ADDIS ABABA UNIVERSITY COLLEGE OF NATURAL SCIENCES

Study on the Electrophysiological and Behavioral Responses of *Bactrocera dorsalis, B. zonata, B. cucurbitae, B. oleae and C. capitata* to protein lures

A Thesis presented to the School of Graduate Studies of Addis Ababa University in partial Fulfilment of the Degree of Masters of Science in Biology

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INSECT SCIENCE STREAM

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Declaration by candidate

I hereby declare that this thesis is my own work and effort and that it has not been submitted anywhere. Where other sources of information have been used, they have been acknowledged.

Signature: ……..

Date: …….

Certificate of approval

I hereby declare that this thesis is from the student's own work and effort, and all other source of information used have been acknowledge. This thesis has been submitted with my approval.

Supervisor: Dr. Habte Tekie

Signature: …….

Date: …….

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List of Abbreviations

ME	Methyl eugenol
CL	Cue lure
TML	Trimedlure
RK	Raspberry ketone
ECF	E-coniferyl alcohol
DMP	2-allyl-4,5dimethoxy phenol
SPSS	Statistical Package for Social Sciences
CAS	Chemical Abstract Service

Abstract

The family of Tephritidae are economically important agricultural pests. Among them *Bactrocera* is the most devastating genus known that damages fruits and lads to economic losses. To control these pests female targeted attractants are essential. Previous work has shown that females of fruit flies are attracted to protein baits. The objective of the present work was to identify volatiles from a mixture of five different lures (brewery yeast, baker's yeast, torula yeast, GF-120 and anamed) that elicit antennal response of our experimental species. The experiment was carried out on five species of fruit flies (*Ceratitis capitata*, *Bactrocera dorsalis*, *Bactrocera zonata*, *Bactrocera cucurbitae* and on *Bactrocera oleae)*. We scored the similarity and dissimilarity of these species in the antennal response to volatiles from mixed fermentation and protein lures using gas chromatography electorantennography detection (GC-EAD). Nine compounds were identified that elicited an antennal response. Of these nine compounds in *C. capitata*, seven compounds in *B. zonata*, six compounds in *B. dorsalis* and *B. cucurbitae* and two compounds in *B. oleae* gave an antennal response. The active compounds were identified using gas chromatography coupled to mass spectrometry (GC-MS). In olfactometer assay flies were attracted to brewery yeast and a mixture of fermentation and protein lures made. The findings are discussed in the potential of developing a bait that attracts these fruit flies.

Key words: *protein baits, antennal response, GC-EAD, GC-MS, Bactrocera and Olfactometer*

1. Introduction

Fruit flies jeopardize production of fruits and vegetables worldwide (Vayssières *et al*., 2009). So fruits and vegetables importing and exporting countries are giving increasing attention to fruit fly management to reduce its effect in the quality and quantity, in return can affect nutritional status of fruits and vegetables (Drew, 1992 and Cugala *et al*., 2009). The family Tephritidae, which cause immense economic losses are very destructive group of insects occur all over the tropics and subtropics of the world (Drew, 1992 and Vayssières *et al*., 2009). Female fruit flies that lay eggs under the skin of fruits and vegetables are responsible for direct damage. The eggs hatch into larvae that feed in the decaying flesh of the fruits (Ekesi and Billah, 2007). Infested fruits and vegetables quickly rot and become inedible or drop to the ground. Most of the fruit fly species are polyphagous and attack several fruits and vegetables.

There are about 950 species and 150 genera of fruit fly (Tephritidae) known in Africa, attacking wild fruits and flowers. The Asian species *Bactrocera cucurbitae* (Diptera:Tephritidae), *Bactrocera zonata* and *Bactrocera invadens* are among the exotic alien invasive fruit fly species in Africa even though Africa is known to be the origin of a number of fruit flies (Lux *et al*., 2003; Drew *et al*., 2005 and Mwatawala *et al*., 2006). The hosts of these flies include crops such as citrus, mango, apples, cucurbits, tomatoes, and many others (Cugala and Mangana, 2009).

Bactrocera dorsalis, B. cucurbitae. B. olea, B. zonata and Ceratitis capitata are economically important, Therefore my study focused on the above mentioned five species of fruit flies. In controlling fruit flies different techniques are used. Among the several techniques used for fruit fly control, para pheromones and protein bait sprays are the most commonly used. Protein bait can be sprayed by mixing with insecticides, which is environmentally friendly relative to applying full

dosage of insecticides and also attracts both male and female (Sabine, 1992). Pheromones, in contrast, attract only one sex. They are species or genera specific, volatile semiochemicals and can be applied environmentally friendly, with no effect on beneficial organisms. They function by weakening sexual communication and mate annihilation among pests.

1.1 Tephritidae attractant

As a rule of thumb in *Bactrocera*, males released pheromone attracts potential searching females (Fig 1). In addition, recent study has shown that naturally occurring male lures may function as precursors in pheromone synthesis (Tan *et al*., 2011). As mentioned above these pheromones are species or genera specific.

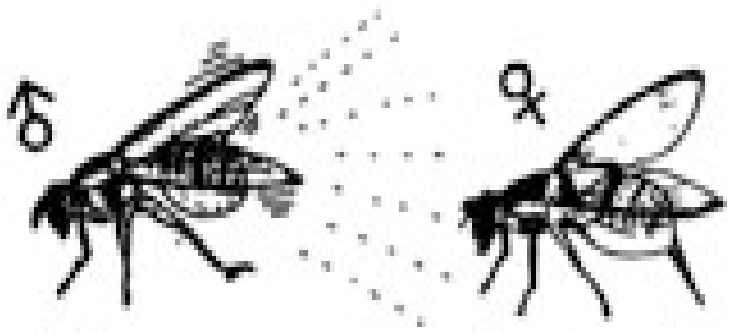


Figure. 1 Male releasing pheromone that attracts the female (Tan *et al*., 2014)

1.1.1 *Anastrepha* pheromones

Different economically important species of the genus *Anastrepha* males take the lek on leaves and attract females to the area through aural, visual and olfactory signals (Aluja *et al*., 2000). In the case of olfactory signals, males emit volatiles from different parts of the insect, like everted pleural pouches, mouth, and anal membranes, and then scattered in the air with the help of rapid

wing vibration (Nation 1972, 1990). Increasing the evaporative surface area of the volatile components can facilitate pheromone attractiveness to the female (Sivinski *et al*. 1994).

Under laboratory condition mature and virgin females of *A*. *fraterculus* and *A*. *obliqua* were found to be attracted to freshly dissected salivary glands of males (Lima *et al*., 2001). This pheromone attracts females to the males but not to the exact point sources (Robacker 1988), so after reaching vicinity of the male females need to use other ways to find the male like acoustic and/or visual signals (Webb *et al*., 1983, Sivinski and Calkins 1986). The effort to develop pheromone-based traps for *Anastrepha* is very little because of the complex nature of the pheromonal blend.

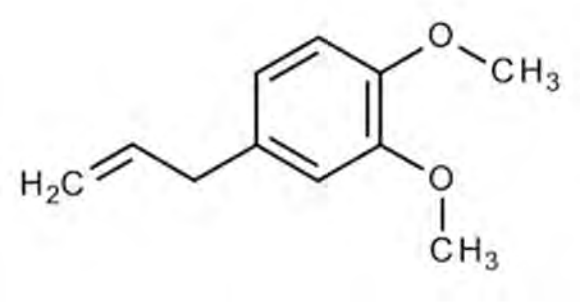
1.1.2 *Bactrocera* pheromones

Males of many *Bactrocera* species are attracted to methyl eugenol (ME), a compound found in a wide diversity of plant species and known to be a pheromonal precursor. *B. cucurbitae* and *B. tryoni* are attracted to cue-lure (CL)/raspberry ketone (RK) and *B. latifrons* are attracted to neither CL/RK nor ME (Tan and Nishida, 2012). Like the most economically important tephritid species, *Bactrocera* males are responsible for sex pheromone emission or "calling" while resting on vegetation and detection and subsequent mate searching is by females.

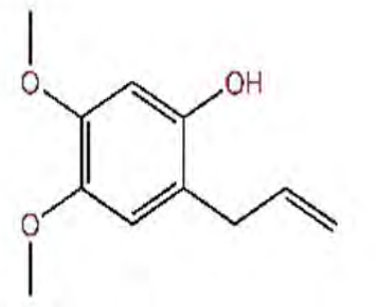
1.1.2.1 *Pheromone of B. dorsalis and B. zonata species*

B. dorsalis females are attracted to both live males and male rectal gland extract (Kobayashi *et al*., 1978). The main components of the glands are E-coniferyl alcohol (ECF) and 2-allyl-4,5dimethoxy phenol (DMP) and Z-3.4-dimethoxycinnamyl alcohol, only in some males (Tan and Nishida, 1996). Feeding ME to different species of *B. dorsalis* complex was shown to increase attractiveness of males by converting the consumed ME to the important components of the male pheromones, ECF and DMP (Nishida *et al*., 1988a & b, Obra and Resilva, 2013). For example, Wee *et al*., 2007 has shown that females are more attracted to ME fed males than unfed, not only

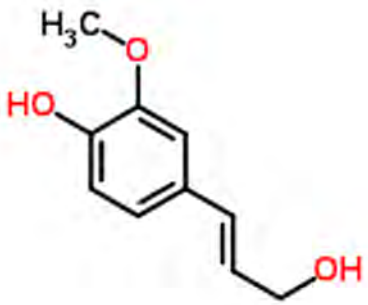
by feeding synthetic but also feeding on natural floral or fruit sources of ME (Shelly 2000a, 2001a). ME and flower-fed males were found to provoke more frequently and attract greater total numbers of females as compared to non-fed males.



Methyl eugenol (ME)



DMP



ECF

Figure. 2 structure of ME, DMP and ECF

In *B. zonata* ME also acts as a pheromone precursor. Like *B. dorsalis* it is transformed into two male sex pheromonal components, DMP and Zconiferyl alcohol (ZCF) (Tan *et al*., 2014).

1.1.2.2 Pheromone of *Bactrocera oleae*

This species is quite different from other *Bactrocera* species in that females attract males for mating (Haniotakis, 1974). The major components of the female sex pheromone are 1,7-dioxaspiro [5.5] undecane (also called olean), o-pinene, n-nonanal, and ethyl dodecanoate (Baker *et al*. 1980, Mazomenos and Haniotakis 1981). Olean was also isolated from males (Mazomenos and Pomonis

1983). Baker *et al*., (1982b) has also identified two other pheromone components, hydroxyl spiroacetals, from *B. oleae* females. Apart from the attraction of males to female Carpita *et al*., (2012) identified a compound, (Z)-9-tricosene, from male rectal gland extracts which was attractive to female. Actually not only the rectal gland extracts, but also whole body extract attract females (Mavraganis *et al*., 2010). Studies (Haniotakis 1974, Mazomenos and Haniotakis 1981, 1985) have shown male attraction to natural or synthetic components or whole blends of the female pheromone in *B. oleae*. Laboratory and field investigation demonstrated that olean was more attractive than the remaining three components, o-pinene, n-nonanal, and ethyl dodecanoate, but that the blend of all four components was more attractive than olean alone.

(*R*)-Olean
[(*R*)-1,7-dioxaspiro[5.5]undecane]

(*S*)-Olean
[(*S*)-1,7-dioxaspiro[5.5]undecane]

Figure. 3 Stereo enantiomers of (R)- and (S)-Olean found in *B. oleae* sex pheromone (Tan *et al*., 2014)

1.1.3 *Ceratitis* Pheromones

The detailed description of emission of volatiles associated with the attraction of females to males was given by Fe'ron at different period, 1959 and 1962 in *C. capitata*. Female attraction to males was also supported from laboratory work by Jang *et al*., (1998) and field (Shelly 2000c) studies. For the medfly, Ohinata *et al*., (1977) and Nakagawa *et al*., (1981a) provided the first quantitative demonstration of the long-range, female attraction to calling male in the field by recording female

captures in male-baited traps. Methyl (E)-6-nonenoate and (E)-6-nonen-1-ol – were thought to be the components of pheromone females are attracted to (Jacobson *et al*., 1973). Ohinata *et al*., (1977, 1979) identified 15 carboxylic acids in addition to the two main components. Later Baker *et al*. (1985) put the three, ethyl (E)-3-octenoate, geranyl acetate, and (E, E)-a-farnesene, to be the main components of the male pheromones.

1.1.4 Male Lures

Male lures can be two type, manmade and plant borne. The manmade are cue lure (CL), trimedlure (TML), fluorinated methyl eugenol analogs, and raspberry ketone-formate (RKF). α- copaene, ME, RK, and zingerone are plant-borne lures. The chemical structures of certain pheromones are simple. Thus, male lure synthesis is relatively cheap for certain species. Since male lures are very potent attractants in most cases using them for mass trapping program is better than developing male sex pheromone as baits (Vargas *et al*., 2010a). So surveys and detection of invasive species, delimitation of an infestation, and control or eradication is made frequently using male lures as baits via the male annihilation technique. Below different male lures are discussed.

