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Behavioural response of melon fly, *Zeugodacus cucurbitae* (Coquillett) (Diptera: Tephritidae) to cue lure and protein fed conspecifics body volatiles

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ABSTRACT: *Zeugodacus cucurbitae* (Coquillett), is an important pest of several vegetable crops. Detection and monitoring of this pestiferous species rely heavily upon male lure or parapheromone cue lure. Consuming natural male lure compounds by males of tephritid fruit flies enables them to mate more successfully within their female choice mating systems. Here, we studied the olfactory behavioural response of mature male and female melon flies to body volatiles of cue lure fed and unfed mature male melon flies and protein fed immature male and female, protein unfed mature male and female melon flies body volatile. Olfactometer assay results revealed that female melon flies were highly attracted towards the cue lure fed male flies body volatile and male melon flies towards the cue lure unfed male flies body volatile. Our study also revealed the chemical composition of body volatiles collected from the aforesaid treatments and N-(3-Methylbutyl) acetamide was found to be abundant in the body volatile samples.

Keywords: Cue lure, behaviour, *Z. cucurbitae*, body volatile, olfactometer

INTRODUCTION

Zeugodacus cucurbitae (Coquillett), a tephritid fruit fly is an important economic pest of several vegetable crops. Detection and monitoring of this pestiferous species rely heavily upon male lure or attractant such as raspberry ketone or its man-made analogue cue lure (Shelly, 2010). In general, for the surveillance of pestiferous tephritid fruit flies, semiochemicals are greatly used (Jang and Light, 1996). The original observation that males of *Bactrocera* are attracted to particular chemicals, named male lures or parapheromones was made nearly a century ago (Cunningham, 1989 and Metcalf, 1990). Behavioural manipulation using chemicals specifically, male lures or parapheromones for the management of tephritid fruit flies has been well documented in the genus *Bactrocera* (Shelly *et al*., 2010). Methyl eugenol (ME) and Cue lure/Raspberry ketone (CL/RK) is used worldwide to detect the initial infestations of *Bactrocera dorsalis* (Hendel) and *Z. cucucrbitae* (= *B. cucurbitae*) (Chambers *et al*., 1974 and Jang and Light, 1996) and they are reported as powerful male attractants for certain *Bactrocera* species since long back (Howlett, 1915 and Steiner, 1952). Adding to this, along with toxicant ME and CL was used to eradicate island populations of this species in a procedure termed "male annihilation" (Steiner *et al*., 1965 and Steiner *et al*., 1970). Although, these male lures are used worldwide, the functionality of ME and CL/RK in the natural history of *Bactrocera* flies is poorly explored.

In nature, diversity of plant species produces raspberry ketone (Nishida *et al*., 1993, Tan and Nishida, 1995 and Tan, 2009) and males of *Bactrocera* species responding to CL/RK have been observed visiting the flowers of orchids that produce and release RK (Nishida *et al*., 1993; Clarke *et al*., 2002 and Tan and Nishida, 2005). Alike ME-responders, in CL responsive species males exhibit greater attraction to CL than females (Nishida *et al*., 1993 and Weldon *et al*., 2008) and the males attraction increases after attaining sexual maturation (Weldon *et al*., 2008). Attraction towards the natural sources of RK in the males of *Z. cucurbitae* and *Bactrocera caudata* (Fabricius) leads to ingestion and accumulation of this compound in the rectal glands of the male (Nishida *et al*., 1993 and Nishida, 2005). In *Bactrocera tryoni* (Froggatt) males that were fed either pure RK (Nishida, 2005) or CL (Kumaran *et al*., 2014) revealed that RK got accumulated and then rapidly hydrolyzed and stored. Further, recent study revealed the chemical composition of droplets of excretions (presumed to be male pheromone volatiles) left by *B. tryoni* males fed on CL during the peak of calling activity (at dusk) with that left by the same type of males but at noon (Kumaran *et al*., 2014). Results disclosed that *B. tryoni* males released RK together with other pheromonal compounds exclusively during pheromone release phase. Interestingly, RK is incorporated into the pheromone without modifications, as opposed to ME which is converted to two metabolites after ingestion (Nishida, 2014). Because RK is stored in the rectal glands and released with other pheromonal compounds and because female attraction to CL fed males gradually increased with decreasing light intensity (Khoo and Tan, 2000), these phytochemicals are strongly suggested to be involved in attraction of females, their courtship, or both (Segura *et al*., 2018).

