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Volatile profiling of food baits targeting female melon fruit fly, *Zeugodacus cucurbitae* (Coq.) (Tephritidae: Diptera) in gourds

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Abstract
Melon fruit fly, *Zeugodacus cucurbitae* (Coq.) (Tephritidae: Diptera), is a serious threat in horticultural crops especially in cucurbits. Development of female-based trap would be more sensible, befitting and more beneficial. Focusing on female fruit flies, three food baits *viz*, Bait 1 (guava-based food bait), Bait 2 (muskmelon-based food bait) and Bait 3 (guava + muskmelon-based food bait) were developed and volatiles collected from the baits were analysed in GC-MS to determine the compounds responsible for attraction and further subjected to heat map analysis and Principal Component Analysis to quantify their distribution and abundance in the baits. Results revealed that compounds namely á-Pinene, à-Phellandrene, o-Cymene, Caryophyllene were predominantly observed in all the three baits whereas compounds such as 2-Methyl-1-butanol, 1-Pentanol and á-Ocimene were predominant in B1, B2 and B3 baits respectively. Many other compounds such as ethyl butanoate, benzothiazole, myrcene, phenylethyl alcohol were found to be unique compounds in each bait.

Keywords: Food baits, *Zeugodacus cucurbitae*, volatile collection, GC-MS, Heat map, Principal Component Analysis

Introduction
Volatiles emanated from the plants and plant parts plays vital role in both attraction of pests as well as defence against pests. Quality and quantity of plant volatiles has greater impacts on host plant selection by insects [11]. Proper and appropriate utilisation of those plant volatile compounds could drastically reduce the plant protection costs. As melon fruit fly is constantly being ranked among the world's destructive pest of horticultural crops [6], their management gains more importance. As of now, the management focuses mainly on population control of male fruit flies by employing synthetic traps like cue lure or natural trapping compound such as raspberry ketone [17]. Shifting the focus towards management of female flies could be more sensible management practice, since it directly reduces the further generation build up [18]. As the female fruit flies pierces the fruit tissue and lays eggs inside the tissue, a brown resinous oozing out was seen on the fruit which reduces their market value. Larvae emerges out from the eggs feed on the inner content of the fruit which causes damage to the tissue and also invade microorganisms [9, 21, 11]. Managing with insecticides is difficult since the larvae grows inside the fruit and pupates in the soil. Thus, managing the adult population is more important. Extended use of insecticides leads to development of resistance and resurgence in pests. When compared to insecticidal spray, attract and kill strategy could be more advantageous when focusing on managing the adult population. Hundreds of chemicals in the environment could be sensed by the insects through their olfactory reception. Perceived chemicals greatly influence their behavioural response [3, 20]. Female fruit fly mostly uses the host plant volatiles as their olfactory cues for locating their respective host plants for feeding, mating and oviposition [4, 13, 12]. Focusing on female melon fruit fly management, food baits were developed. For more insight into it, current study deals with collection of volatiles from the food baits which were further subjected to gas chromatography- mass spectrometry (GC-MS) analysis for determining the compounds responsible for the attraction of fruit flies.

Materials and Methods
Food baits
Focusing towards trapping of female fruit flies, food baits *viz*, Bait 1(B1) –Guava (30g) + Cane Sugar (3g) + Yeast (0.3g) + Food Grade Alcohol (10ml), Bait 2(B2)-Muskmelon (40g) +

Cane Sugar (4g) + Yeast (0.4g) + Food Grade Alcohol (10ml) and Bait 3(B3)- Guava (20g) + Muskmelon (20g) + Cane Sugar (4g) + Yeast (0.4g) + Food Grade Alcohol (10ml) were developed with slight modification on trap designed earlier by [1]. Fruits and the bait additives were purchased from the market. Fruits were washed, peeled and grinded. To the pureed pulp, respective quantity of bait additives such as cane sugar, yeast and food grade alcohol were added and allowed for 48 hrs of fermentation. These baits were further used for volatile collection.