1.1.4.1 Methyl Eugenol

Nowadays, detection, control and eradication of any pestiferous tephritid species are dependent on the most recognized and powerful male lure called ME. The chemical is found naturally in more than 450 plant species of different families from a trace to over 90 %. It occurs in oil, flower, leaves, roots stem or whole plant extracts (Tan and Nishida 2012). It was first discovered as a fruit fly attractant by Howlett (1912, 1915), who observed males of *B. dorsalis* and *B. zonata* responding to ME-containing citronella grass, *Cymbopogon nardus* (L.) Rendle. Steiner (1952) further documented the strong attraction of *B. dorsalis* male to ME and noted their vigorous feeding on

the chemical. ME was also found to elicit the greatest feeding response compared to other 34 chemicals analogous to it (Metcalf *et al*., 1975). Carcinogenicity of ME to mice, rats and microbes is the drawback with the use of ME (Miller *et al*., 1983, Schiestl *et al*., 1989). The good thing is ME does not pose a significant cancer risk in humans (Long *et al*., 1963) rather has some benefits to human health, e.g., reduction of cerebral ischemic injury (Choi *et al*., 2010) as well as anti-anaphylactic properties (Kim *et al*., 1997) and it is also component of our diet, e.g., flavoring in baked goods and candy and in most spices (Smith *et al*., 2002).

1.1.4.2 Zingerone

Zingerone (4-(4-hydroxy-3-methoxy-phenyl)-2-butanone, 4-hydroxy-3 methoxybenzyl- acetone, (vanillylacetone) is a phenyl butanoid and has pungency of ginger due to it. Males of ME and RK responsive *Bactrocera* species, particularly, *B. dorsalis* and *B. cucurbitae* are attracted to Zingerone (Tan and Nishida, 2000&2007). Even though, it is relatively very weak attraction in comparison to ME and RK (Tan and Nishida, 2007). As consumed by *B. dorsalis* males Zingerone is converted to zingerol, as a component of male sex pheromone (Tan and Nishida, 2007). A recent work has shown zingerone's potential in attracting mates in *B. cucurbitae* and *B. tryoni* (Kumaran *et al*., 2013).

1.1.4.3 Trimedlure

Since its discovery approximately 50 years ago (Beroza *et al*., 1961), TML (tertbutyl 4(and 5)-chloro-trans_2-methylcyclohexane-1-carboxylate) has become widely adopted as the chief male attractant used in detection and surveillance programs for *C. capitata* (Jang *et al*., 2001). Traps baited with TML captured as many *C. capitata* males.

1.1.5 Food baits

Protein contained foods are essential for fruit flies egg development and to reach sexual maturity (Mazor *et al*., 2002). It's been reported that both sexes of fruit flies are attracted to protein baits (Sabine, 1992). Since females are the main reason for increasing the population number of these pests, attractive baits are required for trapping female fruit flies (Mazor *et al*., 2002). Yeast protein, yeast outolysate and traps baited with fermented sugar and liquid solutions of protein are among the protein baits used in monitoring and control of fruit flies (Epsky *et al*., 1999, Sabine, 1992).

2. Objective

2.1 General objective

- The main objective of this study is to contribute in developing lure for fruit flies, *B. dorsalis, B. zonata, and B. oleae, B. cucurbitae,* and *Ceratitis capitata*

2.2 Specific objectives

- To identify volatiles from baits or lures attractive to female fruit flies of various species
- To identify which compounds from the complex mixtures/lures elicit responses on female antenna using GC-EAD
- To identify electrophysiological active compounds using GC-MS from baits which are found to be attracting females
- To identify differences and commonalities in the response of closely related and distantly related species to the complex mixtures
- To study the behavioural response of the flies to different protein baits using an olfactometer

3. Materials and methods

3.1 Experimental insects

The pupae of all flies used in these experiments were brought from International Atomic Energy Agency (IAEA) division of nuclear techniques in food and agriculture, Austria, Vienna. Adult flies were kept in 30x30x30cm cages provided with water and mixture of yeast and sugar (three hand of sugar and one hand of yeast) (Biasazin *et al*., 2014). The colony was maintained at a temperature of 26-29°C, 50-60% relative humidity, and 12:12 hr. light and dark photoperiod. All the experiments were done in Swedish University of Agricultural Sciences (SLU) Alnarp, Sweden.

3.2 Protein and fermentation lure sources

Saccharomyces cervisae is an important yeast species which has been a very useful tool in bread, beer and wine making for man kinds. The yeast species is produced in a way which is suitable to make the products. For example, the wine and beer making strain is genetically selected to produce more alcohol and less carbon dioxide since alcohol is important product. The reverse is true for baker's yeast making strain because carbon dioxide is the important product. We also used products made of this yeast species which are baker's yeast and brewery yeast. We are not sure which species is used the torula yeast we had used in our experiment.

Two of the fruit fly attractants used (anamed and torula yeast) in this experiment were purchased from ISCA technologies and brewery yeast was brought from St. George brewery factory Addis Ababa, Ethiopia and transported to the Swedish University of Agricultural Sciences (SLU) Alnarp, Sweden. Gf-120 and baker's yeast was provided by SLU.

For volatile collection of baker's yeast, 2g of Baker's yeast and 2g of sugar were mixed with 4ml of water in a glass used for aeration while the other three lures (Gf-120, anamed and brewery yeast)

used directly for odor collection. Since torula yeast is the form of pallets, three torula yeast pallets were dissolved in one liter water for volatile collection.

3.3 Volatile collection

Odors were collected from five types of protein baits (Brewery yeast, Baker's yeast, Torula yeast, Anamed and Gf-120). The aeration was held for three hours for all the baits. The compounds in the baits were trapped by passing charcoal-filtered and humidified air through a Porapak-Q mesh 50-80 adsorbent trap, in each column 0.05 g of adsorbent (Porapak Q) were used. The adsorbent (Porapak Q) were contained inside the Teflon tube by two stoppers, one in each end, made from polypropylene wool and thick walled Teflon tube. The adsorbent were rinsed with 2 ml n-hexane before the experiment and used for aeration from baits contained inside a wash bottle. The collected compounds on the adsorbent were extracted with 1 ml of n-hexane into glass vials (size 1.5 ml). Immediately after collection the samples were stored at -20 °C for gas chromatography coupled with electroantennography detection (GC-EAD) and gas chromatography coupled with - mass spectrometer (GC-MS) analysis. The wash bottle used for aeration was rinsed with acetone and burned in an oven at 350°C for 8 hours before the next aeration. The odor collection protocol were the same for all the lures.

For electrophysiological studies the extracts taken from the baits were pooled (mixed) by taking 500µl of each sample and were kept for 24 hours in a glass vial for thorough mixing.

3.4 Gas chromatography - electroantennographic detection (GC-EAD)

GC-EAD was used to detect compounds that elicit antennal response of the flies. The set up was linked to the 6890N gas chromatography (Agilent Technologies Inc., Santa Clara, CA, USA) with an HP-5 column (diameter 0.25 and length 30m). The carrier gas was hydrogen (1.5 ml/min). The

oven temperature was held at 40°C for 3 min, then increased at 10°C /min to 280°C and held for 5 min.

For EAD recordings, the insect was mounted in a plastic pipette tip with the head protruding from the aperture. The ground electrode was inserted in the head and the recording electrode was placed on the antenna. Most of the time two positions are preferred for EAD: the tip (distal) and the base (proximo-medial) of the antenna, as positional differences yield differences in EAD amplitudes but in this experiment only distal recordings were covered (Biasazin *et al*., 2014) . The micro-electrodes were filled with saline solution creating a saline bridge between the antenna and the Ag/AgCl electrodes. The preparations were held in a humidified continuous air stream of 1.5 l /min.

3.5 GC-Mass spectrometry (GC-MS)

For identification of the compounds from the mixed lures, a gas chromatography coupled with a mass spectrometer was used. Extracted compounds from mixed lures in a mass spectrum were obtained on a 6890N gas chromatography linked to a 5975 mass spectrometer (Agilent Technologies Inc., Santa Clara, CA, USA) equipped with a HP5 column. The carrier gas was Helium. The temperature was set at 30°C for 2 min and increased with 10°C /min until reaching 300°C, and held for 5 min. 2µl of the sample was injected manually. Peaks were identified using NIST 05 and Wiley 175 library.

3.6 Synthetic blends

After the compounds were identified in GC-MS, synthetic blends of the compounds were tested on the insect antennae for conformation. Dilutions of the blends down to 1µg/µl were prepared in a hexane from the stock solutions.

Butyl acetate, isoamyl acetate, styrene, myrcene and ethyl hexanoate (purity 99.5, 98, 99, ?, 99% respectively) were purchased from Sigma Aldrich, 3 methyl-1 butanol (purity 98.5%) and 2 phenyl ethanol (purity 98 %) were provided by Acros organics and Merck schuchardt.

3.7 Behavioral bioassay

Bioassays were carried out under laboratory conditions at the most active period of the day (9 a.m. - 3 p.m.) at a temperature of 25°C and 43% relative humidity for both male and female insects. A glass Y-tube olfactometer of 3 cm diameter with two arms of 20 cm length each was used. Humidified air was purified with activated charcoal and blown into each lateral arm (1 l/min). The humidified air was blown through odorless teflon tube connected to the two arms of the Y-tube, in between a junction of glass. In one arm a filter paper free of treatment and taken as a control, and in the other, the tested lure was enclosed. In the same set up both arms were enclosed with different lures and preference between the lures was determined. To avoid a directional bias, the odor source were alternated after every five trial and the Y-tube set up was placed inside a box, covered with cartoon and white mesh. The Y- tube was changed after every ten trials.

Adult female and male insects in a laboratory were starved for 24 hours before use. The insects were then tested individually by placing them in the downwind end of the Y-tube. A total of 120 individuals were tested per odor source, 60 males and 60 females. The behavioral choice made by each individual was recorded by distinguishing three modalities: choice for compound in one arm or the other, this is when both arms are enclosed with extracts, choice for control, or no choice. No choice was defined as the insect remaining in the common arm and a choice was defined as the insects walking further than two thirds of one arm and remain in that area for 6 minutes. At the end of each experiment the Y-tubes were cleaned with acetone and burned in an oven at 350°C for 8 hrs. For the behavioral bioassay, *B. zonata* and *B. dorsalis* were used; they were tested for 5µl

of brewery yeast and mixtures of five baits, brewery yeast, baker's yeast, torula yeast, gf-120 and anamed. For the preparation of the mix 1gm of anamed and 1ml of the rest of the compounds were used.

3.8 Statistical analysis

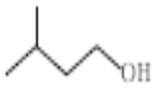
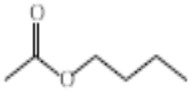
For all the behavioral bioassay non-parametric, binomial test, of SPSS version 20 were used. The result is considered significant when ($P < 0.05$) and not significant when ($P > 0.05$).

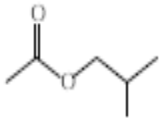
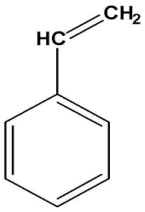
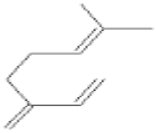
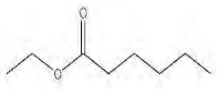
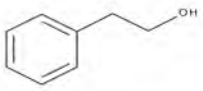
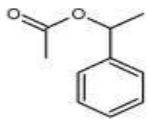
4. Result

4.1 EAD identified compounds from mixed lures

Visual observation of EAD responses showed 9 responses to the mixed protein bait (Table. 1). The traces of each compound in the mixed bait and to which compounds each species gave response are listed in Table 2. For few of the responses (2-3) we could not find neither a matching MS peak nor a good match (quality) in the libraries. These were excluded from further analysis.

Table 1 Antennal active compound

Compound	CAS NO	Ret time	KI	Area	Amount	Structure
Unidentified						
Unknown						
3-Methyl-1butanol	123-51-3	5.673		46544	15.99263	
Butyl acetate	123-86-4	6.841	814	86025	29.5584	

Isoamyl acetate	123-92-2	7.977	876	57400	19.72278	
Styrene	100-42-5	8.364	897	180575	62.04602	
Unidentified						
β-Myrcene	123-35-3	10.295	993	157148	53.99644	
Ethyl hexanoate	123-66-0	10.404	999	557381	191.5175	
2-Phenylethanol	60-12-8	12.822	1120	855	0.29378	
α-Methylbenzyl acetate	93-92-5	14.38	1201	2479014	851.7954	

CAS NO= Chemical Abstract Service Number

KI= Kovats retention index

Ret time= Retention time

4.2 Lure sources of the identified compounds

In the experiment each lures (brewery yeast, baker's yeast, torula yeast, anamed and GF-120) were injected singly in GC-MS to trace the source of the compounds that are identified.

Table 2 Antennal active compounds in different lures and insect species

compounds	*B.dorsalis*	*B. zonata*	*B. oleae*	*B.cucurbitae*	*C.capitata*	Source
1. Unknown	*	*			*	1,2,3,4,5
2. 3-Methyl-1butanol	*	*		*	*	2,3
3. Butyl acetate	*	*			*	5
4. Isoamyl acetate	*	*	*	*	*	2,5
5. Styrene	*	*		*	*	5
6. β-Myrcene		*		*	*	2
7. Ethyl hexanoate	*	*	*	*	*	2,5
8. 2Phenyl ethanol					*	2,3
9.α-Methylbenzyl acetate				*	*	5

*represents antennal active compound that elicit response in the species and the numbers, 1- 5, represent the presence of the compounds in the five protein baits.