Consuming natural male lure compounds by males of tephritid fruit flies enables them to mate more successfully within their female choice mating systems. However, still it remains unclear what benefits females derive from mating with lure-fed males. When the CL and zingerone fed *B. tryoni* males mated with females, it revealed that there is an increased fecundity of mated females but this direct fitness benefit was not apparent with *B. dorsalis* fed with ME or *Z. cucurbitae* fed with CL (Inskeep *et al*., 2019). Nevertheless, role of ME in increasing copulatory success was noticed in male *B.dorsalis* (Reyes - Hernandez *et al.,* 2018).

The inclusion of protein or yeast hydrolysate into the diet reported to increase the males sexual competitiveness, increase the number of sperm stored by females and decrease the probability of female remating (Gavriel *et al*., 2009; Perez-Staples *et al*., 2008; Taylor *et al*., 2013 and Yuval *et al*., 2007). Studies revealed that, significant enhancement in the mating success of *B. dorsalis* males when fed on ME and a protein-rich diet (Orankanok *et al*., 2013; Shelly and Nishida, 2004 and Shelly *et al*., 2005). ME and protein incorporation into the diet of adult flies increase the mating competitiveness of the Oriental fruit fly, *B. dorsalis*. Exposing males to ME or protein is a promising post-teneral treatment for males being released in the sterile insect technique (SIT) (Reyes-Hernandez *et al*., 2018). However, the effect of this post-teneral treatment on male reproductive organs or the male ejaculate is unknown.

The goal of the present study was to evaluate behavioural response of both male and female *Z. cucurbitae* to CL fed and unfed male melon flies body volatile and also to the protein fed immature male and female flies body volatile and protein unfed mature male and female flies body volatile. Males of the melon fruit fly are strongly attracted to this plant-borne compound, which they ingest and use in the synthesis of the sex pheromone. Previous studies conducted on *B. dorsalis* in the laboratory and small field-cages have shown that males given ME produce a more attractive pheromone for females and have a higher mating success rate than males denied ME. In this study, we wanted to study if the body volatiles of the fruit fly, *Z. cucurbitae*, fed with CL and protein had any influence on their behaviour. Further, to identify the chemical composition of the body volatile collected from melon flies with different treatments.

MATERIALS AND METHODS

Insect Culture

The melon fly, *Z. cucurbitae* was reared on ridge gourd (*Luffa acutangula* L.) in the laboratory. Fruits were exposed for 24 h to mixed populations of fruit flies (100 pairs) for oviposition. Fruits exposed to melon flies were kept separately in plastic container (27 cm length × 24 cm width) containing fine sterilized sand as pupation medium. The sand was sieved after 10 days to facilitate pupal collection. The collected pupae were placed at ambient room condition (27 ± 2 °C, 75 % RH and 12:12 h dark: light cycle) in a wire mesh cage for adults to emerge. The emerged adults were provided with sugar and yeast extract (Himedia) *ad libitum*. Mature males and females are used as test insects in all olfactometer assays.

Body volatile collection through air entrainment

Body volatiles from different stages such as CL fed mature male (♂ MV-CF), CL unfed mature male (♂ MV-CUF), protein fed immature male (♂ IMV-PF), protein unfed mature male (♂ MV-PUF), protein fed immature female (♀ IMV-PF), protein unfed mature female (♀ MV-PUF) melon flies (Fig. 1) using a customized air entrainment system. Before volatile collection, all the glassware were washed with liquid detergent, rinsed with distilled water and acetone and then dried in an oven at 180 °C for 2 h. The Porapak Q columns used for volatiles collection were washed with redistilled diethyl ether and heated at 100 °C for 2 h under a stream of purified nitrogen to remove contaminants. Flies (n = 50) were placed inside a cylindrical glass vessel and closed with lid having an inlet and outlet port at the top. The flange on the open end of the glass vessel was clipped with binder clips. Activated charcoal filter purified air was pumped into the vessel through the inlet port (400 mL/ min). Volatiles were collected on Porapak Q (50 mg, 60/80 mesh; Supelco, Sigma Aldrich, St Louis, USA) placed in a glass tube (5 mm dia.) inserted into the collection ports on the top of the vessels. Further, pumps drew air (300 mL/ min) through these tubes. All connections were made with PTFE tubing with brass ferrules and fittings (Swagelok, India) and sealed with PTFE tape. Volatiles were collected from fruit for 24 h and the Porapak Q columns were eluted with 750 µl of redistilled diethyl ether. Volatile samples were stored in a freezer (-20 °C) until further use