Volatile collection

After 48 hrs of fermentation, food baits were subjected to volatile collection. Fermented food baits were placed inside the conical flask sealed with rubber cork comprising ports. Stainless steel thermal desorber tube packed with Tenax™ TA (Perkin Elmer®, USA) was inserted into the port and sealed. Under nitrogen stream these Tenax adsorbent were conditioned at 200º c prior to collection. After 2.5 hours of collection, tenax tubes were removed aseptically and further used for GC-MS analysis.

GC-MS analysis

Collected volatiles were analysed in GC-MS (PERKIN ELMER CLARUS SQ8C) customized with thermal desorber with Turbomatrix 150 (Perkin Elmer®, USA). Following specifications were followed: initial oven temperature was programmed to 40°C for 2 min, then an increase in temperature of 10°C /min run to 250°C hold for 2 min. The detector temperature was maintained at 320°C and source line temperature was maintained at 230°C. Helium was used as carrier gas at a flow rate of 1ml/min. DB-5 MS capillary standard non-polar column of 30 m length, 0.25 mm ID and 0.25 µm film thickness with 5% diphenyl/95% dimethyl polysiloxane as stationary phase was used for the analysis. Using ionization voltage of 70 eV, mass spectral data were recorded. Mass spectrometer has an integral library of compounds which automatically search and matches the spectrum produced by the sample. For the interpretation of sample compounds, the spectrums of the sample were compared with spectral database of National Institute of Standard and Technology (NIST14).

Statistical analysis

Compounds obtained from the GC-MS were centred and scaled which were further subjected to quantification using Heat map analysis and Principal Component Analysis (PCA). Heat map analysis was done to determine the distribution pattern of the volatile compounds among the food baits. Heat map analysis was performed in online software "Heat mapper" with the range of scale 0-8. Principal Component Analysis was done to reduce the dimensions which facilitates better understanding and it aids in significant separation of samples. PCA analysis was performed using prcromp function in R software (R version 3.5.1).

Results and Discussion

Compounds obtained through GC-MS were grouped based on their chemical properties. From these three food baits, total of 53 compounds were finalised by comparing the sample spectrum with NIST library spectrum (Table. 1). These compounds mostly come under the groups like, alcohol, aldehyde, ketone, terpene, ether and carboxylic acid. Abundance and distribution of these compounds among the baits were studied using heat map analysis and Principal Component Analysis.

Table 1: Compounds obtained from the GC-MS reports numbered for heat map and PCA

1.	1-Phenoxy-2-propanol	28.	1-Butanol, 3-methyl-, propanoate
2.	Tetramethylammonium cation	29.	á-Pinene
3.	1,3-Pentadiene	30.	β-Pinene
4.	Benzene	31.	á-Myrcene
5.	4-Methoxy-2-methylbutane	32.	(E)-á-Farnesene
6.	Propanoic acid, 2-methyl-, ethyl ester	33.	Butanoic acid, butyl ester
7.	1,3-Dioxolane, 2,4,5-trimethyl-	34.	à-Phellandrene
8.	1-Pentanol	35.	1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-
9.	2-Methyl-1-butanol	36.	o-Cymene
10.	Disulfide, dimethyl	37.	Limonene
11.	Propanoic acid, 2-methyl-, ethyl ester	38.	β-Thujene
12.	Toluene	39.	trans-á-Ocimene
13.	Diclofop-methyl	40.	á-Ocimene
14.	2-Hexanone	41.	à-Pinene
15.	Ethyl butanoate	42.	ç-Terpinene
16.	Hexanal	43.	Acetophenone
17.	8-Chlorotheophylline	44.	2-Nonanone
18.	3-Methylbutanoic acid	45.	Nonane, 4,5-dimethyl-
19.	3-Hexen-1-ol,	46.	5-ethyl-2-methyl- octane
20.	1-Hexanol	47.	Nonanal
21.	2-Propanone, O-methyloxime	48.	Phenylethyl Alcohol
22.	1-Butanol, 3-methyl-, acetate	49.	Boldione
23.	2-Heptanone	50.	Tetrasulfide, dimethyl
24.	Bicyclo [4.2.0] octa-1,3,5-triene	51.	Benzothiazole
25.	2-Propenoic acid, butyl ester	52.	Caryophyllene
26.	1,4-Dioxan-2-ol	53.	Glycerol 1-palmitate
27.	Acetic acid, hydroxy-, ethyl ester		