1= Gf-120, 2= Brewery yeast, 3= Baker's yeast, 4= Torula yeast and 5= Anamed

Average mini volt of each compounds that elicited antennal response of the experimental insects. From the index we can observe ethyl hexanoate induced a higher response.

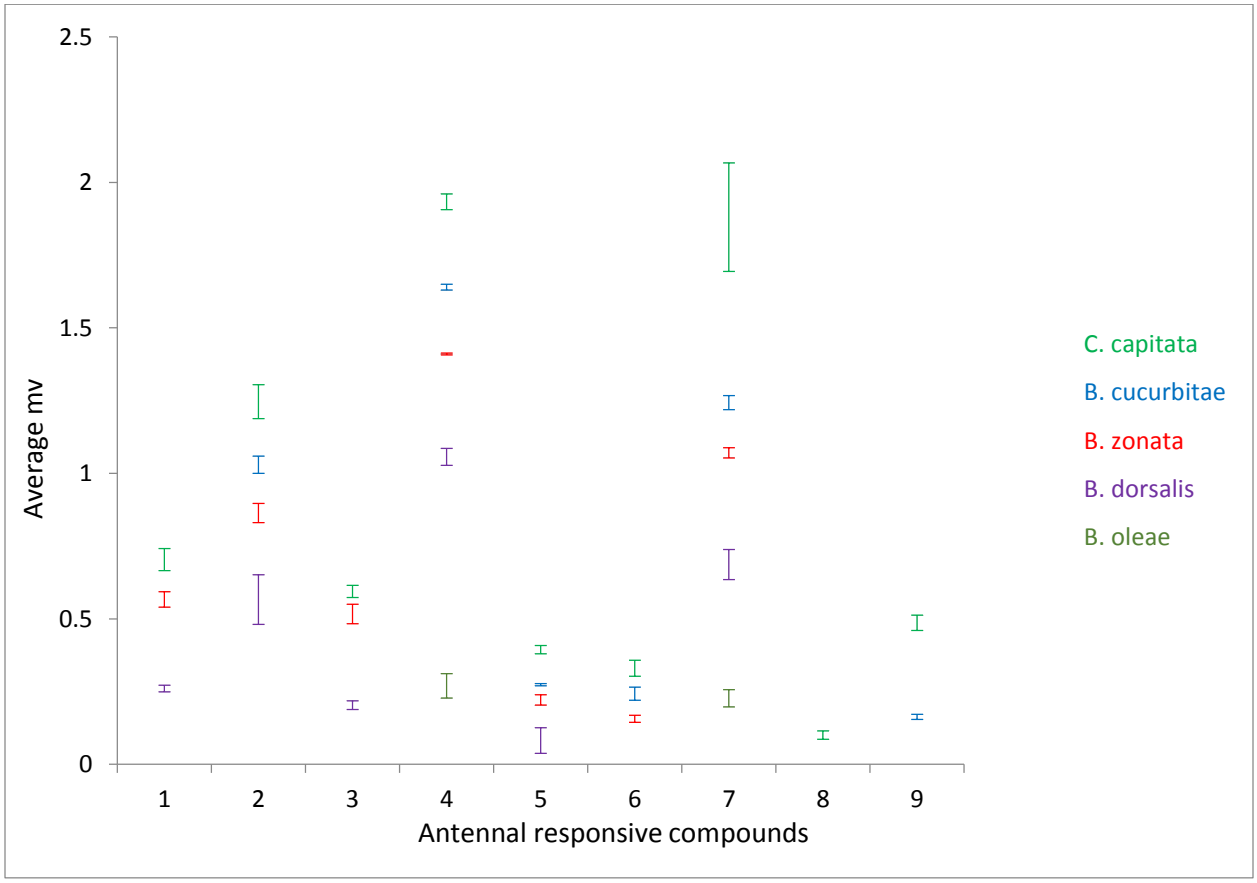


Figure 4 Antennal response of *B. dorsalis, B. zonata, B. cucurbitae, B. oleae and C. capitata* to mixed protein bait (± SE). The numbers correspond to compounds in Table 2.

4.3 Gc- Ead Chromatograph of mixed lures

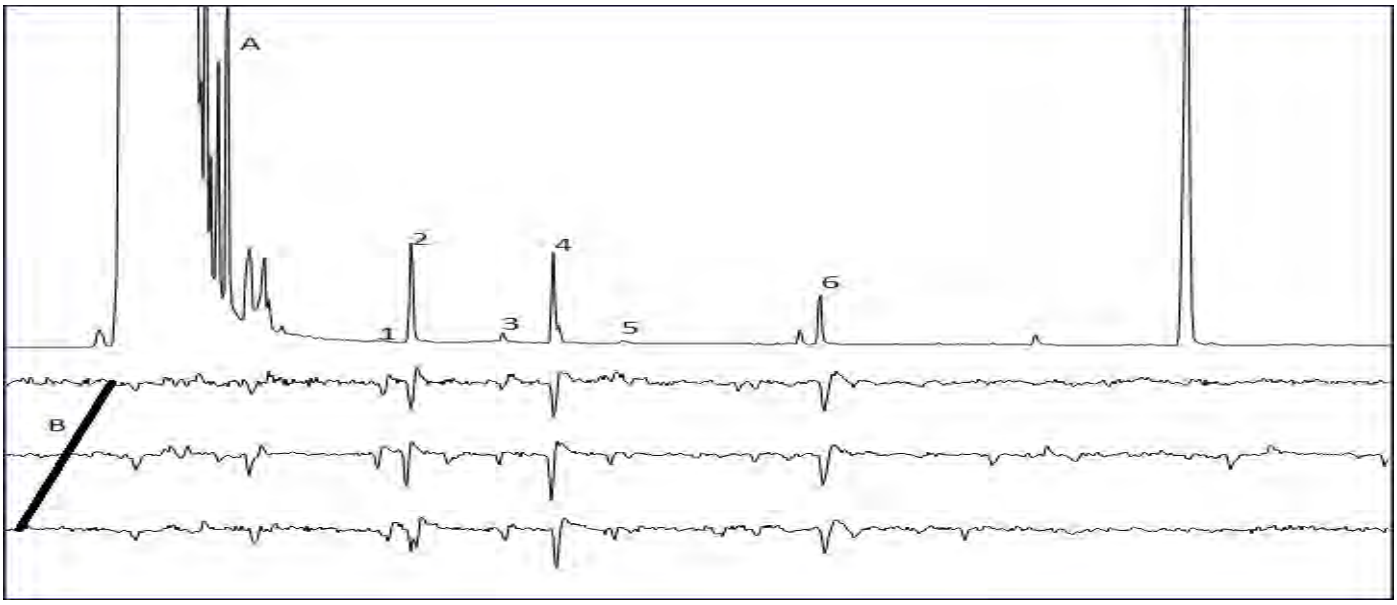


Figure. 5 EAD chromatograph of female *B. dorsalis.* Top trace (A) represents the FID response, the bottom trace (B) the antennal response. Peak numbers correspond to compounds in Table 2.

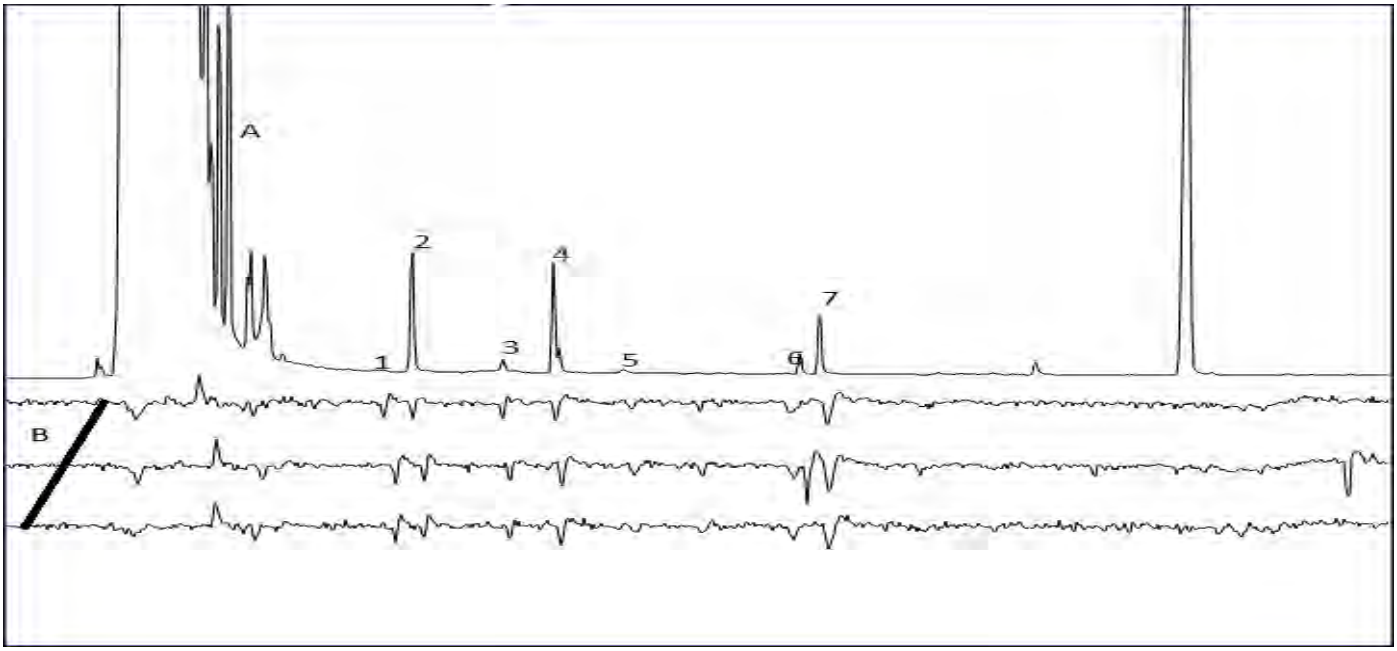


Figure. 6 EAD chromatograph of female *B. zonata.* Top trace (A) represents the FID response, the bottom trace (B) the antennal response. Peak numbers correspond to compounds in Table 2.

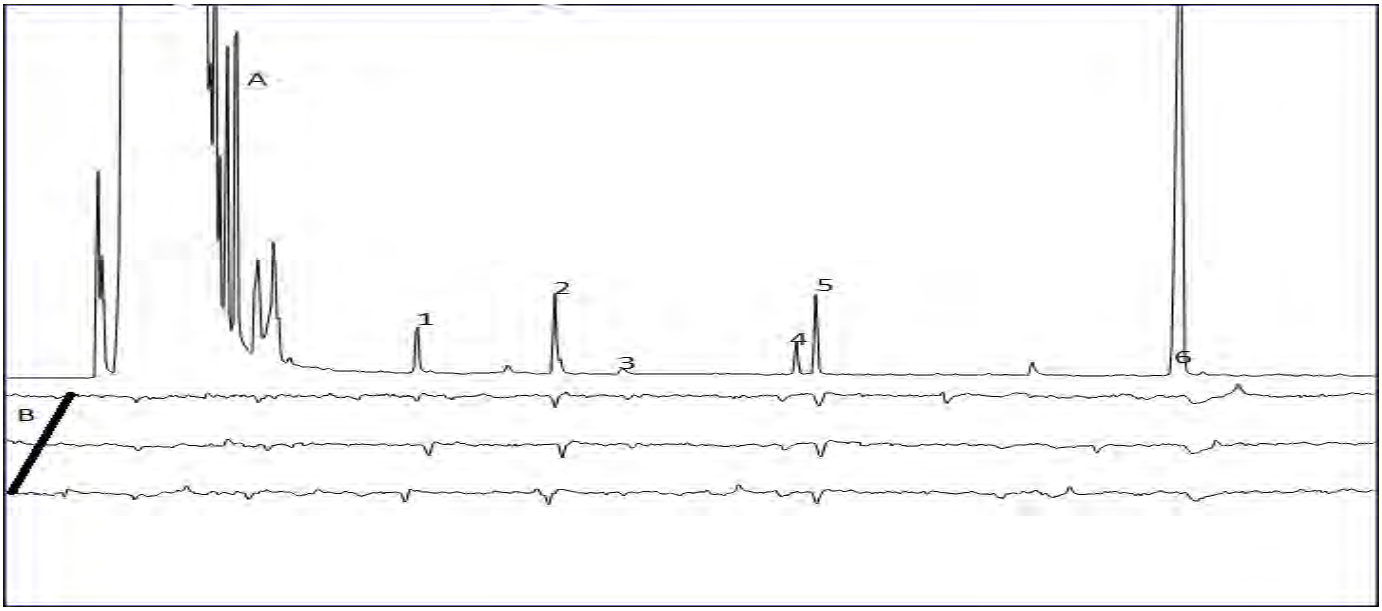


Figure. 7 EAD chromatograph of female *B. cucurbitae.* Top trace (A) represents the FID response, the bottom trace (B) the antennal response. Peak numbers correspond to compounds in Table 2.