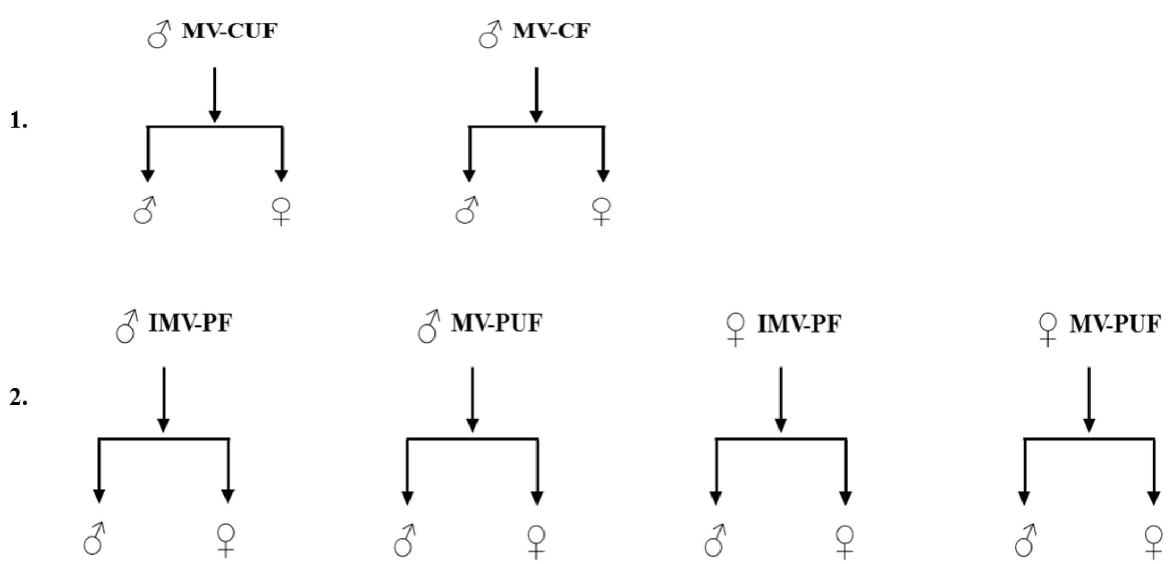


Fig 1. Melon flies body volatile collection with different treatments

MV-CUF = Mature and cue lure unfed melon flies body volatile

MV-CF = Mature and cue lure fed melon flies body volatile

IMV-PF = Immature and protein fed melon flies body volatile

MV-PUF = Mature and protein unfed melon flies body volatile

Test insects used for behavioural biassays are mature males and females

Insect behavioural bioassays

Four-arm olfactometer bioassay

Behavioural response of *Z. cucurbitae* mature males and females towards the CL fed and unfed male conspecific body volatiles, protein fed immature male and female conspecific body volatiles and also protein unfed mature male and female conspecific body volatiles were performed in a circular Perspex four-arm olfactometer (Pettersson, 1970) as suggested by Kamala Jayanthi *et al.* (2012). Prior to the experiment, all glassware used in the bioassay was washed with a non-ionic liquid detergent solution and then rinsed with acetone and distilled water and baked overnight at 100 °C in a hot air oven. Perspex components were washed with non-ionic liquid detergent and rinsed with distilled water and allowed to air dry in a clean room. Experiments were conducted using an olfactometer (9 cm diameter) with central arena which was divided into four side arms leading into four odour fields made up of glass. The bottom of the apparatus was lined with filter paper (Whatman No. 1, 9 cm diameter) to facilitate easy movements of test insect. The olfactometer was placed inside a cabinet (0.62 × 0.62 × 0.62 m) illuminated from above by diffused, uniform lighting using a fluorescent bulb (15 W) and surrounded by black, light proof walls to prevent the influence of any external visual stimuli. The experiments were conducted at the ambient room temperature (27 ± 1 °C). The *Z. cucurbitae* flies were starved for 2 hours and then introduced singly into the central chamber through an entry in the top of the olfactometer. Each fly was allowed 2 min to acclimatize in the olfactometer, after which the experiment was run for 10 min for each replicate. Each replicate involved one treated arm and three control arms (solvent blank). Next, the air was drawn through the central entry on the top of the olfactometer at the rate of 350 mL/min. The central arena of the olfactometer was divided into four discrete odour fields corresponding to each of four glass arms. The apparatus was rotated 90° every two min to avoid any directional bias on insect during the bioassay. The test samples (10 µl) were applied on to filter paper