported to elicit behavioral activity in insects, predators, and parasitoids is methyl salicylate.

In this study, our results corroborate other findings that the melon fly uses olfactory cues for host finding. Additionally, shared host-finding volatiles in cucumber and tomato plants may explain the possible host expansion of the melon fly from its natural host cucumber to tomato plants. Semiochemical lures have been the subject of attention of most researchers investigating the behavior and chemical ecology of fruit flies. Of particular interest are lures that target males because of their unique behavior to respond to floral odors. Examples of lures that have been developed for males include 1-(4-methoxyphenyl) butan-2-one,[44] 4-(3-oxobutyl)phenyl acetate (cuelure),[45] benzyl acetate,[46] 4-(3-oxobutyl)phenyl formate (melolure),[47] and raspberry ketone trifluoroacetate.[47] Additionally, some attempts have been made to develop lures for females. A well-known lure for females is (*E*)-6-nonenyl acetate.[48] Furthermore, other researchers have investigated host plant attraction to discover additional lures for both sexes of the melon fly. For practical purposes, our results suggest the potential for exploitation of this specific group of shared plant chemicals as an attractant along with other identified chemicals in monitoring populations of the melon fly.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jafc.8b03452.

Table S1: Response analyses of female and male melon flies to the seven EAD-active compounds tested singly at different concentrations (PDF)

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Notes

The authors declare no competing financial interest.
