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Volatile emissions of *Ceratitis capitata* adults (Wiedemann) (Diptera: Tephritidae)

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Abstract
The volatiles compounds emission by *Ceratitis capitata* adults and those that were extracted from male and female adults and analyzed by the gas chromatography-mass spectrometer. Gas Chromatography (GC) technique coupled with flame ionization detection (FID), and gas with mass spectrometry (GC-MS) were used for identification VOC's. Head Space-Solid Phase Micro Extraction HS-SPME method with Three-phase fiber 50/30 µm divinylbenzene/carboxen/ polydimethylsiloxane (DVB/CAR/PDMS) was used for VOC's extraction. Twenty nine compounds identified in the emission volatiles, acetoin, toluene, 2,3-hexanedione, hexaldehyde, o-dimethylbenzene, nonane, butanoic acid,4-hydroxy, 2,3,4-trithiapentane, octane, 2,7-dimethyl-, octanal, 4-Methyl-5-hexen-4-olide, acetophenone, 3,3-dimethylstyrene, cosmene, 2,4,6-octatriene,2,6-dimethyl-(E,Z)-, undecane, 2,6-dimethyl-, 1h-Pyrrole-2-carboxylic acid, tridecane, 2,6,10-trimethyltridecane, dimethyl phthalate, cuparene, farnesene, (e)-y-bisabolene, undecane,5-phenyl-, tetradecanoic acid, carboric acid, 2-ethylhexyl octyl ester, n-hexadecanoic acid, 2(3h)-furanone, 5-dodecyldihydro- and octadecanoic acid The results indicated that there were different chemicals emitted from male and female of Medfly. Thus, the VOC's technique could provide a possible tool for understanding the insect response and understand chemical communications between insect-insect interactions in adult stages of Medfly.

Keywords: *Ceratitis capitata,* VOCs, SPME-GC-MS, Fibre

1. Introduction
Mediterranean fruit fly *Ceratitis capitata* (Medfly) is species of invasive insect and polyphagous insect that impact fruit production and export worldwide [1, 2]. Medfly *C. capitate* is the most invasive of all members of the tephritidae species [3]. In the organic production of fruits, the issue is more dangerous, since the law regarding organic farming prevents the use of synthetic methods that include pesticides [4]. Females of medfly cause extensive damage on commercial produced fruits form more than 260 species of plants [5], by puncturing the fruits during oviposition time and the feeding larvae inside the fruit that damage the fruit in generally. Females attracted by males pheromones and they choose the males to mate based on complex chemical stimuli and volatiles [6, 7]. The control of pests needs to develop diagnostic tools and a precise pest detection technique for control management strategies. Also, genetic studies need the investigation of the evolutionary relationship between the species [8]. For these reasons, farmers are trying to limit the problems by avoiding infection by Medfly. Results obtained in the field and laboratory tests explain different susceptibilities to fruit fly damages [9]. Identification of the VOC's released by different stages of Medfly can help us to understand the chemical communications between insect-insect interactions in various stages of Medfly [10]. Recently, Head Space-Solid Phase Micro Extraction (HS-SPME) method coupled with flame ionization detection (FID) and gas chromatography with mass spectrometry (GC-MS) has been used successfully to examine volatile compounds (VOC's) [11]. VOCs have been widely used to detect stored grain insects and aggregation pheromone in pests [12-16]. Some of the complex phormones are remained unknown [17]. Based on these studies, it needs to develop a precise pest-detection technique, detect tools and management strategies of Medfly and investigation of their evolutionary relationships as well as the characterization of the adult stage of Medfly [2]. The study aimed to analyze the chemical composition of the volatile organic compounds emitted by the males and females. It also helps to understand the communication signals between males and females during mating time.

2. Materials and methods

2.1 Insect culture

All the flies reared under the following conditions: 23±2 °C and 75±5% RH, and 12:12-h (L:D) [1]. Adults were placed in (40cm length ×40cm height ×40cm depth) screen cages, and each cage contained medfly food made from crystaline sugar (Bidvest, Australia), yeast hydrolysate (Australian Biosearch) in the ratio of 4:1 and water 50 mL. About 10-12 days after adult emergences from pupae and mating of adults flies, eggs were collected every day, which is deposited on to the mesh side and fallen into the water tray kept adjacent to the cage.