strip (Whatman No. 1, 3 cm length, 1 cm breadth) using the micro pipette (Gilson) and placed inside the treatment arm after the solvent evaporation. Another filter paper strip with solvent (10 µl diethyl ether) served as a control in the remaining arms. Twelve replicates (n = 12) were carried out for each treatment. Observations on the time spent into each arm by the fly were recorded manually on Olfa program (F. Nazzi, Udine, Italy).

Statistical analysis

The mean time spent in treated and control regions were compared using a paired *t*-test. Analysis was carried out using GraphPad Prism software (Ver. 7.02) for Mac OS X.

GC-MS analysis

Chemical composition of Porapak Q elutes were analysed using Gas Chromatography-Mass Spectroscopy. The mass spectral analysis was completed on Agilent 7890 B Gas Chromatography apparatus coupled MSD (Agilent 5977 B). The samples were separated on a capillary column (HP-5 MS) of 30 m length and 0.25 mm ID and 0.25 µm film thickness with Helium as a carrier gas at a flow rate of 1 mL/min. The Oven temperature of instrument was programmed to 60-280 °C with ramping at 10 °C/min for 40 min. The MS detector was maintained at 280 °C and was in full scan mode (70 eV) and AMU ranged from 40 to 450. 1 µl of each sample was injected in an injection port maintained at 270 °C with a split less mode ratio of 1:20 (40 mL/min). Compounds were identified by comparing mass spectra of detected compounds using spectral libraries (NIST 2014 version).

RESULTS

Behavioural response of melon flies to CL fed and unfed mature male melon flies body volatile

First, we studied the attraction of CL fed and unfed mature male melon flies body volatile to mature male and female flies of *Z. cucurbitae*. There was no significant attraction to the body volatiles of CL fed mature male flies ($t = 0.15$; $df = 11$; $P = 0.8840$) by mature male *Z. cucurbitae* (Fig. 2a). However, CL unfed mature male flies body volatile elicited positive behavioural response in mature male *Z. cucurbitae* as they spent significantly more time in the olfactometer arm containing this volatile ($t = 4.64$; $df = 11$; $P = 0.0007$) (Fig. 2b). When we tested the attractiveness of mature female flies of *Z. cucurbitae* to these volatiles, female *Z. cucurbitae* spent significantly more time in the CL fed mature male flies body volatile treated arm of the olfactometer ($t = 6.77$; $df = 11$; $P < 0.0001$) (Fig. 2c). However, no significant difference was found in the response of mature female flies to the body volatile of CL unfed mature male flies ($t = 0.72$; $df = 11$; $P = 0.4885$) (Fig. 2d).