Heat map analysis

Heat map analysis was done to determine the distribution pattern of the compounds in food baits (Fig. 1). Range of compound distribution varies from 0-8 scale. Darker yellow exhibited the maximum amount of distribution whereas the darker blue represented the lower amount of distribution.

Similar colour patterns throughout the row depicted the same amount of distribution of that particular compound in all three baits. 2-Methyl-1-butanol (9) seem to have higher amount of distribution in Bait1(guava) followed by 1,3-pentadiene (3) and β-Thujene (38) compounds. Similar to the current results, Methyl-1-butanol was reported to exhibit attraction response in female Caribbean fruit fly, *Anastrepha suspensa* [5, 2]. 1-Pentanol (8) has higher proportion of distribution in Bait2 (muskmelon) followed by Benzene (4), o-Cymene (36) and ç-Terpinene (42). In concordance with our results, O-Cymene was reported in male pheromones of *Anastrepha fraterculus* and *A. suspensa* which attracts the female fruit flies [11]. 4-Methoxy-2-methylbutane (5) was distributed in larger amount in the Bait3 (guava + muskmelon) followed by 1-Pentanol (8) and á-Ocimene (40). As similar to the current findings, volatiles emitted by the *A. suspensa* were analysed and the results reported that ocimene compounds were released by the fruit flies as pheromones [15, 10].

Terpenes plays major role in attracting the insects as they greatly influence the olfactory perception of the fruit flies. α-phellandrene, β-phellandrene, β-ocimene, á-Ocimene were major group of terpenoid compounds reported from the volatiles collected and analysed from cucumber. These compounds contributed the major proportion in synthetic blend formulated for attraction of *Zeugodacus cucurbitae* which were also reported as EAD active [11]. Similar results were obtained in the current findings i.e, compounds like á-Ocimene (40), á-Pinene (41), o-Cymene (36), Caryophyllene (52) were commonly found in all three baits which might probably contributes major part in attraction of fruit flies towards the baits.

Comparing with other two baits, B3 (Guava+ Muskmelon) seem to have a greater number of compounds in higher proportion which were reported earlier for attraction of fruit flies. For instance, compounds like Caryophyllene (52), á-Ocimene (40), à-Phellandrene (34), á-Pinene (41), á-Myrcene (31) were in higher proportion in comparable with other two baits. Similar to the current results, Myrcene and ocimene compounds were observed in 'Alphonso' variety of mango, which produced positive attraction response for *B. dorsalis* [7]. Similarly, (E)-caryophyllene was observed in tomato plant volatile which reported to have attraction response on melon fruit fly [11]. These compounds were reported from cucumber volatiles which were found to have attraction response on *Zeugodacus cucurbitae*.