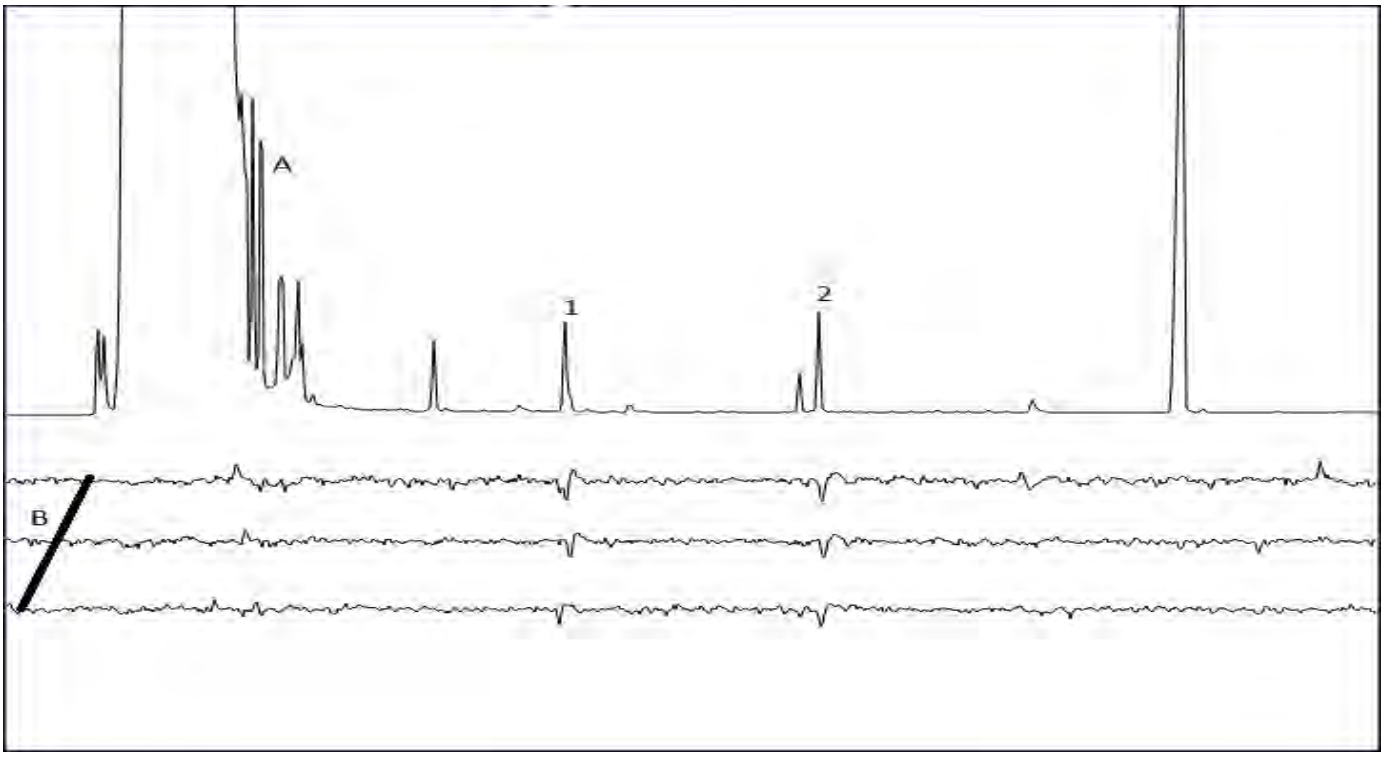


Figure. 8 EAD chromatograph of female *B. oleae.* Top trace (A) represents the FID response, the bottom trace (B) the antennal response. Peak numbers correspond to compounds in Table 2.

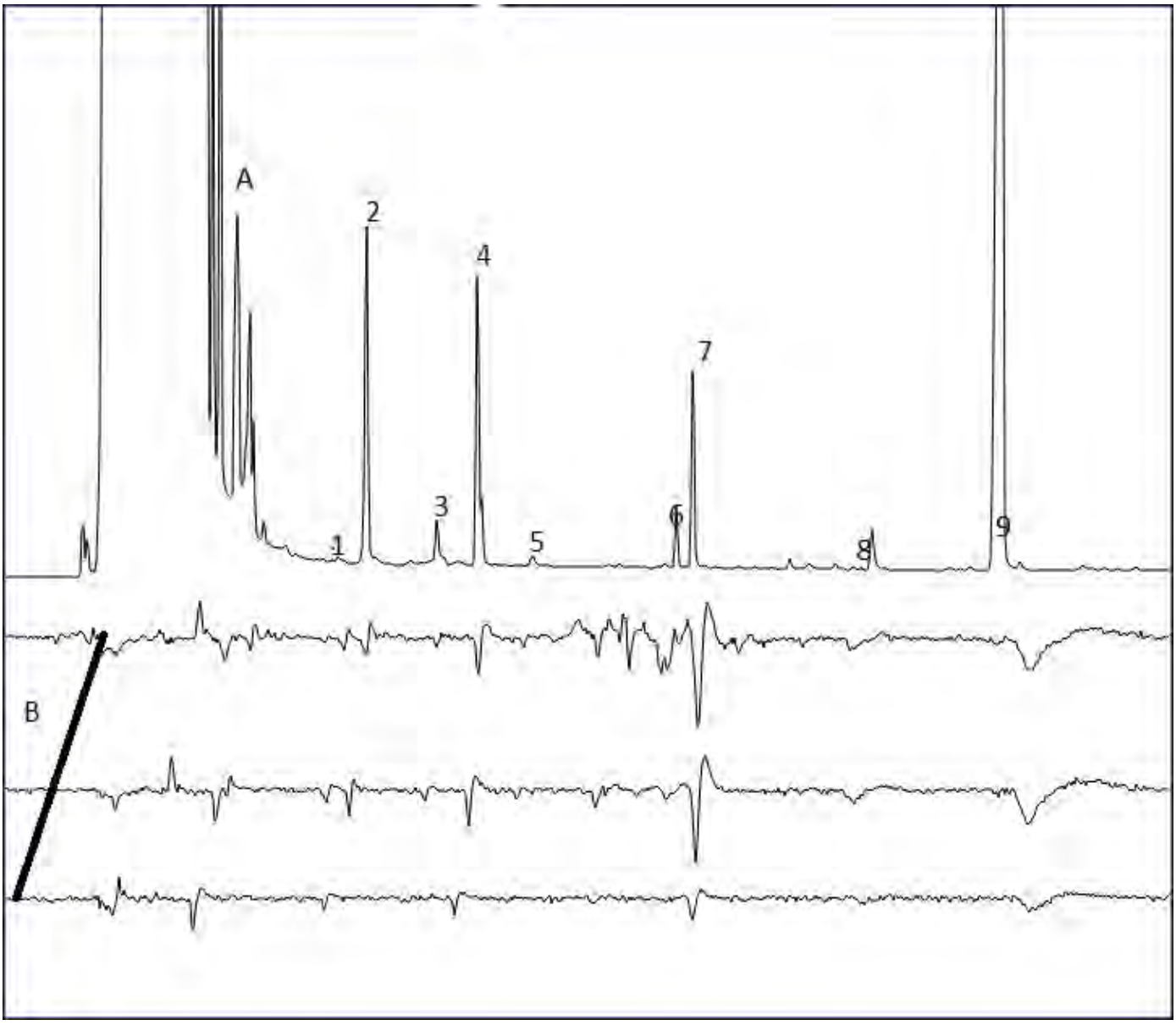
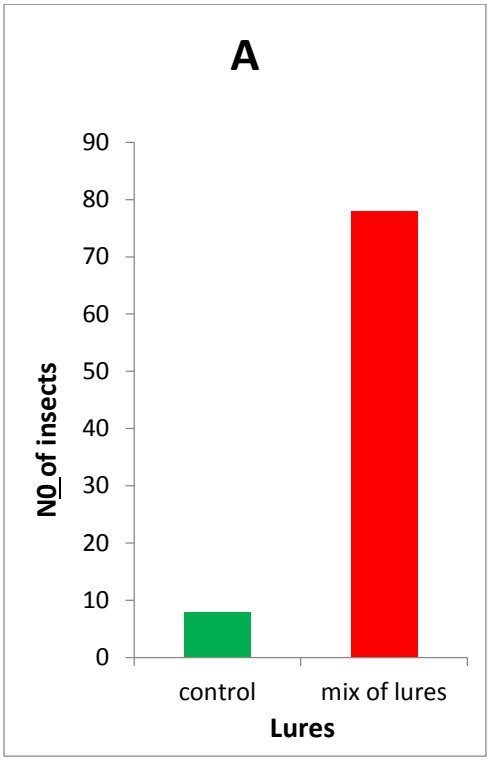
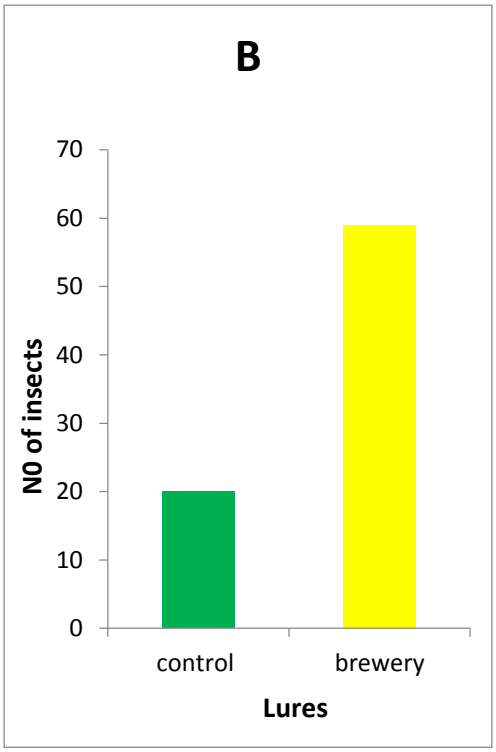


Figure. 9 EAD chromatograph of female *C. capitata.* Top trace (A) represents the FID response, the bottom trace (B) the antennal response. Peak numbers correspond to compounds in Table 2.

4.4 Behavioral bioassay result

B. zonata and *B. dorsalis were used* for behavioral bioassay. The result indicated that *B. zonata* was highly attracted to both brewer's yeast and mix of lures over the control (P<0.001 for both against control), but the comparison between mix of lure and brewery were not significant (P>0.53 for B. dorsalis and P>0.07 for B. zonata) in both species although there is a slight preference between them. The result from both sexes were pooled.





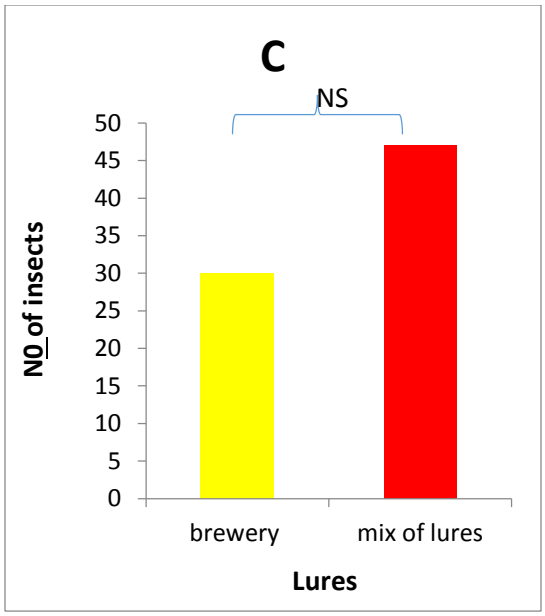


Figure 10 Preference of *B. zonata* in olfactometer bioassay. (A) Mix of lures over control, (B) brewery over control, (C) mix of lures over brewery. NS represents not significant.

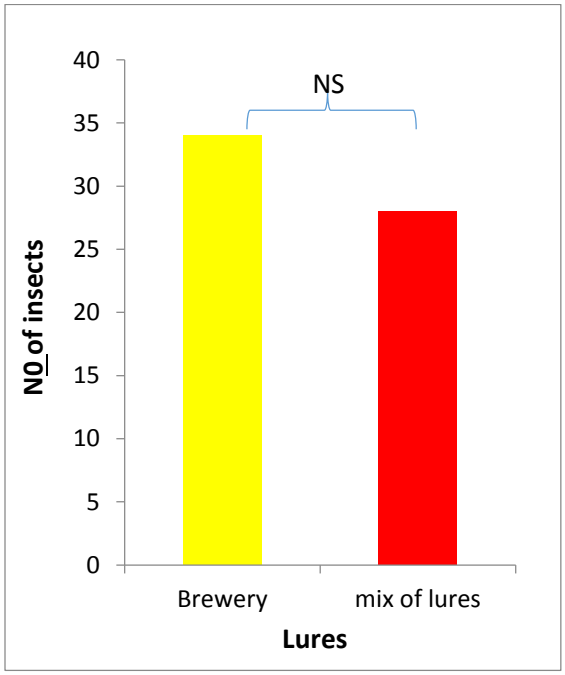


Figure 11 Preference of *B. dorsalis* in olfactometer bioassay. Mix of lures over brewery. NS represents not significant.