2.2 Preparation of samples for analysis

The samples prepared for 20 males, 20 females and companied (10M/10F) adults in 36 mL jar. Each sample was replicated three times, and all samples were conditioned for four h at 26 ◦C.

2.3 Equipment and methods for collection of VOCs

VOCs were collected by solid phase micro extraction with SPME fibre 50/30 µm Carboxen/DVB/PDMS (2 cm) (Sigma-Aldrich, USA) coating. Samples were collected by inserting the fibre into the jar and exposing to the headspace for four h. The chemicals extracted by HS-SPME were analyzed with an Agilent Technologies gas chromatograph 7829A (serial number CN14272038) fitted with an HP-5MS column non-polar (30m x 0.25mm, 0.25 µm, RESTEK, catalogue no. 13423), with a flame ionization detector (FID). The carrier gas was Helium (He) with a constant flow rate of 1.1 mL/min, injection temperatures 250 ˚C, detector temperature 290 ˚C and the oven temperature was 50 ˚C increased to 250 ˚C. The GC-FID was operated in the splitless mode. Total run was 45 mins, and each flask was sampled three times. For identification the compounds, GC-MS 7820A gas chromotocharphy equipped with a mass spectrometer detector 5977E (Agilent Technologies, USA), the HP-5MS column was non-polar (30 m x 0.25 mm, film thickness 0.25 µm, catalogue NO 95051) (Santa Clara, CA 95051 product) was used. The carrier gas 99.999% was helium supplied by (BOC, gas, Sydney, Australia). The GCMS operation was as following conditions: temperature injector port was 250 ˚C. The initial oven temperature was 50 ˚C and increased to 250 ˚C by (5 ◦C/min), Column Flow rate was 0.7 ml per min, which splitless was 20 ml/min at 1.5 min. The total run of instrument time was 45 mins. The volatiles were identified by comparsion of the mass spectrum with the database the US National Institute of Standards and Technology (NIST) 2014 with retention index RI confirmation samples.

2.4 Limit of detection (LOD)

The limit of detection (LOD) was evaluated with alkane standard C7-C30 (Supelco, Bellefonte, USA). The stock of standard for concentration was prepared by adding four µl of the standard into 1L Erlenmeyer flasks. After that, samples were diluted to ppb from ppm and ppt from ppb levels by transferring one mL of headspace by syringe into another flask. After 1 hour of sealing time with 50/30 ìm Carboxen/DVB/PDMS (2 cm) (Sigma- Aldrich, Bellefonte, USA) fibre at the room temperature, the SPME fibre was injected into GC-FID with 250 ˚C injection port. Each level was repeated two times as per previous research.

2.5 Statistical analysis

Data were analyzed by Excel 2010. For the variation in standard deviation (SD) of VOCs areas, three replicate were used and compared with the average of area readings. Least significant differences (LSD≤0.05) have been used to compare data between the Sex.

3. Results and discussion

3.1 Limit of detection

The GC-FID response of the stock and diluted alkanes standard decreased form ppm level to ppt level (Fig 1). Some of the alkanes detected less than ppt level with SPME method. Octane, decane, undecane, pentadecane, and heptadecane can be detected in ppt level (Fig 1).

3.2 GC-FID analysis

The main peaks from the adult stage were 29 compounds. These compounds have been recorded as identify by GC-MS. The results showed that 14 compounds from Males, 20 compounds from females (Fig. 2). To identify the chemicals, the peak area of each compound with respect to the total integrated peaks was calculated to comparison the emission between compounds in the same treatment [17]. Jang *et al*. (2) reported that some short-chain aldehydes at trace levels that released by the female of fruit flies. The current study did not detect some compounds which reported by other studies; however, most of these chemicals are minor chemicals. The reason could be using the different technique for collecting the volatile compounds, or other experimental factors affect like time of taking samples, air flow, GC temperature, etc. [18].