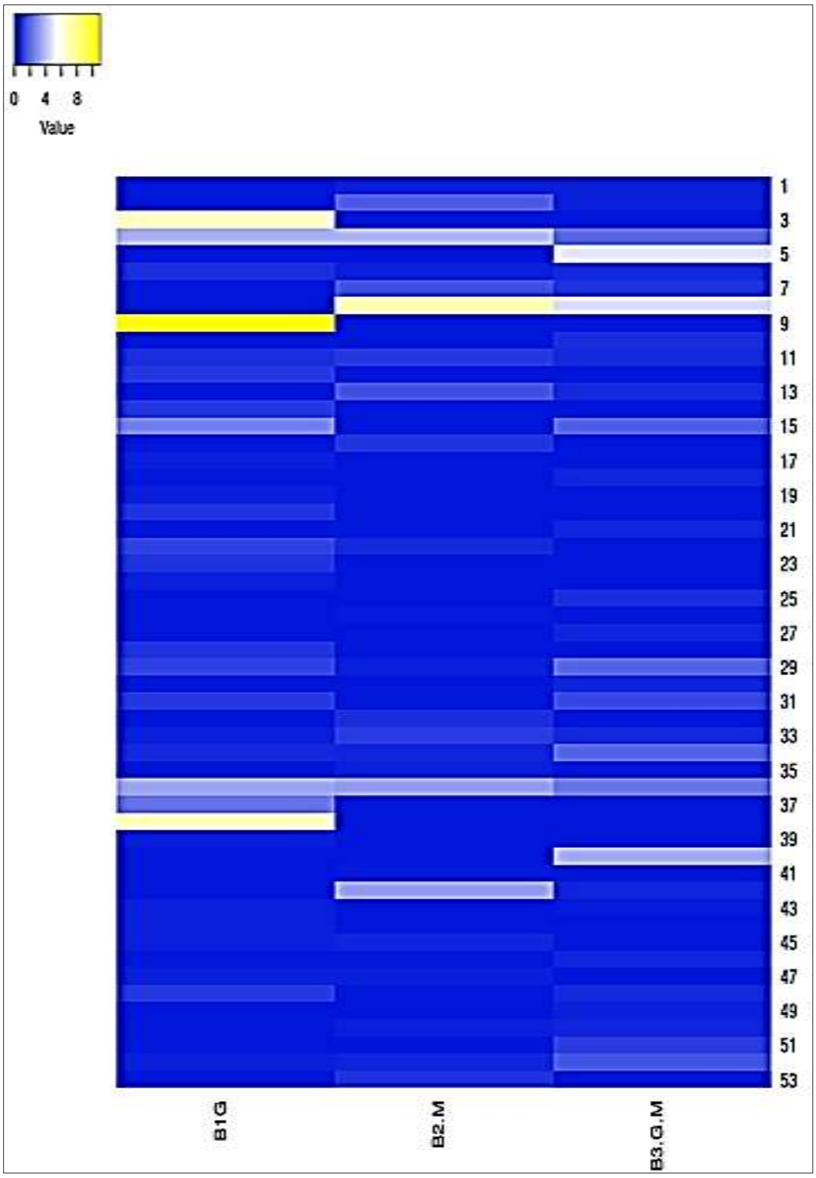

Fig 1: Heat map of volatiles collected from food baits B1, B2 and B3

Principal component analysis

A total of 53 compounds from all three bait volatiles were numbered and further subjected to Principal Component Analysis (PCA). Based on qualitative and quantitative differences of the compounds obtained from the volatile profiles, the food baits were separated. The scree plot obtained was tends to deviate at PC2 as well as PC1 and PC2 accounted 83.62% of cumulative proportion of variance, which depicts the high reliability of the analysis (Fig. 2). Biplot obtained from the analysis shown that compounds namely 1,3-Pentadiene (3), 2-Methyl-1-butanol (9), 2-Nonanone (44), 2-Heptanone (23), β-Thujene (38), 1-Hexanol (20) were present alone in B1 (guava) in a higher proportion of distribution. In concordance with the current findings, 2-Heptanone was identified from the host plant volatiles produced from coffee and this compound was found to be the prime compound responsible for attraction of mature female fruit fly, *Ceratitis capitata* [14]. For attraction of melon fruit fly, *Zeugodacus cucurbitae* pureed cucumber was used and the volatile compounds listed contains 1- Hexanol among the blend of compounds [11]. Ethyl butanoate (15) was found to be more associated with B1 in terms of quantitative distribution. Identification of [7] *agrees* with the current findings as the author reported that Ethyl butanoate was found to be EAD active compound for *B. dorsalis* which was observed in 'Chausa' variety of mango [7]. Limonene was found specifically in B1. Similar to the current results, Volatile compounds such as limonene and pinene emitted from the host plants found to have strong impact on mating and oviposition site selection behaviour of female fruit fly [16]. Volatile compounds detected from male pheromone extracts of *A. ludens* reported to have limonene which has the potential of attracting the female fruit fly [10, 15]. Benzene (4) and o-Cymene (36) were commonly observed in all three baits but based on the proportion of occurrence the compounds were more associated with B1 and B2. Nonanal (47) was recorded in both B1 and B2. Compounds like nonanal, α-pinene and p-cymene were reported from the pheromone released from both *B. oleae* and *R. cerasi* [16].