5. Discussion

For fruit fly control different parapheromones, plant semiochemicals and food type attractant were studied previously (Tan *et al*., 2014, Light and Jang 1996). However, most of female targeted control methods are restricted to food type attractant (Light and Jang 1996). In the present study a mixture of five different lure were tested (brewery yeast, torula yeast, baker's yeast, gf-120 and anamed). Although it's not exactly the same lures, similar kinds like fermented sugar, hydrolyzed protein and yeasts were tested previously (Epsky *et al*., 1999, Sabine, 1992 and Light and Jang 1996).

The research discovered nine compounds which were detected on GC-EAD and later identified on GC-MS. For the experiment, five important species of fruit flies (*B. zonata*, *B. dorsalis*, *B. oleae*, *B. cucurbitae* and *C. capitata*) were tested. The result obtained in these studies has shown that *C. capitata* was the only species that responded for all the nine compounds (Fig.9). However, on GC-EAD when the synthetic blends of these nine compounds tested on *C. capitata*, six compounds, Butyl acetate, Isoamyl acetate, Styrene, β-Myrcene, Ethyl hexanoate, and α-Methylbenzyl acetate were found to be responsive and we were able to confirm indeed the compounds were the one elicited the antennae. Recent studies have shown that some of the compounds induced antennal response in other fruit flies. For instance, isoamyl acetate, which is found in wine and vinegar, induced antennal response in *Drosophila suzukii* (Cha *et al*., 2012).

3-methyl 1-butanol in the synthetic blend has failed to elicit antennal response. In the present study the unknown compound were identified in each of the individual lures and three of the tested

species had respond to it except *B. cucurbitae* and *B. oleae*. In contrast to our study, 3-methyl 1-butanol, from mango, it is known to elicit antennal response in *B. dorsalis* (Jayanthi *et al*., 2012) .

2-phenyl ethanol is the other compound that didn't elicit antennal response on the antennae of female *C. capitata*. In agreement with our result Siderhurst and Jang (2006) found that ethanol extracts failed to elicit antennal response in *B. dorsalis*. It might be better to test both compounds (3-methyl 1-butanol and 2-phenyl ethanol) with the best purity (100%) and verify the result. From the compounds that were identified, isoamyl acetate and ethyl hexanoate were the only two compounds detected eliciting antennal response in all five species including the specialist fruit fly *B. oleae* (Fig.8). In fact, these are the only two compounds that *B. oleae* responded to.

As mentioned in the result, beyond the nine compounds identified in the mixed lure, a few other compounds were observed inducing antennal response. The identity of these compounds is not known and are also not included in our result. No apparent GC peak was seen in the regions where these EAD responses were observed. However, GC-MS revealed several nitrogen containing compounds, although not a good match in the libraries was not found yet. Some nitrogen containing compounds may not give good FID peaks, which would explain the discrepance between the FID and MS readings.

In the olfactometer assay the result demonstrated that both males and females were attracted to odors of both brewery and mix of lures over the blank (Fig. 10). This might be due the occurrence of compounds, in the brewery and mix of lures, which are important component of fruit flies diet. This agrees with the work done by Siderhurst and Jang (2006) on *Terminalia catappa,* a tropical tree, fruit extracts of which have been found to be attractive to both sexes of *B. dorsalis*. In addition, when mix of lures is paired with brewery in the bioassay, *B. zonata* slightly preferred mix of lures over brewery but was not significant in the other hand *B. dorsalis* was slightly attracted

to brewery than the mix of lures even though the difference was not significant. For the sake of time and number of insect*, B. zonata* and *B. dorsalis* were chosen among the rest of the species for the behavioral assay, the lures were also mixed for the same reason.

According to the result fruit flies were attracted to mixture of fermentation products, brewery and baker yeast, and protein lures, Torula yeast, GF-120 and anamed. Similarly a previous study had shown that fermentation products and protein lures can be used for *Bactrocera control* and other important fruit flies like *Drosophila* species (Landolt *et al*. 2012). For example volatiles from fermentation process like ethanol, acetic acid, ethyl acetate, and acetaldehyde found to attract *Drosophila melanogaster* (West 1961). Torula yeast and hydrolysate protein are also used in monitoring and population suppression (Episky *et al*., 1999). Female targeted trapping system, using long lasting synthetic attractants is developed, these synthetic attractants are Ammonium Acetate, Putrescine and Trimethylamine (Episky *et al*., 1999). Actually these synthetic attractant in combination attract more flies than the protein baits (torula yeast and protein hydrolysate) (Katsoyannos *et al*., 1999a). The compounds that are identified in this experiment can be tested as an attractant like the above mentioned synthetic food lures. The major importance of synthetic food lure is more target specific compared to food lures which trap other tephritid and non-targeted flies. GF-120 (one of the lure in our mixed protein baits) baited with spinosad is also used in combination with three synthetic attractants (Ammonium Acetate, Putrescine and Trimethylamine) for *Ceratitis capitata control* (Cristofaro *et al*., 2007). So studying the behavioural response of fruit flies to mixed protein lures and fermentation products can be a step ahead in developing a novel attractant.

6. Conclusion

- Most of the identified compounds that elicited antennal response were sourced from brewer's yeast and anamed.
- Isoamyl acetate and ethyl hexanoate were the compounds that elicited antennal response in all five species and induced a higher response.
- The olfactometer study demonstrate that *B. zonata* and *B. dorsalis* were attracted to both brewery and mix of lures over the control. However, there were no significant preference when brewery was tested against mix of lures in both species. Although it is not significant, the test showed that *B. zonata* slightly preferred mix of lures but *B. dorsalis* preferred brewer's yeast.

7. Recommendation

This research can be a valuable source for further studies in developing baits that selectively capture these fruit flies. Isoamyl acetate and ethyl hexanoate which were identified in all five species EAD result, can be used as attractant bait in fields to trap and kill as methyl eugenol used with spinosad. Since isoamyl acetate and ethyl hexanoate induced a higher response and found in many fruit odors, it would be much help in constructing these attractant baits. Carrying out behavioural bioassay for each of the lures in each species of the insect and performing GC-EAD for the synthetic blend for each insect species are suggested for further study in order to be accurate in developing super attractant.

8. References

Aluja, M., Pin˜ero, J., Ja´come, I., Diaz-Fleischer, F., and Sivinski, J. (2000). Behavior of flies in the genus Anastrepha (Trypetinae: Toxotrypanini). In: *Fruit flies (Tephritidae): phylogeny and evolution of behavior*, Aluja, M. and Norrbom, AL. (eds.). CRC Press, Boca Raton, pp 375–406.

Baker, R., Herbert, R.H., Grant, G.G. (1985). Isolation and identification of the sex pheromone of the Mediterranean fruit fly, *Ceratitis capitata* (Wied.). *J. Chem. Soc. Chem. Commun*. **12**:824–825.

Baker, R., Herbert, R., Howse, P.E., Jones, O.T., Franke, W. and Reith, W. (1980). Identification and synthesis of the major sex pheromone of the olive fly, *Dacus oleae*. *J. Chem. Soc. Chem. Commun.* **1**:52–53.

Baker, R., Herbert, R.H. and Parton, A.H. (1982b). Isolation and synthesis of 3- and 4-hydroxy-1, 7dioxaspiro [5.5] undecanes from the olive fly (*Dacus oleae*). *J. Chem. Soc. Chem. Commun.* **11**:601–603.

Beroza, M., Green, N. and Gertler, S.I. (1961). New attractants for the Mediterranean fruit fly. *J. Agric. Food Chem.* **9**:361–365.

Biasazin, T.D., Karlsson M.F., Hillbur, Y., Seyoum, E. and Dekker, T. (2014). Identification of Host Blends that Attract the African Invasive Fruit Fly, *Bactrocera invadens*. *J. Chem. Ecol*. **40**:966–976.

Carpita, A., Canale, A., Raffaeli, A., Saba, A., Benelli, G., Raspi, A. (2012). (Z)-9-tricosene identified in rectal gland extracts of *Bactrocera oleae* males: first evidence of a male-produced female attractant in olive fruit fly. *Naturwissenschaften* **99**:77–81.

Cha, D.H., Adams, T., Rogg, H. and Landolt, P., (2012). Identification of Field Evaluation of Fermentation Volatiles from Wine and Vinegar that Mediate Attraction of Spotted Wing Drosophila, Drosophila suzukii. *J. Chem. Ecol*., 38

Choi, Y.K., Cho, G.S., Hwang, S., Kim, B.W., Lim, J.H., Lee, J.C., Kim, H.C., Kim, W.K. and Kim, Y.S. (2010). Methyleugenol reduces cerebral ischemic injury by suppression of oxidative injury and inflammation. *Free Radical Res*. **44**:925–935.

Cristofaro, M., Fenio, A., Tronci, C., Porto, M.E., Cristofaro, A., Baldacchino, F. and Tabilio, M. R. (2007). Improved attractants for Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) and Olive fruit fly*, Bactrocera oleae* (gmelin): two years data evaluating the responses of wild fly populations in 3 southern Italy locations. *Proc.Int.Atom.Agn*. Vienna **1574**:51-60.

Cugala, R. and Mangana, S. (2009). *Establishment and Maintenance of Fruit Flies Pest Free Area (FF-PFA)*. Department of Plant Protection (NPPO) Mozambique Faculty of Agronomy and Forest Engineering, Eduardo Mondlane University, Maputo. pp 3-11.

Cugala, D., Santos, L. and Albano, G. (2009). *Assessment of the Fruit Fly, Batrocera invadens, Pest Status and the Establishment of the Parasitoid, Fopius arisanus in Mozambique*. Faculty of Agronomy and Forest Engineering, Eduardo Mondlane University, Main University, Maputo. pp 313-316.

Drew, R.A.I., Tsuruta, K. and White, I.M. (2005). A new species of pest fruit fly (Diptera: Tephritidae: Dacinae) from Sri Lanka and Africa. *Afr. Entomol*. **13**:149–154.

Drew, R. A. I. (1992). *Overview of Fruit Flies*. International Training Course Fruit Flies. MARDI, Kuala Lumpur. 4th-15th May 1992. 5 pp.

Ekesi, S., and Billah, M.K. (2007). *A Field Guide to the Management of Economically Important Tephritid Fruit Flies in Africa* (2nd ed.), Nairobi, Kenya: ICIPE Science Press.

Epsky, N.D., Hendrichs, J., Katsoyannos, B.I., Vasquez, L.A., Ros, J.P., Zumreoglu, A., Pereira, R., Bakri, A., Seewooruthun, S.I. and Heath, R.R., (1999). Field evaluation of female-targeted trapping systems for *Ceratitis capitata* (Diptera: Tephritidae) in seven countries. *J. Econ. Entomol.* **92**: 156-164.

Fe´ron, M. (1959). Attraction chimique du male de *Ceratitis capitata* Wied. (Dipt. Trypetidae) pour la femelle. *CR. Acad. Sci. Ser. D. Sci. Nat*. (Paris) **248**: 2403–2404.

Fe´ron, M. (1962.) L'instinct de reproduction chez la mouche Mediterranean des fruits Ceratitis capitata Wied. (Dipt. Trypetidae). Comportement sexuel. Comportement de ponte. *Rev. Path. Veg. Entomol. Agric. Fr*. **41**:1–129

Haniotakis, G.E. (1974). Sexual attraction in the olive fruit fly, *Dacus oleae* (Gmelin). *Environ. Entomol*. **3**:82–86.

Howlett, F.M. (1912). The effects of oil of citronella on two species of *Dacus*. *Trans. R. Entomol. Soc .Lond.* **60**:412–418.

Howlett, F.M. (1915). Chemical reactions of fruit flies. *Bull. Entomol. Res*. **6**:297–305.

Jacobson, M, Ohinata, K., Chambers, D.L., Jones, W.A. and Fujimoto, M.S. (1973). Insect sex attractants. Isolation, identification, and synthesis of sex pheromones of the male Mediterranean fruit fly. *J. Med. Chem.* **16**:248–251.

Jang, E.B., McInnis, D.O., Lance, D.R. and Carvalho, L.A. (1998). Mating-induced changes in olfactory mediated behavior of laboratory-reared normal, sterile, and wild female Mediterranean fruit flies (Diptera: Tephritidae) mated to conspecific males. *Ann. Entomol. Soc. Am*. **91**:139–144.

Jang, E.B., Raw, A.S. and Carvalho, L.A. (2001). Field attraction of Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) to synthetic stereo selective enantiomers of the ceralure B1 isomer. *J. Chem. Ecol*. **27**:235–242.

Jayanthi, K. P. D., Woodcock, C. M., Caulfield, J., Birkett, M. A, and Bruce, T. J. A. (2012). Isolation and identification of host cues from mango, *Mangifera indica*,that attract gravid female oriental fruit fly, *Bactrocera dorsalis*. *J. chem. ecolo*, **doi**: 10.1007/s10886-012-0093-y.