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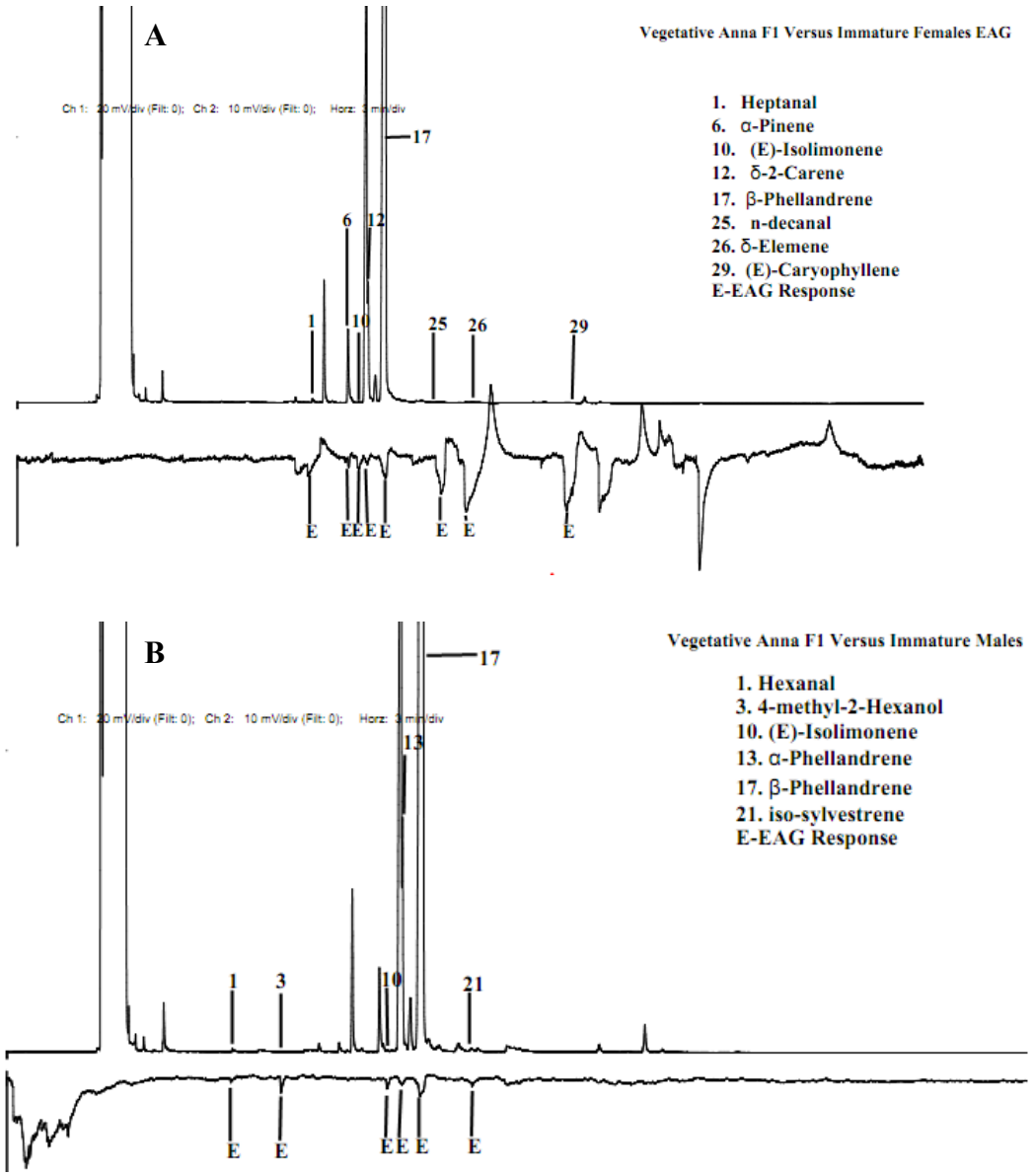
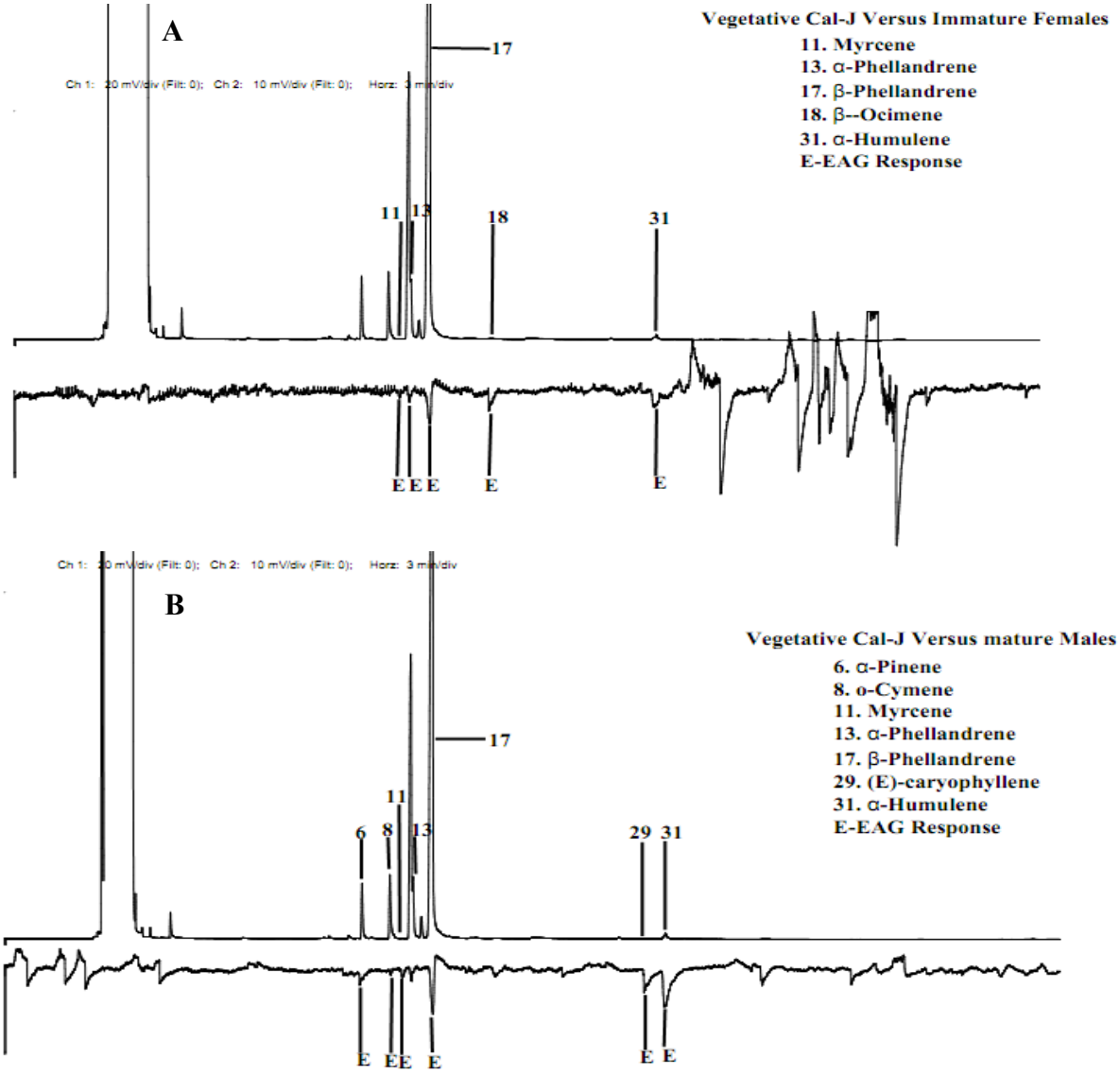
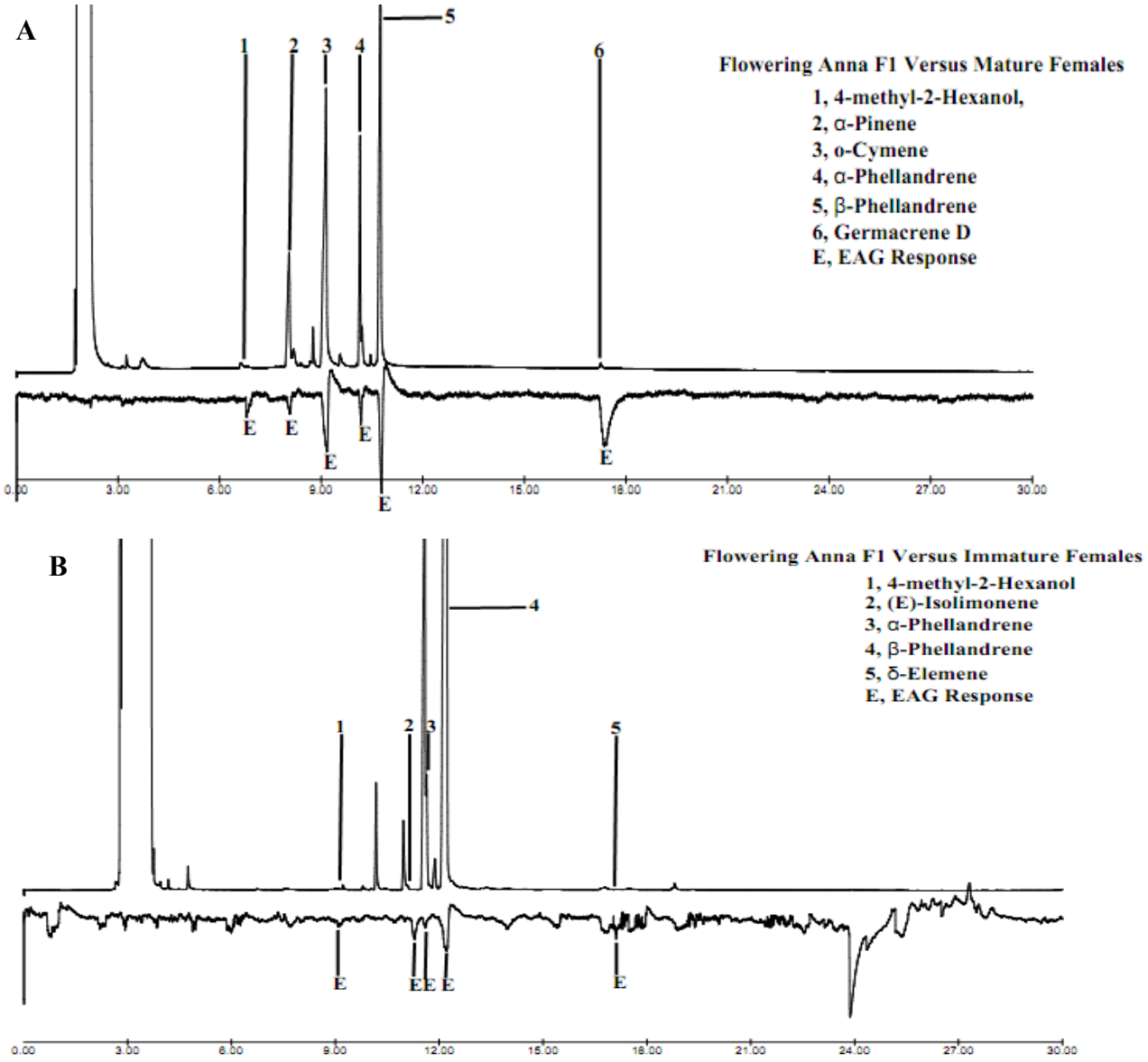
Appendix II: EAG of Immature females (A) and Males (B) against Vegetative Anna F1 tomato volatiles



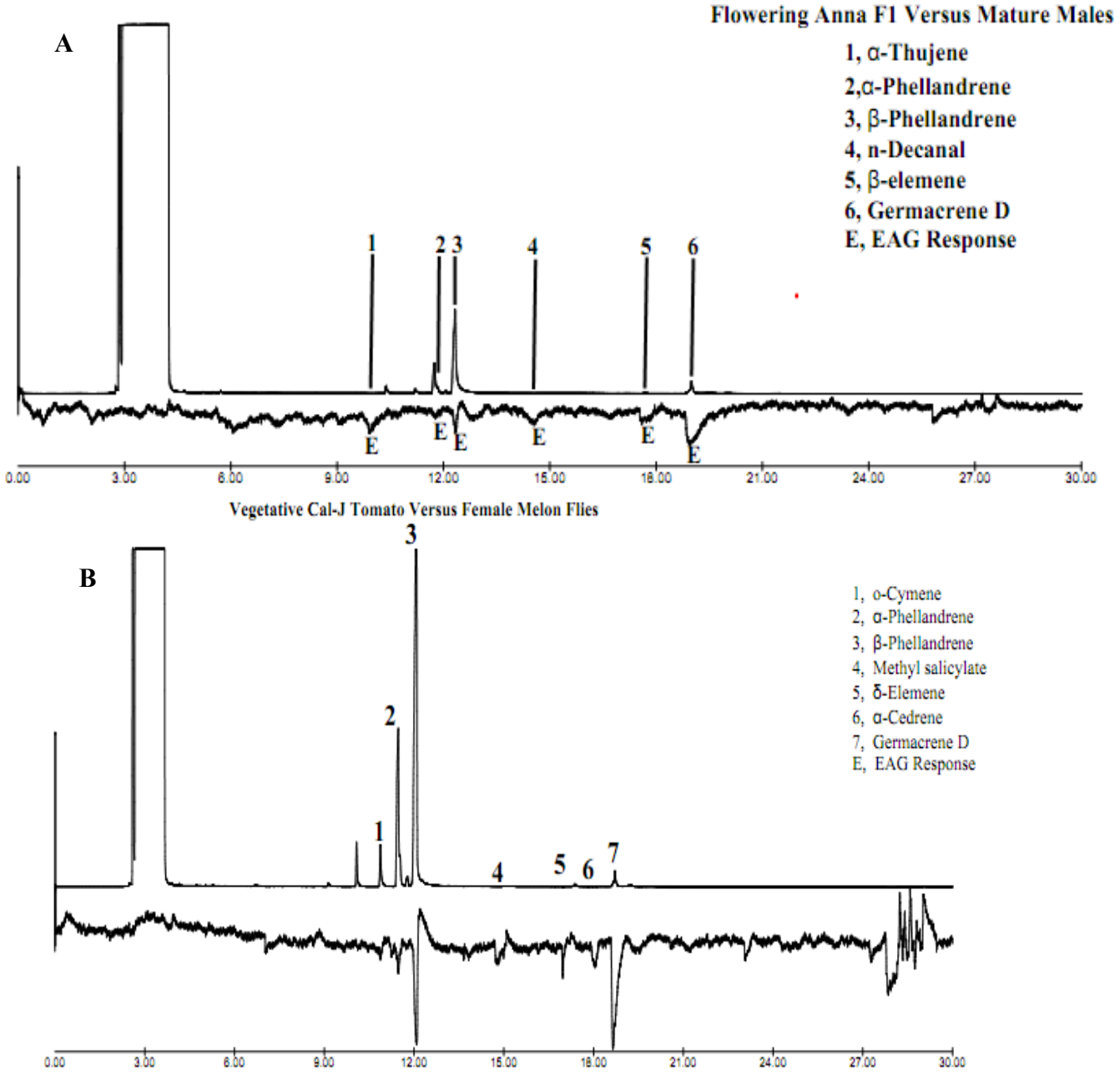
Appendix III: EAG of Immature females (A) and Males (B) against Vegetative Cal J tomato volatiles