3.3 VOCs from males and females

After recording the compounds, we used GC-MS to identify the chemicals name, and some were not detected in GC-MS samples especially in females. These compounds like 2,3-hexanedione, o-dimethylbenzene, nonane, 2,3,4-trithiapentane, octanal, acetophenone, 2,4,6-octatriene,2,6-dimethyl-(E,Z)-, 1h-Pyrrole-2-carboxylic acid, 2,6,10-trimethyltridecane, dimethyl phthalate, farnesene, (E)-Y-bisabolene, undecane,5-phenyl-, carboric acid, 2-ethylhexyl octyl ester and 2(3H)-furanone, 5-dodecyldihydro-(Fig. 2). The techniques applied in this current research (headspace collection of insect volatiles, GC-FID, and GC-MS) allowed for the identification of volatiles produces by Medfly. SPME fibre technique could be used for the qualitative analyses of the fruit fly emanation [5]. Recently, some of the studies worked on headspace, solvent extraction and analytical techniques, which explained the differences in Medfly volatiles profile 18. Acetophenone group has been described before released by females of *Dendroctonus spp* [19]. The main constituents of male chemicals include (E,E)-R-farnesene, geranyl acetate, and ethyl (3E)-3-octenoate, which is consistent with the results of the same kind of studies [20]. Some of the chemicals in this research have been described previously as being part of volatile constituents of fruit flies, and some were not described before. These include acetophenone, eicosen [8, 21-23]. In fact, this type of experiment was carried out with *C. capitata,* and the conditions (age, temperature, RH, light, sex, etc.) could also be an influence on the detected VOCs.

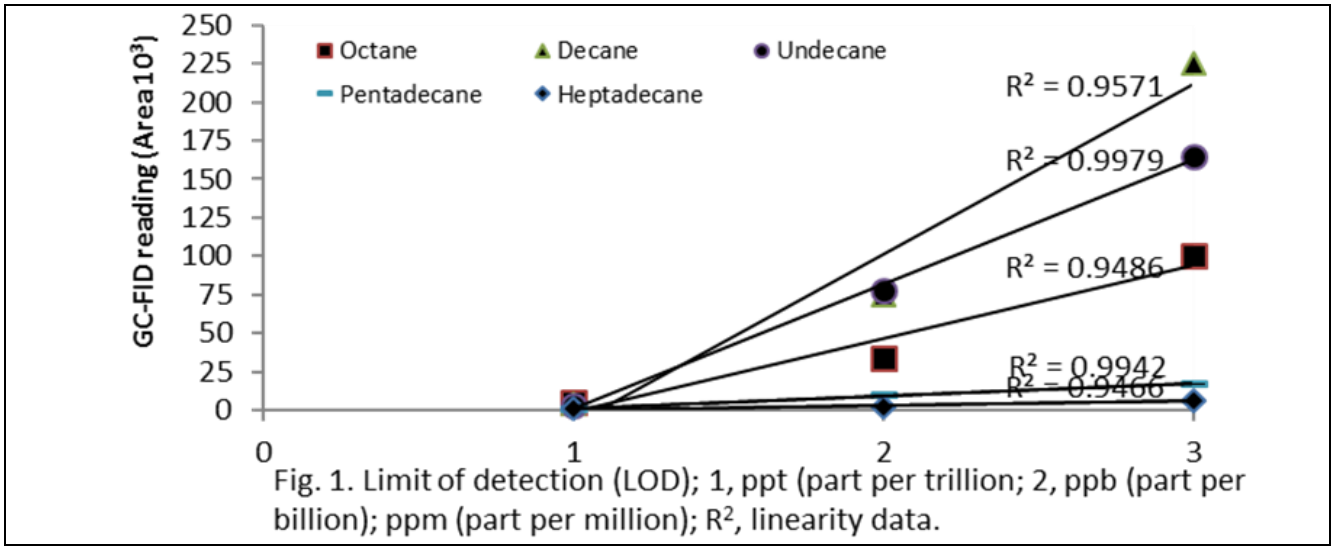

Fig. 1. Limit of detection (LOD); 1, ppt (part per trillion; 2, ppb (part per billion); ppm (part per million); R^2, linearity data.