Katsoyannos, B. I., Papadopoulos, N. T., Heath, R. R., Hendrichs, J., and kouloussis, N. A. (1999a) Evaluation of synthetic food-based attractants for female Mediterranean fruit flies (Diptera:Tephritidae) in McPhail type traps. *J. Appl. Entomol*. **123**: 607-612.

Kim, H.M., Lee, E.H., Kim, C.Y., Chung, J.G., Kim, S.H., Lim, J.P., and Shin, T.Y. (1997). Antianaphylactic properties of eugenol. *Pharmacol*. *Res*. **36**:475–480.

Kobayashi, R.M., Ohinata, K., Chambers, D.L. and Fujimoto, M.S. (1978). Sex pheromone of the oriental fruit fly and melon fly: mating behavior, bioassay method, and attraction of females by live males and by suspected glands of males. *Environ*. *Entomol*. **7**:107–112.

Kumaran, N., Balagawi, S., Schutze, M.K. and Clarke, A.R. (2013). Evolution of lure response in tephritid fruit flies: phytochemicals as drivers of sexual selection. *Anim*. *Behav*. **85**:781–789.

Landolt, P., Adams, T. and Rogg, H. (2012). Trapping spotted wing Drosophila, Drosophila suzukii (Matsumura) (Diptera: Drosophilidae), with combinations of vinegar and wine, and acetic acid and ethanol. *J. Appl. Entomol*. **136**: 148–154.

Light D.M. and Jang, E.B. (1996). Plant volatiles evoke and modulate tephritid behavior. In: *Fruit fly pests: a world assessment of their biology and management*. McPheron BA, Steck GJ (eds). St. Lucie Press, Delray Beach, pp 123–133.

Lima, I.S., Howse, P.E., Do Nascimento, R.R. (2001). Volatile substances from male *Anastrepha fraterculus* Wied. (Diptera: Tephritidae): identification and behavioural activity. *J. Braz. Chem. Soc.* **12**:196–201.

Long, E.L., Nelson, A.A., Fitzhugh, O.G., Hansen, W.H. (1963). Liver tumors produced in rats by feeding safrole. *Arch. Pathol*. **75**:595–604.

Lux, S.A., Copeland, R.S., White, I.M., Manrakhan, A. and Billah, M.K. (2003). A new invasive fruit fly species from the *Bactrocera dorsalis* (Hendel) group detected in East Africa. *Ins. Sci. and its Applin.* **23**: 355–360.

Mwatawala, M.W., De Meyer, M., Makundi, R.H. and Maerere, A.P. (2006). Biodiversity of fruit flies (Diptera, Tephritidae) in orchards in different agro-ecological zones of the Morogoro region, Tanzania. *Fruits* **61**:321-332.

Mavraganis, V.G., Papadopoulos, N.T. and Kouloussis, N.A. (2010). Extract of olive fruit fly males (Diptera: Tephritidae) attract virgin females. *Entomol. Hellenica*. **19**:14–20.

Mazomenos, B.E. and Haniotakis, G.E. (1981). A multicomponent female sex pheromone of *Dacus oleae* Gmelin: isolation and bioassay. *J. Chem. Ecol*. **7**:437–444.

Mazomenos, B.E. and Haniotakis, G.E. (1985). Male olive fruit fly attraction to synthetic sex pheromone components in laboratory and field tests. *J. Chem. Ecol*. **11**:397–405.

Mazomenos, B.E. and Pomonis, G.J. (1983). Male olive fruit fly pheromone: isolation, identification and lab bioassays. In: **Fruit Flies of Economic Importance**. Cavalloro, R. (ed.) A.A. Balkema, Rotterdam, pp 96–103.

Mazor, M., Peysakhis, A. and Reuven, G. (2002). Release rate of ammonia – a key component in the attraction of female Mediterranean fruit fly to protein-based food lures. *International Organization for Biological and Integrated Control of Noxious Animals and Plants Bulletin*, **25**: 1-6.

Metcalf, R.L., Mitchell, W.C., Fukuto, T.R. and Metcalf, E.R. (1975). Attraction of the oriental fruit fly, Dacus dorsalis, by methyl eugenol and related olfactory stimulants. *Proc. Natl. Acad. Sci.* **72**:2501–2505.

Miller, E.C., Swanson, A.B. and Phillips, D.H. (1983). Structure-activity studies of the carcinogenicities in the mouse and rat of some naturally occurring and synthetic alkenylbenzene derivatives related to safrole and estragole. *Cancer Res*. **43**:1124–1134.

Nakagawa, S., Harris, E.J. and Keiser, I. (1981b). Performance of capilure in capturing Mediterranean fruit flies in Steiner plastic or cardboard sticky traps. *J. Econ. Entomol*. **74**:244–245.

Nation, J.L. (1972). Courtship behavior and evidence for a sex attractant in the male Caribbean fruit fly, *Anastrepha suspensa*. *Ann. Entomol. Soc. Am*. **65**:1364–1367.

Nation, J.L. (1990). Biology of pheromone release by male Caribbean fruit flies, *Anastrepa suspense* (Diptera: Tephritidae). *J. Chem. Ecol*. **16**:553–572.

Nishida, R., Tan, K.H. and Fukami, H. (1988a). Cis-3.4-dimethoxycinnamyl alcohol from the rectal glands of male Oriental fruit fly, *Dacus dorsalis*. *Chem. Express* **3**: 207–210.

Nishida, R, Tan, K.H., Serit, M., Lajis, N.H., Sukari, A.M., Takahashi, S. and Fukami, H. (1988b). Accumulation of phenylpropanoids in the rectal glands of male oriental fruit fly, *Dacus dorsalis*. *Experientia* **44**:534–536.

Obra, G.B. and Resilva, S.S. (2013). Influence of adult diet and exposure to methyl eugenol in the mating performance of Bactrocera philippensis. *J. Appl. Entomol.* **137**: 210–216.

Ohinata, K., Jacobson, M., Nakagawa, S., Fukimoto, M. and Higa, H. (1977). Mediterranean fruit fly: laboratory and field evaluations of synthetic sex pheromones. *J. Environ. Sci. Health* **12**: 67–78.

Ohinata, K, Jacobson, M, Nakagawa, S, Urago, T, Fujimoto, M. and Higa, H. (1979). Methyl I-6 nonenoate: a new Mediterranean fruit fly male attractant. *J. Econ. Entomol*. **72**:648–650.

Robacker, D.C. (1988). Behavioral responses of female Mexican fruit flies, *Anastrepha ludens*, to components of male-produced sex pheromone. *J. Chem. Ecol*. **14**:1715–1726.

Sabine, B. N. E. (1992). *Pre-harvest Control Methods*. International Training Course Fruit Flies. MARDI, Kuala Lumpur. 4th-15th May 1992. 20 pp.

Schiestl, R.H.W.S., Chan, R.D., Gietz, R.D.M. and Hastings, P.J. (1989). Safrole, eugenol and methyleugenol induce intrachromosomal recombination in yeast. *Mutat*. *Res*. **224**: 427–436.

Shelly, T.E. (2000a). Flower-feeding affects mating performance in male oriental fruit flies (Diptera: Tephritidae). *Ecol. Entomol*. **25**:109–114.

Shelly, T.E. (2000c). Male signaling and lek attractiveness in the Mediterranean fruit fly. *Anim. Behav.* **60**: 245–251.

Shelly, T.E. (2001a). Feeding on papaya flowers enhances mating competitiveness of male oriental fruit flies, Bactrocera dorsalis (Diptera: Tephritidae). *Proc. Hawaiian Entomol. Soc.* **35**:41–47.

Siderhurst, M. S., and Jang, E. B. (2006). Female-biased attraction of oriental fruit fly, *Bactrocera dorsalis* (Hendel), to a blend of host fruit volatiles from *Terminalia catappa* L. *J. Chem. Ecol.* **32**: 2513-2524.

Sivinski, J. and Calkins, C. (1986) Pheromones and parapheromones in the control of tephritids. *Fla*. *Entomol*. **69**:157–168.

Sivinski, J.M., Epsky, N. and Heath, R.R. (1994). Pheromone deposition on leaf territories by male Caribbean fruit flies, *Anastrepha suspensa* (Loew) (Diptera: Tephritidae). *J. Insect Behav.* **7**:43–51.

Smith, R.L., Adams, T.B., Doull, J., Feron, V.J., Goodman, J.I., Marnett, L.J., Portoghese, P.S., Waddell, W.J., Wagner, B.M., Rogers, A.E., Caldwell, J. and Sipes, I.G. (2002). Safety assessment of allylalkoxybenzene derivatives used as flavouring substances – Methyl eugenol and estragole. *Food Chem. Toxicol*. **40**:851–870.

Steiner, L.F. (1952). Methyl eugenol as an attractant for oriental fruit fly. *J. Econ. Entomol.* **45**: 241–248.

Tan, K.H. and Nishida, R. (1996). Sex pheromone and mating competition after methyl eugenol consumption in Bactrocera dorsalis complex. In: *Fruit Fly Pests – a World Assessment of their*

Biology and Management, McPheron BA, Steck GJ (eds.). St. Lucie Press, Delray Beach, pp 147–153.

Tan, K.H. and Nishida, R. (2000). Mutual reproductive benefits between a wild orchid, Bulbophyllum patens, and Bactrocera fruit flies via a floral synomone. *J. Chem. Ecol*. **26**:533–546.

Tan, K.H. and Nishida, R. (2007). Zingerone in the floral synomone of *Bulbophyllum baileyi* (Orchidaceae) attracts Bactrocera fruit flies during pollination. *Biochem. Syst. Ecol*. **31**:334–341.

Tan, K.H. and Nishida, R. (2012) Methyl eugenol – its occurrence, distribution, and role in nature, especially in relation to insect behaviour and pollination. *J. Insect Sci.* **20**: 1–74.

Tan, K.H., Tokushima, I., Ono, H. and Nishida, R. (2011). Comparison of phenylpropanoid volatiles in male rectal pheromone gland after methyl eugenol consumption, and molecular phylogenetic relationship of four global pest fruit fly species – *Bactrocera invadens*, *B. dorsalis, B. correcta* and *B. zonata*. *Chemoecol*. **21**: 25–33.

Tan, K.H, Nishida, R., Jang, E.B. and Shelly, T.E. (2014). Pheromones, Male lures and trapping of Tephritid fruit flies. In: *Trapping and Detection, Control and Regulation of Tephritid fruit flies*. Shelly, T., Eric, N.E., Jang, B. J., Flores, R. and Vargas, R. (eds).pp. 18-31.

Vargas, R.I., Shelly, T.E., Leblanc, L., Pin˜ero, J.C. (2010a). Recent advances in methyl eugenol and cue-lure technologies for fruit fly detection, monitoring, and control in Hawaii. *Vitam. Horm*. **83**:575–595

Vayssie'res, J.F., Sinzogan, A., Korie, S., Ouagoussounon, I. and Thomas Odjo, A.S. (2009). 'Effectiveness of Spinosad Bait Sprays (GF-120) in controlling Mango infesting Fruit Flies (Diptera: Tephritidae) in Benin'. *J. Econ. Entomol*. **102**: 515-521.

Webb, J.C., Burk, T. and Sivinski, J. (1983). Attraction of female Caribbean fruit flies, *Anastrepha suspensa* (Diptera: Tephritidae), to the presence of males and male-produced stimuli in field cages. *Ann. Entomol. Soc. Am.* **76**: 996–998.