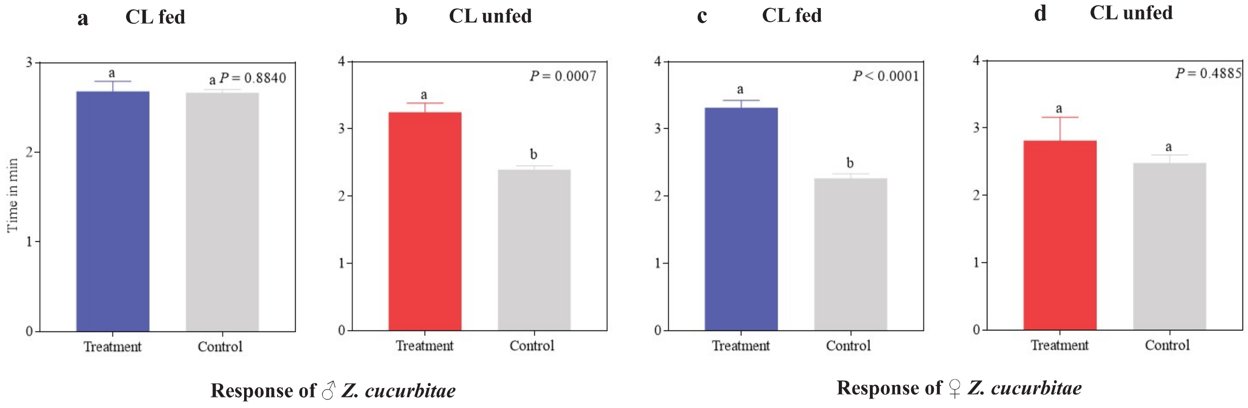
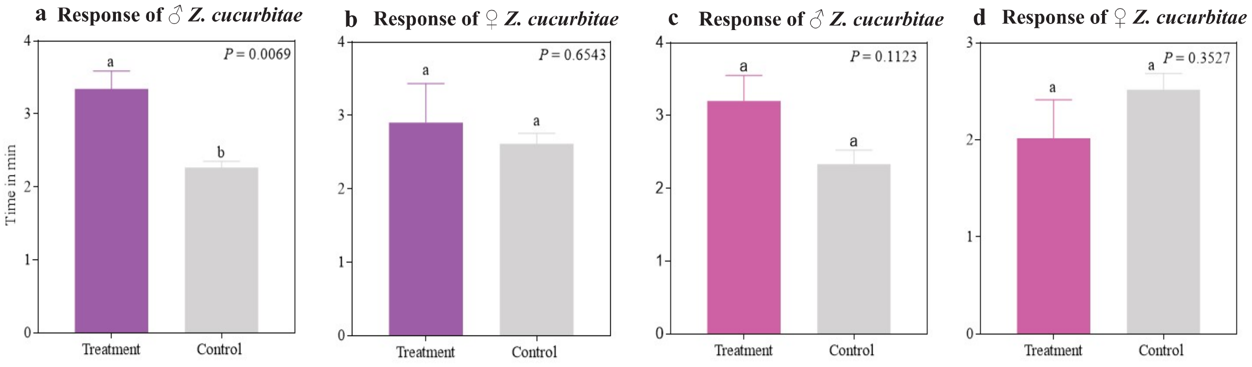


Fig 2. Behavioural response of mature male and female *Z. cucurbitae* to the CL fed and unfed mature male melon flies body volatile. *Z. cucurbitae* male flies showed a. less attraction to CL fed mature male melon flies body volatile (treatment = 2.68 ± 0.11 min; control = 2.66 ± 0.04 min; mean ± SEM) and b. highly attracted to CL unfed mature male melon flies body volatile (treatment = 3.25 ± 0.14 min; Control = 2.40 ± 0.06 min) whereas, c *Z. cucurbitae* female flies were highly attracted to CL fed mature male melon flies body volatile (treatment = 3.32 ± 0.11 min; Control = 2.27 ± 0.07 min) and d. showed less attraction to CL unfed mature male melon flies body volatile (treatment = 2.81 ± 0.35 min; control = 2.48 ± 0.12 min)

Behavioural response of melon flies to protein fed immature and protein unfed mature melon flies body volatile

Next, we studied the attractiveness of *Z. cucurbitae* mature male and female flies to the body volatile of protein fed immature and protein unfed mature melon flies. The mature male flies of *Z. cucurbitae* were highly attracted towards the body volatiles of protein fed immature males ($t = 3.13$; $df = 11$; $P = 0.0069$) and spent significantly more time in the olfactometer arm containing this volatile (Fig. 3a). Whereas, there was no significant difference in the preference of mature female flies of *Z. cucurbitae* towards the body volatiles of protein fed immature males as they spent lesser time in the olfactometer arm containing this volatile ($t = 0.46$; $df = 11$; $P = 0.6543$) (Fig. 3b). However, both males and females of *Z. cucurbitae* showed less attraction towards the body volatile of protein unfed mature males and spent significantly less amount of time, male ($t = 1.73$; $df = 11$; $P = 0.1123$) (Fig. 3c) and female ($t = 0.97$; $df = 11$; $P = 0.3527$) (Fig. 3d) in the olfactometer arm containing this volatile.