Hexanal (16) and 1,4-Dioxan-2-ol (26), (E)-á-Farnesene (32) and à-Pinene (41) were found uniquely in B2 (muskmelon) in a greater proportion. As similar to the current findings, (E)-á-Farnesene was reported from the list of compounds released by male Mediterranean fruit fly which attracts the female fruit fly [16][8]. 1,3-Dioxolane, 2,4,5-trimethyl- (7) was found in both B2 and B3 baits whereas the proportion was greater in B2. 1-Pentanol (8) was commonly found in both B2 and B3 but the closer association was recorded in B2 since the compound presence was more in B2 when compared with B3.

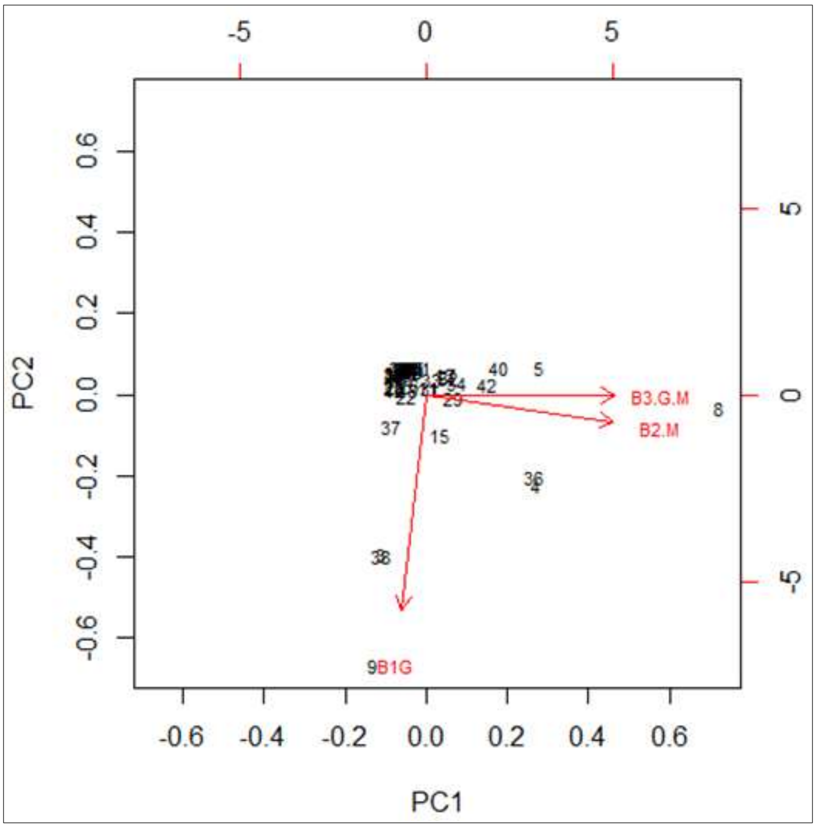

Fig 2: Scree plot for the compounds analysed from food baits through GC-MS

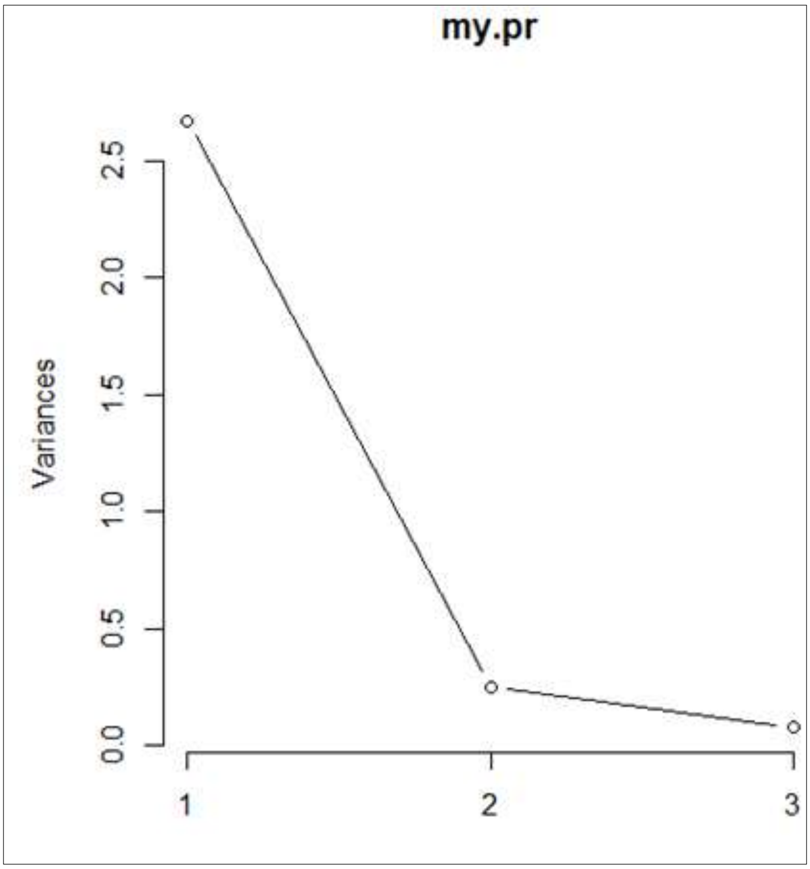

Fig 3: Biplot for the compounds analysed from food baits through GC-MS

Monoterpene such as ç-Terpinene (42) was found abundant in B2 which might contribute a vital role in female fruit fly attraction. 5-Ethyl-2-methyl octane (46), 4-Methoxy-2-methylbutane (5), 3-Methylbutanoic acid (18), 2-Propanone, O-methyloxime (21), 2-Propenoic acid, butyl ester (25), Boldione (49) and Benzothiazole (51) were specifically associated with B3. Terpene compounds such as β-Pinene (30), á-Myrcene (31) and á-Ocimene (40) were found uniquely in B3 whereas à-Phellandrene (34), Caryophyllene (52) were found abundant in B3 which seem to be contributed more towards trapping of female fruit fly (Fig. 3). Similar to the current results obtained, Benzothiazole was observed in 'Chausa' variety of mango which was identified as attractive compound for *B. dorsalis* [7]. Similarly, Compounds such as hexanal, α-pinene, o-cymene, α-phellandrene γ-terpinene, n-nonanal were reported from host plant volatiles of both tomato and cucumber plants which were used in the synthetic blends. These compounds were reported as EAD active compounds for attraction of melon fruit fly [11].

Conclusion

Total of 53 compounds were identified from the food baits developed targeting the female melon fruit fly, *Zeugodacus cucurbitae* (Coq.). Among which, compounds namely 2-heptanone, α-pinene, p-cymene, Benzothiazole, á-Myrcene, á-Ocimene à-Phellandrene, Caryophyllene, Limonene, 1,3-pentadiene and Pentanol were found to be more abundant in baits and also found to have more attraction properties towards female melon fruit fly. These results will be more helpful in developing the synthetic blend for the management of female melon fruit fly in gourds. From the list of compounds obtained, further studies on behavioural response of insects to be done for the development of synthetic bait traps.

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Conflict Of Interest

All the authors declared that there is no conflict of interest in the current study.

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