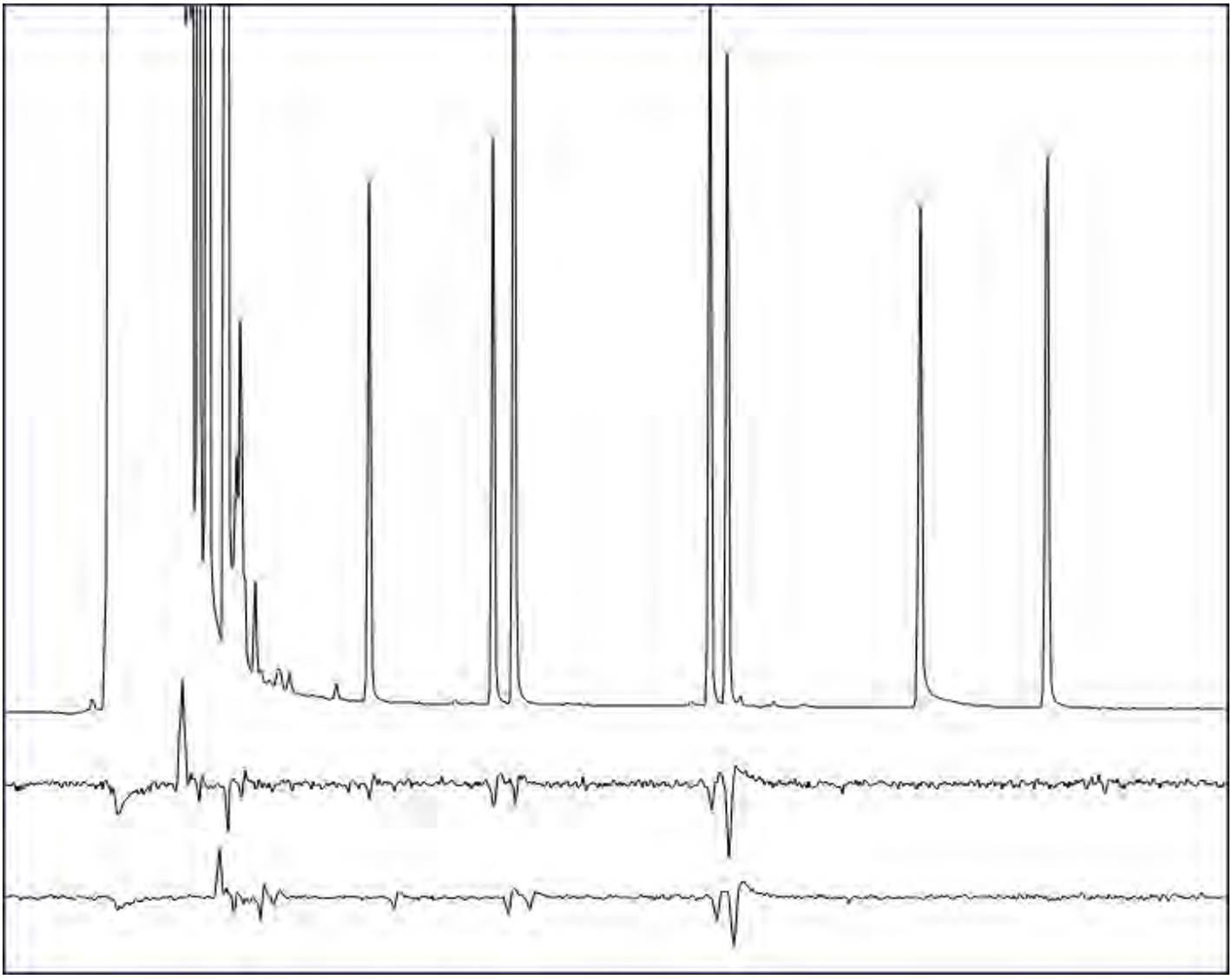
Wee, S.L., Tan, K.H. and Nishida, R. (2007). Pharmacophagy of methyl eugenol by males enhances sexual selection of *Bactrocera carambolae* (Diptera: Tephritidae). *J. Chem. Ecol*. **33**: 1272–1282.

West, A. S. (1961). Chemical attractants for adult Drosophila species. *J. Econ Entoml.* **54**:677-

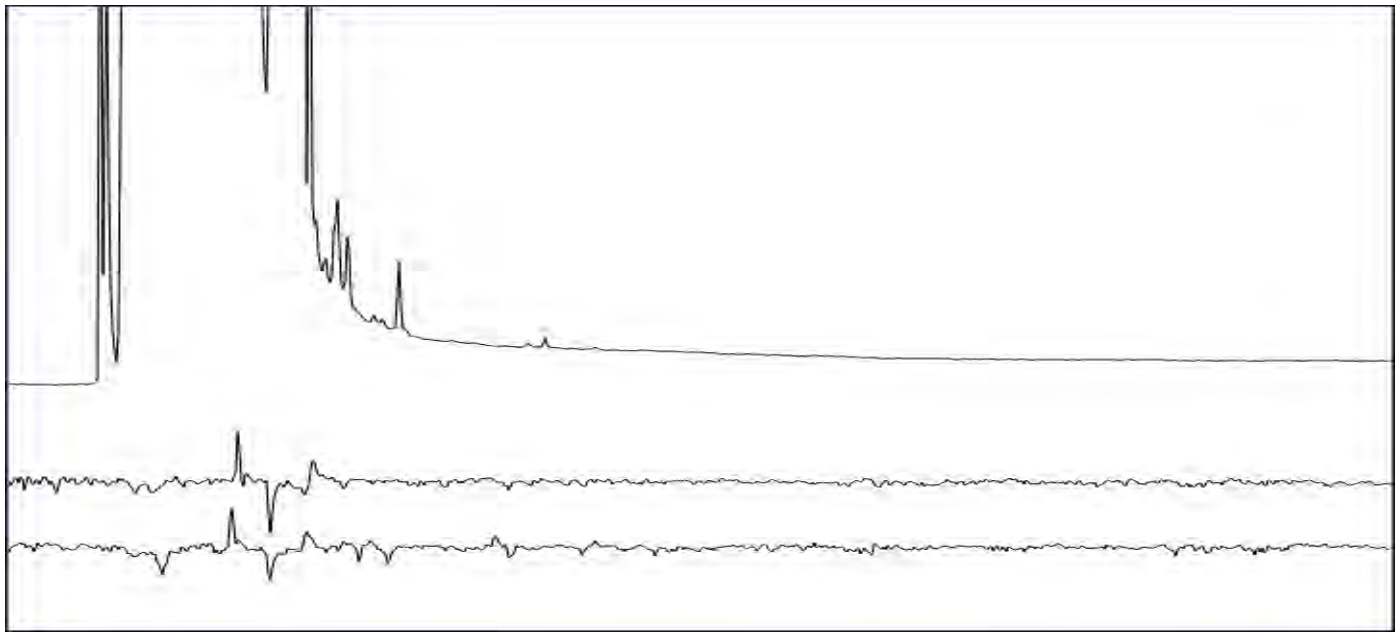
681.

9. Annexes

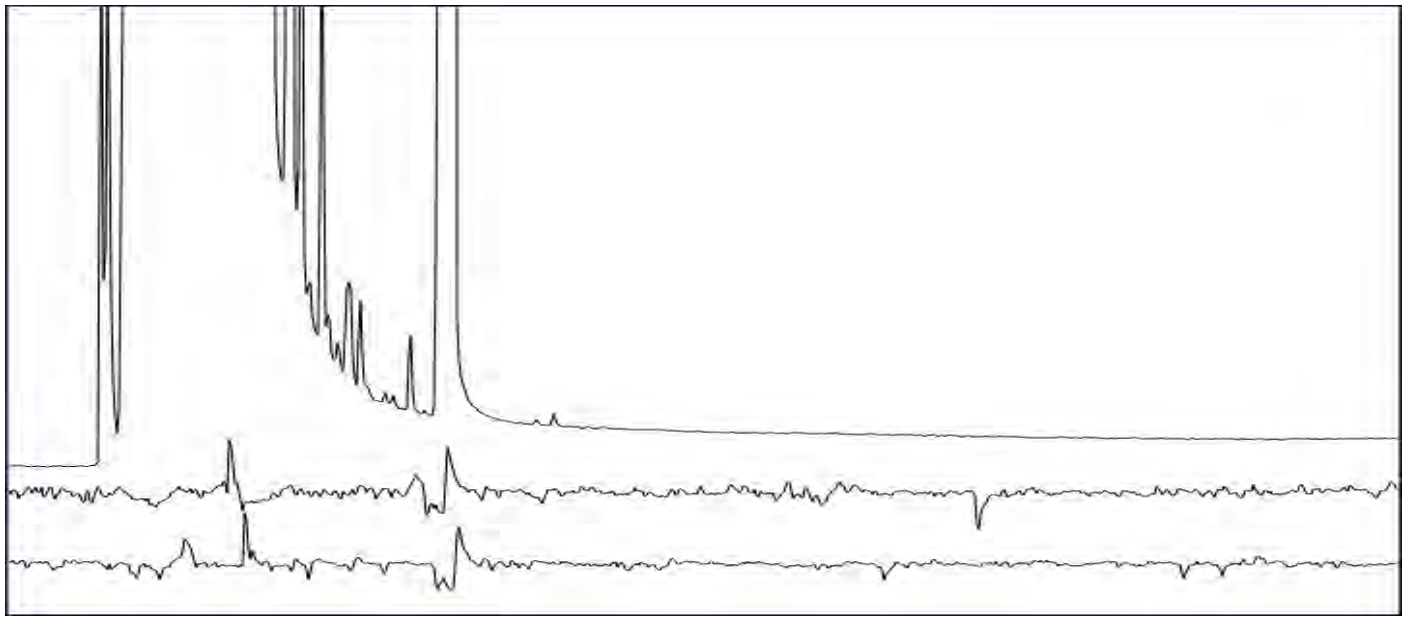
Annex 1 EAD chromatograph of female *C. capitata* for mix of synthetic blends



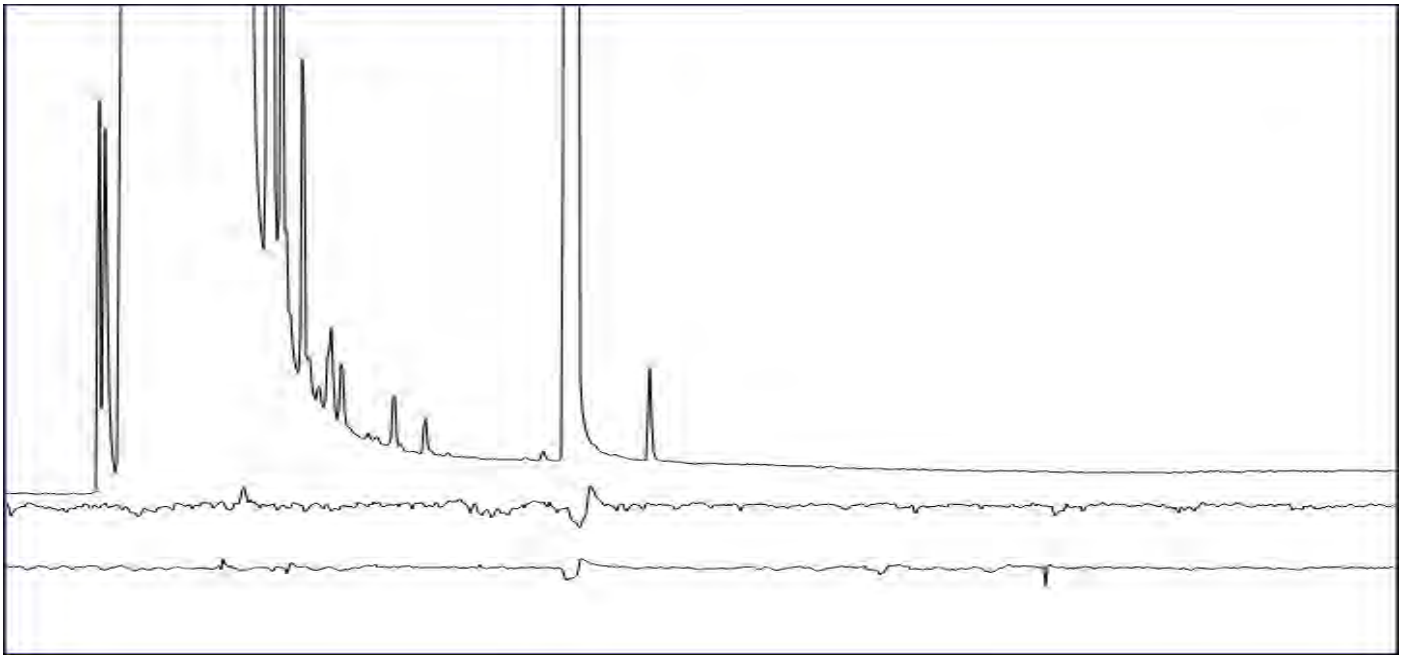
Annex 2 EAD chromatograph of *C. capitata* for 3-methyl 1-butanol



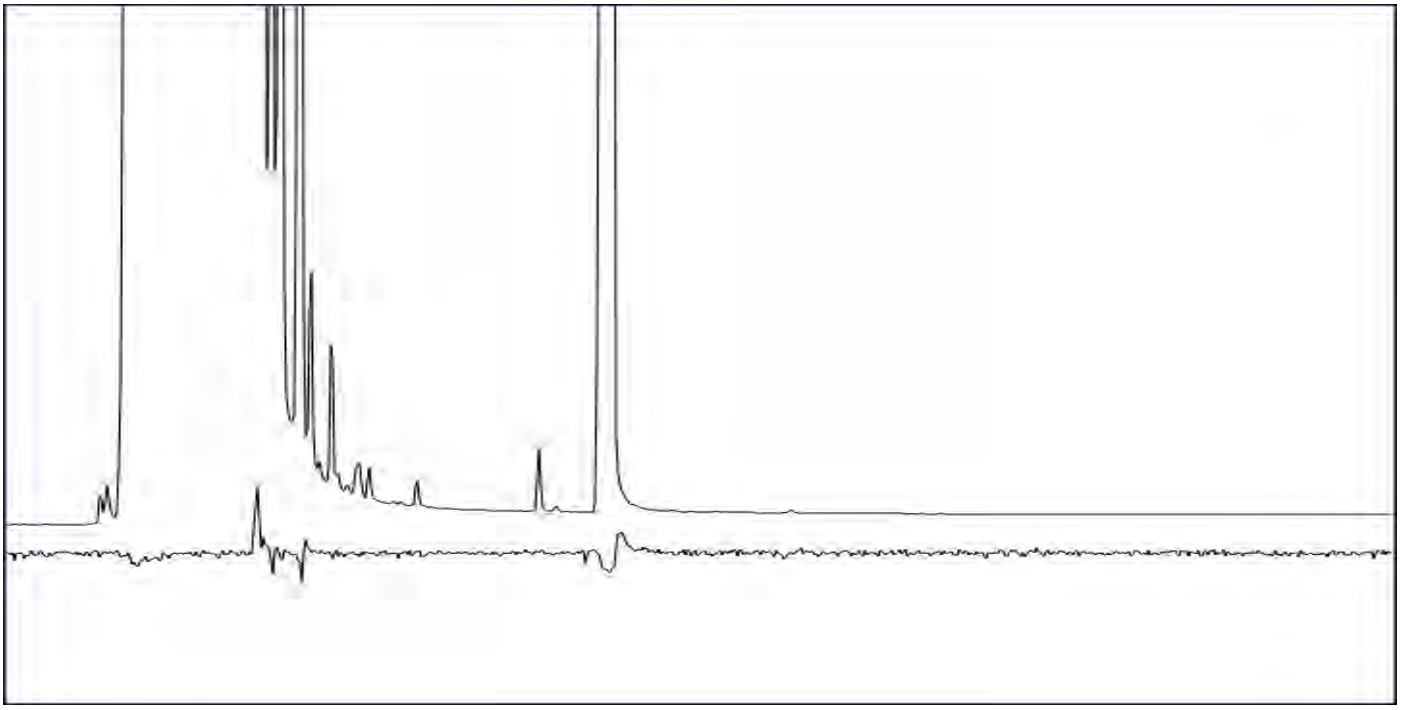
Annex 3 EAD chromatograph of *C. capitata* for butyl acetate