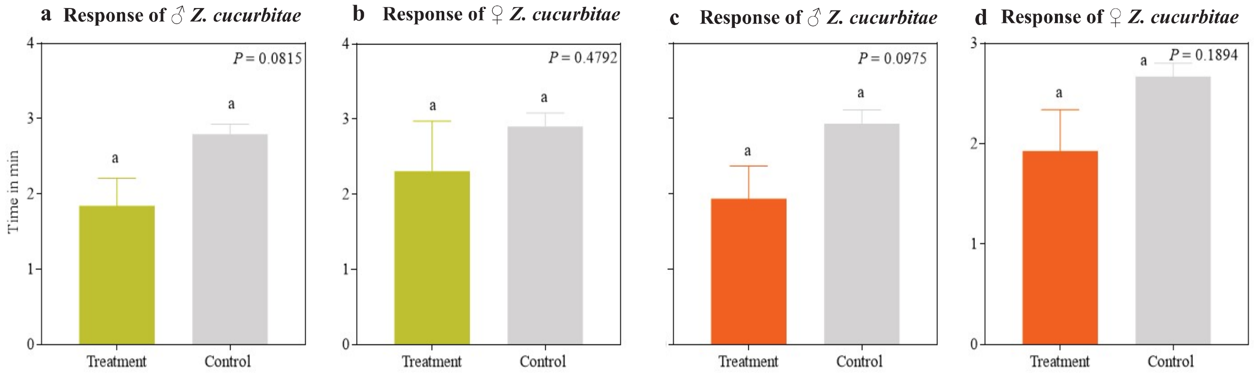
Further, the body volatile of protein fed immature female and protein unfed mature male did not elicit any positive behavioural response in the *Z. cucurbitae* mature male and female flies. The mature male flies of *Z. cucurbitae* showed less attraction towards the protein fed immature female melon flies body volatile ($t = 1.92$; $df = 11$; $P = 0.0815$) (Fig. 4a) and also to the body volatile of protein unfed mature female melon flies ($t = 1.81$; $df = 11$; $P = 0.0975$) and spent significantly less time in the olfactometer arm containing this volatile (Fig. 4c). There was no significant difference in the preference of mature female flies of *Z. cucurbitae* towards the treated arm of the olfactometer containing the body volatile of protein fed immature female melon flies ($t = 0.73$; $df = 11$; $P = 0.4792$) (Fig. 4b) and also to the protein unfed mature female melon flies ($t = 1.40$; $df = 11$; $P = 0.1894$) compared to control and they spent significantly less time in the olfactometer arm containing this volatile (Fig. 4d).



Protein fed immature male body volatile

Protein unfed mature male body volatile

Fig 3. Behavioural response of mature male and female *Z. cucurbitae* to the protein fed immature male and protein unfed mature male melon flies body volatile. a *Z. cucurbitae* male flies were highly attracted to protein fed immature male melon flies body volatile (treatment = 3.34 ± 0.25 min; control = 2.27 ± 0.08 min; mean ± SEM). b Whereas, *Z. cucurbitae* female flies showed less attraction to protein fed immature male melon flies body volatile (treatment = 2.90 ± 0.53 min; control = 2.62 ± 0.14 min) c However, *Z. cucurbitae* male flies showed less attraction to protein unfed mature male melon flies body volatile (treatment = 3.20 ± 0.35 min; control = 2.33 ± 0.19 min) and d. *Z. cucurbitae* female flies showed less attraction to protein unfed mature male melon flies body volatile (treatment = 2.02 ± 0.40 min; control = 2.52 ± 0.17 min).



Protein fed immature female body volatile

Protein unfed mature female body volatile

Fig 4. Behavioural response of mature male and female *Z. cucurbitae* to the protein fed immature female and protein unfed mature female melon flies body volatile. a *Z. cucurbitae* male flies showed less attraction to protein fed immature female melon flies body volatile (treatment = 1.84 ± 0.37 min; control = 2.79 ± 0.13 min; mean ± SEM) and b. *Z. cucurbitae* female flies also showed less attraction to protein fed immature female melon flies body volatile (treatment = 2.31 ± 0.67 min; control = 2.90 ± 0.18 min) c. Whereas, *Z. cucurbitae* male flies showed less attraction to protein unfed mature female melon flies body volatile (treatment = 1.94 ± 0.43 min; control = 2.93 ± 0.18 min) and d. *Z. cucurbitae* female flies showed less attraction to protein unfed mature female melon flies body volatile (treatment = 1.93 ± 0.41 min; control = 2.67 ± 0.13 min).