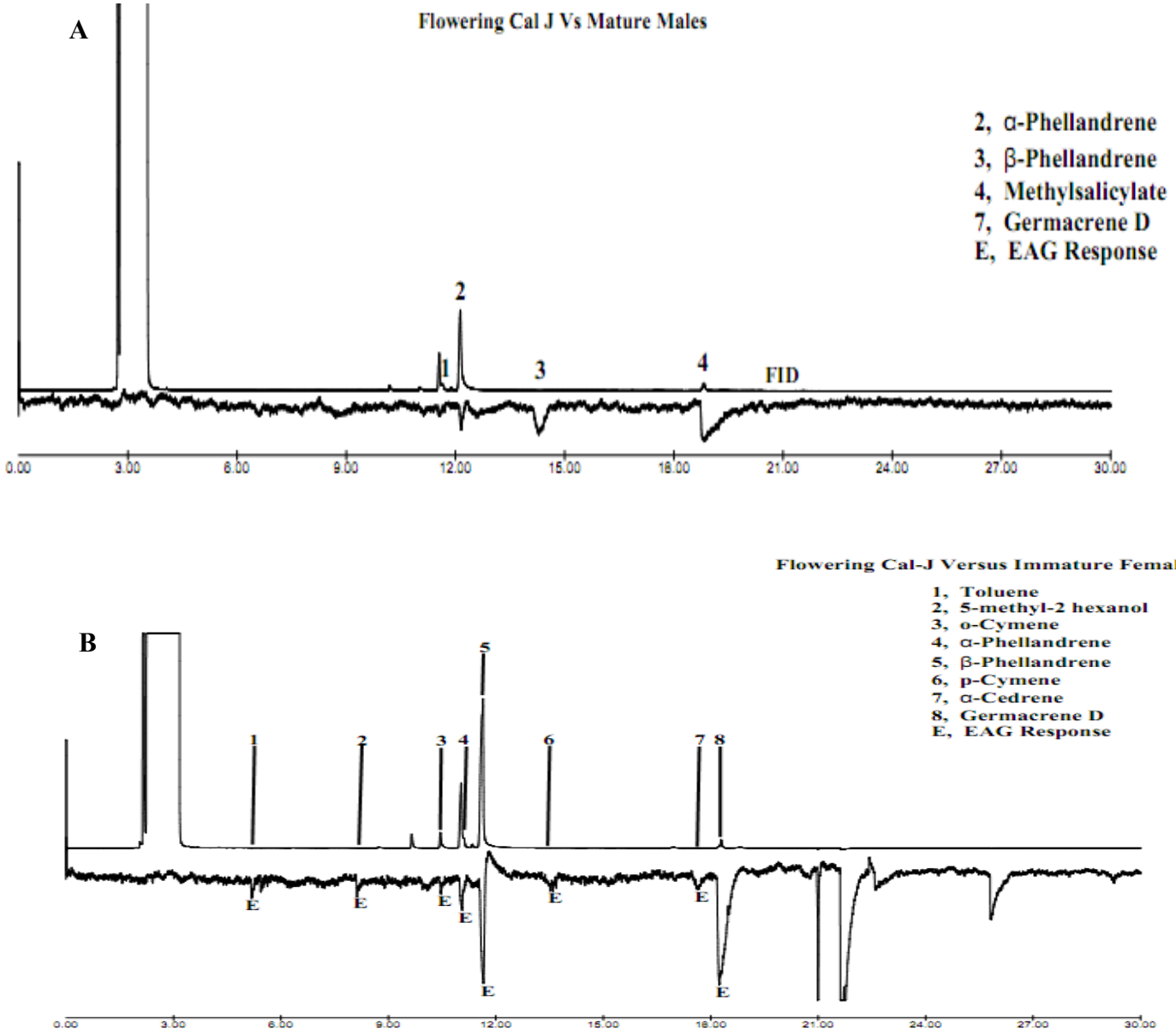
Appendix IV: EAG of Mature females (A) and Immature Females (B) against Flowering Anna F1 tomato volatiles



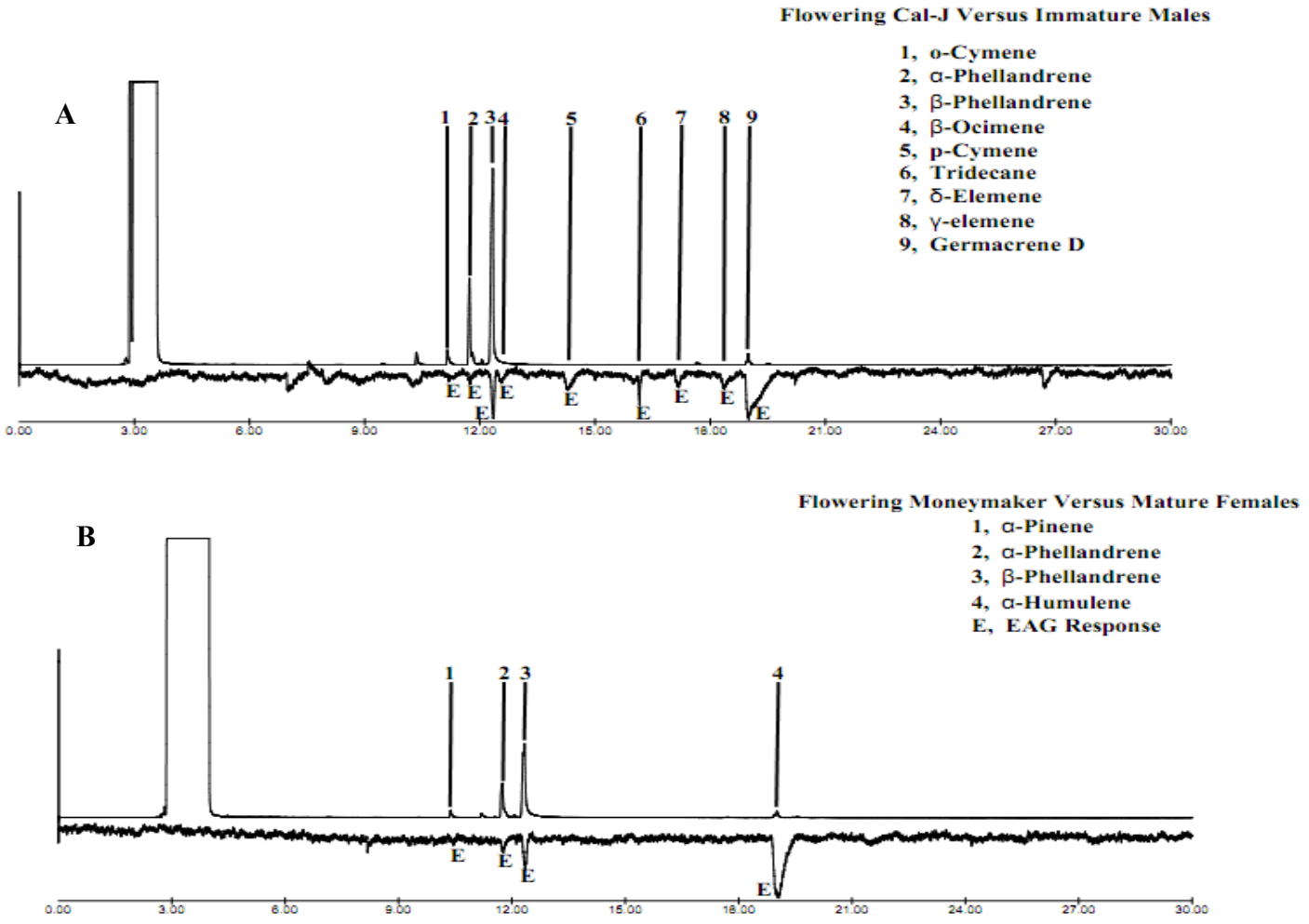
Appendix V: EAG of Mature Males (A) and Mature Females a (B) against Flowering Anna F1 and Vegetative Cal J Respectively tomato volatiles



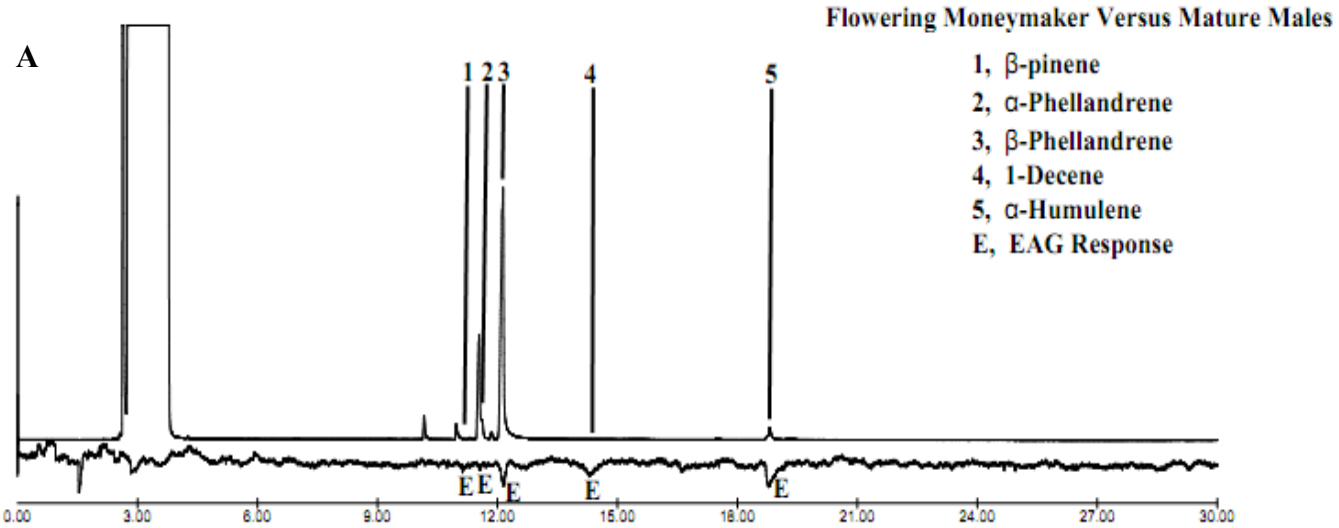