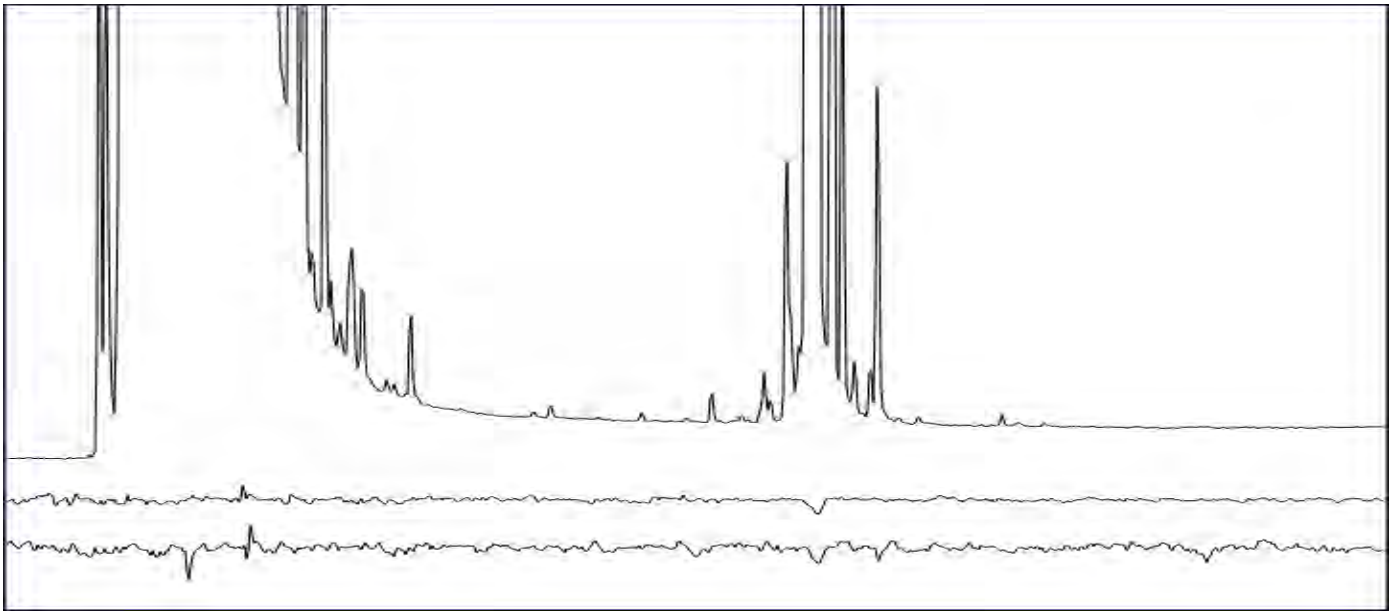
Annex 4 EAD chromatograph of *C. capitata* for isoamyl acetate



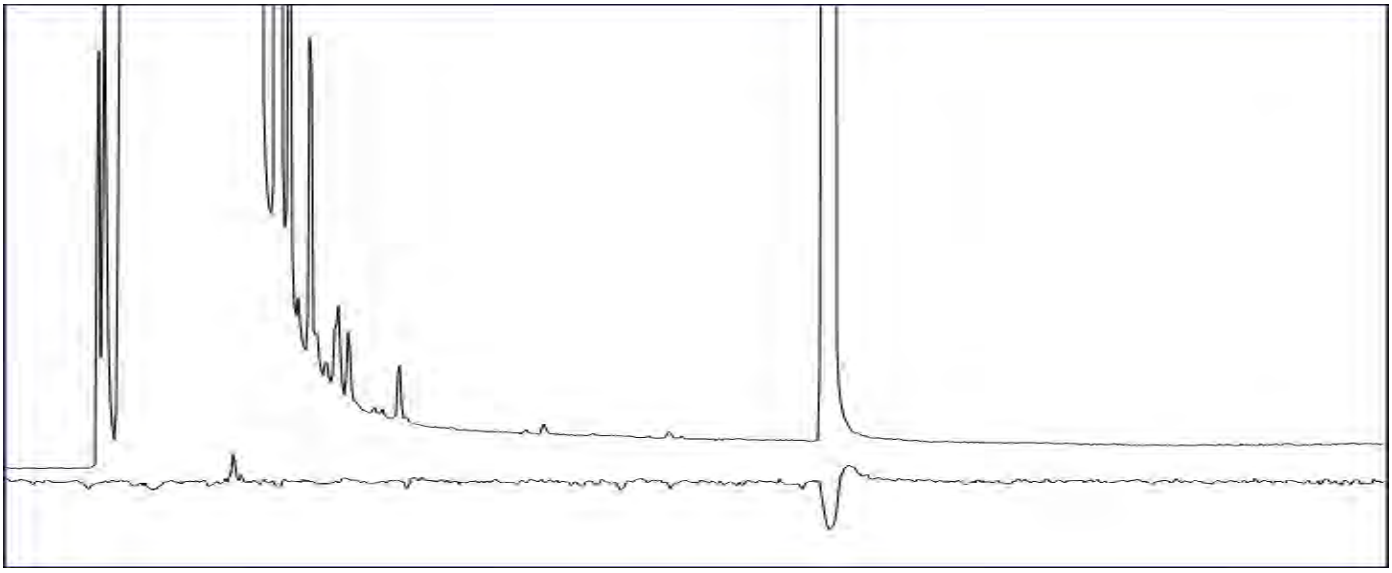
Annex 5 EAD chromatograph of *C. capitata* for styrene



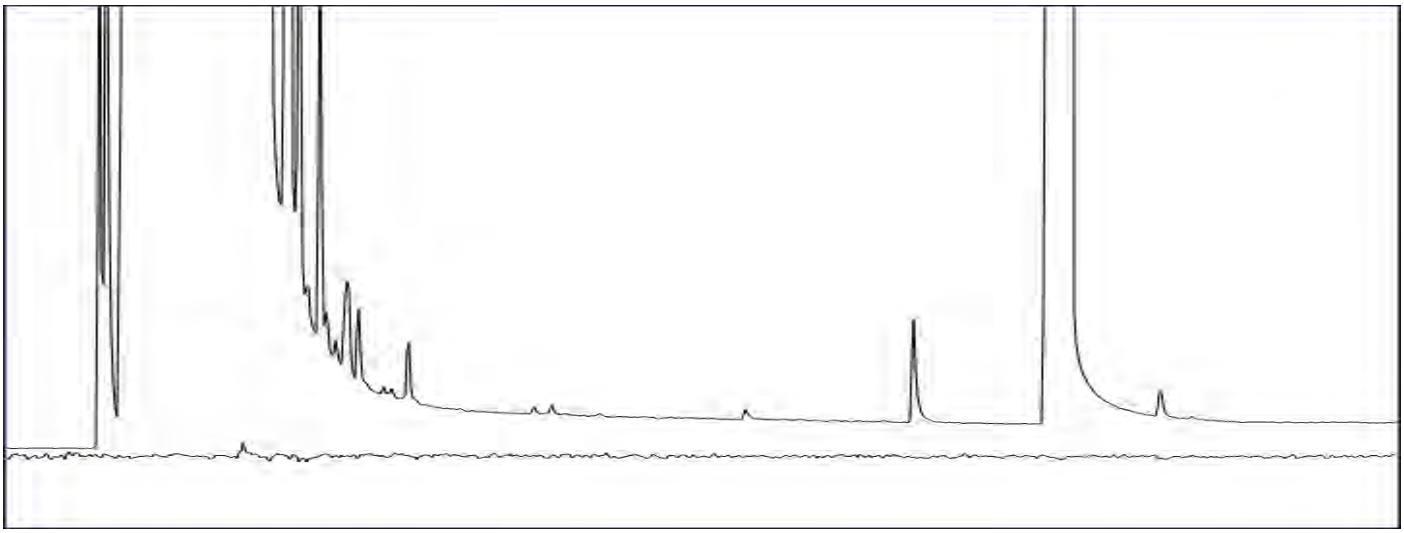
Annex 6 EAD chromatograph of *C. capitata* for β-Myrcene



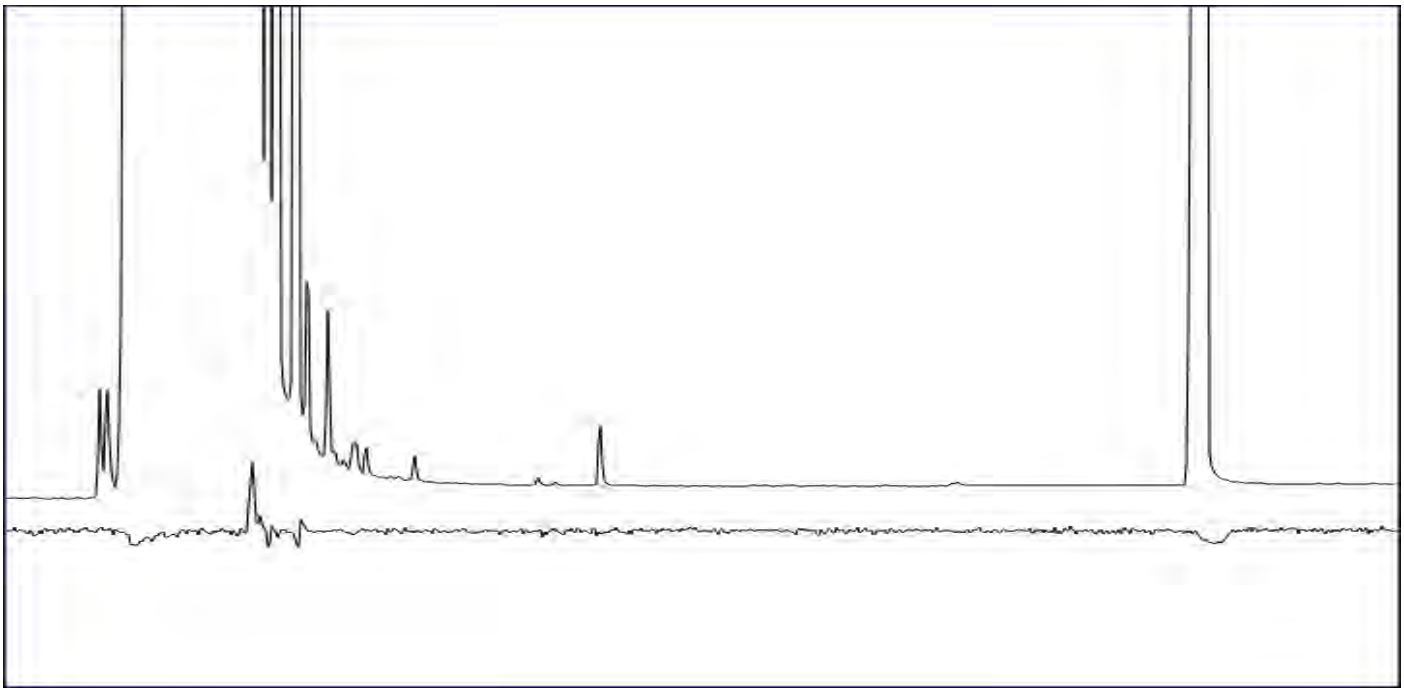
Annex 7 EAD chromatograph of *C. capitata* for ethyl hexanoate



Annex 8 EAD chromatograph of *C. capitata* for 2-phenyl ethanol



Annex 9 EAD chromatograph of *C. capitata* for alpha methylbenzyl acetate



Annex 10 GC-MS identification