Next, we conducted the GC-MS analysis of body volatiles collected from CL fed and unfed mature male melon flies, protein fed immature male and female melon flies and also protein unfed mature male and female melon flies. We found that there is a qualitative and quantitative difference in the volatile profiles between CL fed and unfed mature male melon flies body volatile (Table 1). In case of CL fed male body volatiles, the CL found to be abundant in comparison to other compounds whereas N-(3-Methylbutyl) acetamide was predominately found in the body volatiles of cue lure unfed mature male *Z. cucurbitae*. There was variation in the chemical composition of body volatiles of protein fed immature male and female melon flies and also protein unfed mature male and female melon flies. Altogether, a total of 25 compounds were identified in the *Z. cucurbitae* body volatiles among these treatments.

DISCUSSION

Numerous studies revealed the preferential attraction of females towards ME-fed males over ME-deprived males. In wind tunnel assay, Hee and Tan (1998) and Wee *et al.* (2007) observed that females approached ME-fed males more often than ME-deprived males in *Bactrocera papayae* (Drew and Hancock) and *Bactrocera carambolae* Drew & Hancock, respectively. In our study, we found that *Z. cucurbitae* females getting attracted towards CL fed males than CL deprived males. Till date, limited studies have been conducted with CL responding species that involve the melon fly, *Z. cucurbitae* (= *B. cucurbitae*). Khoo and Tan (2000) in a wind tunnel assay, observed that both CL- and zingerone-fed males attracted more females than non-lure-fed males. Shelly and Villalobos (1995) also reported increased female visitation to CL-fed males, apparently resulting from increased signaling activity. Here, it is important to note that the attractiveness of the male sex pheromone may be enhanced by compounds derived from lures, but it is not completely dependent on them. CL have been shown to influence male copulatory success. In *Z. cucurbitae*, the effect of CL is short-lived, In the laboratory test performed it is found that, CL fed males had mating benefit over unfed males on the same day or one day after CL feeding but not three days after feeding (Shelly and Villalobos, 1995). Similar results were also reported under field cage studies, as CL fed males achieved higher mating advantage than control males 3 d (but not 5 d) after feeding (Shelly and Nishimoto, 2016). Recent study revealed that, CL mediated mating enhancement of *B. tryoni* males under laboratory conditions (Kumaran *et al.,* 2013).

Table 1. GC-MS profile of *Z. cucurbitae* body volatiles with different treatments (CL fed and unfed mature male; Protein fed immature male and female; Protein fed mature male and female)