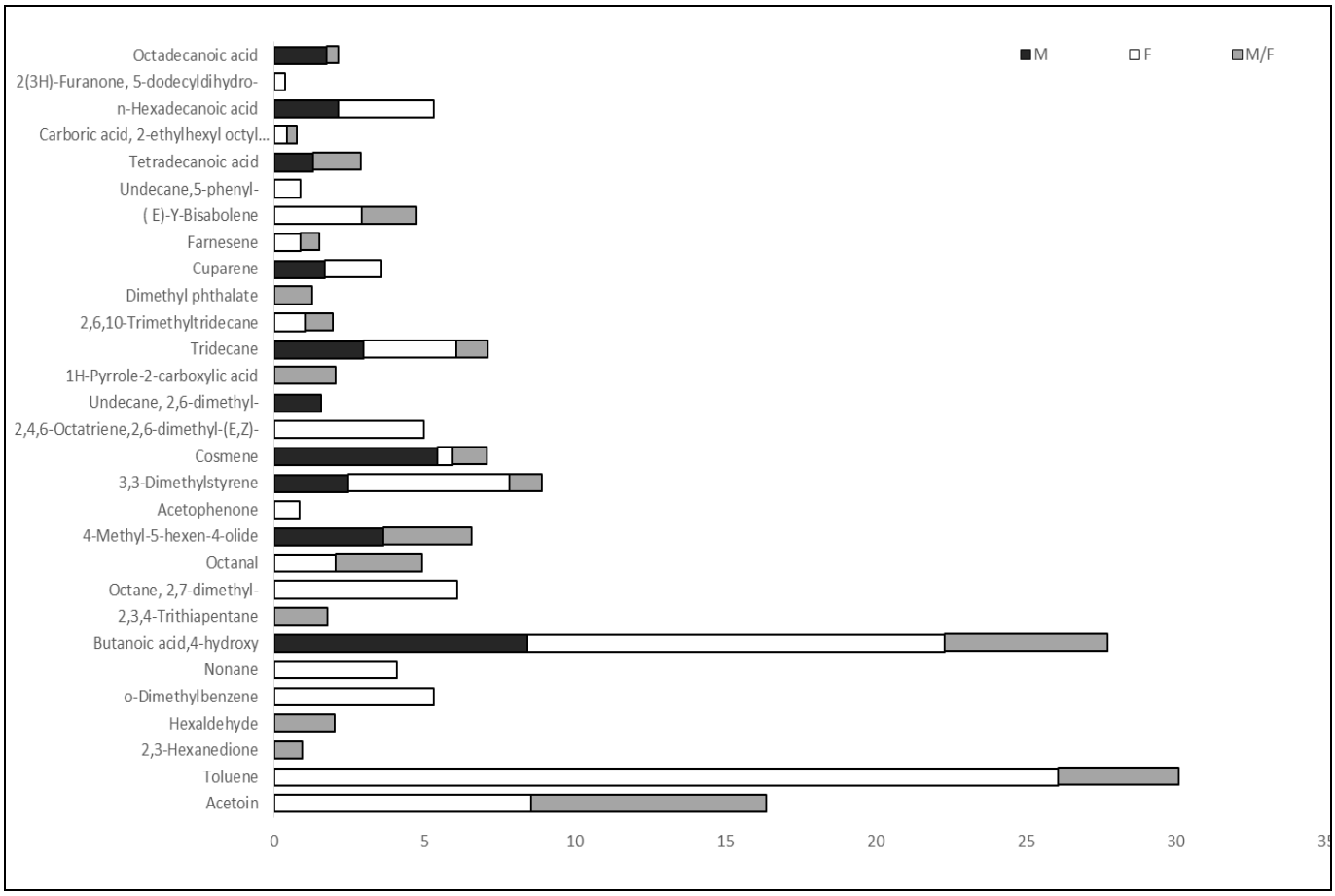

Fig 2: Compounds were released from male and females adults and detemined by GC-FID and GC-MS

4. Conclusion

This technique is a fast, solvent-free and inexpensive that has been developed by analyzed the volatile compounds released by *C. captitata*. The method allowed the separation several of compounds. The SPME procedure was optimized using a 50/30 ìm Carboxen/DVB/PDMS (2 cm). It was found 29 compounds emitted by adults have identified use HS-SPME fibre coupled with GC-MS technique. These compounds were from males, females and combined. The significant compounds, which were acetoin, 2,3-hexanedione; o-dimethylbenzone; nonane; octane,2,7- dimethyl; butanoic acid, 4-hydroxy; 2,3,4- trithiapentane; hexaldehyde; and octanal from adult stage. The application of an experimental design with different sex is reported in this research. The results showed these treated have a key for changing the profile chemicals of medfly in different stages. From these results; we analyzed the chemical composition of the volatile organic compounds emitted by adult stage of Medfly. Also, to understand the communication signals between males and females during mating time. This method can be used for further experimental, explain the chemicals released by Medfly and select these volatile compounds to be existing tools for understanding oviposition, repellence, and attraction of medfly. HS-SPME technique has been shown to be a rapid, reliable and precise diagnostic tool for control medfly.

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