Appendix VI: EAG of Mature Males (A) and Immature Females (B) against Flowering Cal-J tomato volatiles

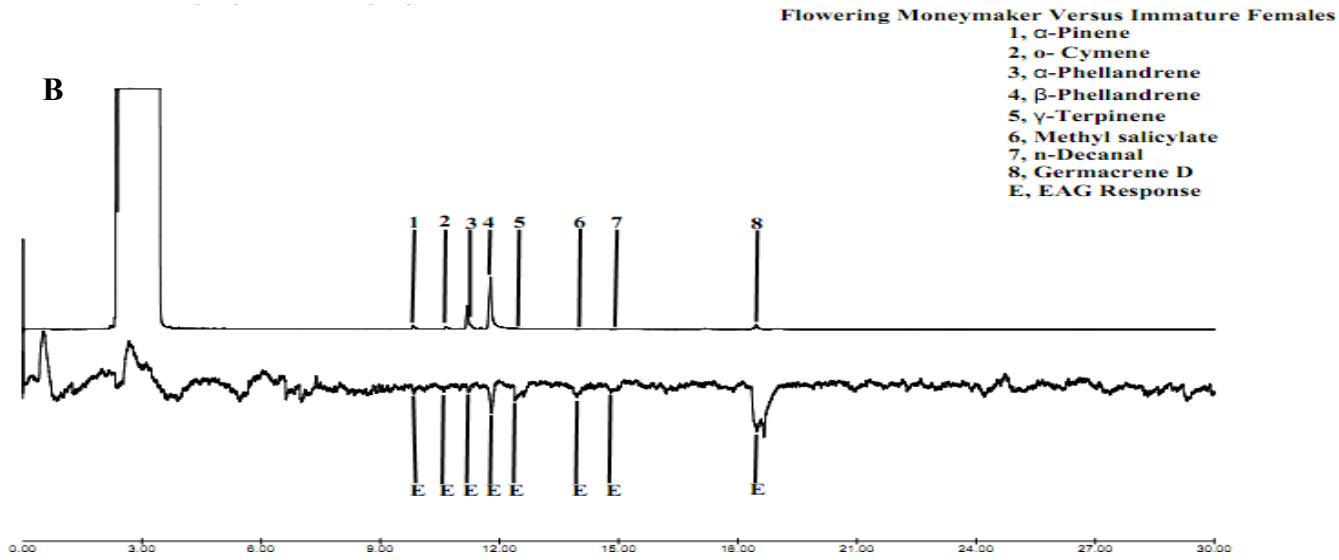


Appendix VII: EAG of Immature males (A) and Mature Females (B) against Flowering Cal-J and MM Respectively tomato volatiles



Appendix VIII: EAG of Mature Males (A) and Immature Females (B) against Flowering MM tomato volatiles





Appendix IX: EAG of Immature males against Flowering MM tomato volatiles