Sl. No.	Compound name	CAS No.	RT	Abundance (%)	
				Mature ♂cue lure fed	Mature ♂cue lure unfed
1	Benzeneethanamine, 4-methoxy-α-methyl-	23239-32-9	9.01	0.00	1.89
2	2-Methylbicyclo[4.3.0]non-1(6)-ene	-	9.76	0.00	2.07
3	Pyrazine, tetramethyl-	1124-11-4	11.27	0.99	23.09
4	N-(3-Methylbutyl) acetamide	13434-12-3	12.57	3.60	70.73
5	Butanamide, N,N-dimethyl-3-oxo-	5810-11-7	16.22	0.56	0.00
6	Propanoic acid, 3-chloro-, 4-formylphenyl ester	-	18.42	0.15	0.00
7	Benzoic acid, p-ethoxy	619-86-3	22.62	0.17	2.21
8	1-Aza-3,12-dioxatricyclo[8.4.0.0(1,10)]tetradeca-4,6,8-triene-2,11-dione	-	23.07	0.47	0.00
9	Cue-lure	3572-06-3	25.58	93.88	0.00
10	N-[2-(3-Methoxyphenyl)-1-methylethyl] acetamide	-	28.56	0.18	0.00
				Immature ♂ protein fed	**Mature ♂ protein unfed**
11	Pyrazine, tetramethyl-	1124-11-4	11.27	14.11	23.80
12	N-(3-Methylbutyl) acetamide	13434-12-3	12.57	50.93	55.06
13	Butanamide, 2,2,3,3,4,4,4-heptafluoro-N-(2-phenylethyl)-	-	15.96	4.57	0.00
14	3,3-Dimethyl-4-methylamino-butan-2-one	-	16.20	0.00	9.17
15	Isobutyramide, N-propyl-	-	16.23	16.35	0.00
16	N-dl-Alanylglycine	1188-01-8	16.75	3.77	2.38
17	Pentanedial	-	19.26	3.25	0.00
18	Ethyl 13-methyl-tetradecanoate	64317-63-1	19.49	0.00	4.51
19	Benzoic acid, p-ethoxy-	619-86-3	22.62	2.70	5.08
20	Benzoic acid, 4-ethoxy-, ethyl ester	23676-09-7	22.76	4.32	0.00
				Immature ♀ protein fed	**Mature ♀ protein unfed**
21	Tetramethyl pyrazine	1124-11-4	11.29	0.00	1.32
22	Acetamide, N-(3-methylbutyl)-	13434-12-3	12.57	52.01	56.07
23	Cyclopentanecarboxylic acid, 2-ethylhexyl ester	-	12.82	35.36	37.04
24	N-n-Butylpropionamide	1187-58-2	14.61	10.46	5.57
25	Cyclopentanecarboxylic acid, octyl ester	100912-19-4	14.85	2.17	0.00

CL mediated mating enhancement seems to be related to an increase in sexual signaling, which in turn associated to enhanced female attraction. On the other hand, GC-MS analysis revealed that cue lure was found more abundant in the CL fed mature male melon flies body volatile. Composition of pheromone released by CL-fed and unfed males in *B. tryoni* revealed that RK was found only in the pheromone of CL-fed males and the abundance of some endogenous pheromone components (*N*-(3-methylbutylacetamide), *N*-hexylpropanamide and *N*-propylbutyramide) was higher in CL-fed males (Kumaran *et al*., 2014). In addition, it is reported that females are more attracted to isolated glands of CL-fed males than control (non- CL-fed) males, strongly suggesting that the greater attractiveness of CL-fed males is related to the quality of the olfactory signal (Kumaran *et al*., 2014). In a study of *B. tryoni*, it is found that females laid more eggs when mating with CL and zingerone-fed males (Kumaran *et al*., 2013). This suggests a simple explanation for mate choice in this species; females select lure-fed males because this results in higher fecundity and presumably more surviving adult offspring. Furthermore, zingerone feeding by males induced weight loss and resulted in the up regulation of genes believed to influence aggression, pheromone synthesis, mating and accessory gland protein synthesis in various *Drosophila* (Drosophilidae) species (Kumaran *et al*., 2014a).

While receiving less attention, male-male attraction has also been documented in the context of tephritid behaviour. In this study, we observed that, *Z. cucurbitae* males were highly attracted towards the CL unfed mature male body volatiles in olfactometer assay which is in contradiction to the earlier reported results. Males are more likely to approach lure-fed males than non-fed males in wind tunnel experiments for both ME (Hee and Tan, 1998 and Wee *et al*., 2007) and CL- (Khoo and Tan, 2000) responding species. At field level, male-male attraction has not been reported for any *Bactrocera* species. It is challenging to detect whether the attraction of other males would have a positive or negative effect on the mating success of males. With limited available field data, males of *Bactrocera* may form mating aggregations (leks) on host or non-host plant species (Shelly and Kaneshiro, 1991 and Drew *et al*., 2008). Among the members of the lek, females may choose fit males based the signalling activity and displays during calling period.

Consuming male lures or in some cases simple exposure to the volatiles improves the mating success of males in a variety of species and improves the attractiveness of the male pheromone blend to conspecific females. Males presumably are attracted to the lures because it enhances their chance of mating success, but less is known about the benefits to the female derived from mating with lure-fed males in this studied species. Taken together, our results provide evidence on attractiveness of *Z. cucurbitae* females to CL fed males in olfactometer assay and also revealed the composition of body volatiles of lure fed males.

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