

Original Research Article

Effects of Male Diet on Cuticular Hydrocarbons in *Drosophila melanogaster*

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ABSTRACT:

Many elements of animal physiology, including as lifespan, general health, and reproductive success, have been studied, and cuticular hydrocarbon variation in *Drosophila*, are considered to be influenced by nutrition, which is one of the most significant environmental factors. A most important role of the insect CHC profile is to water-resistant the cuticle and avoid desiccation. Here we investigate the effect of male diet on cuticular hydrocarbon of *Drosophila melanogaster*, we found that carbohydrate and protein-rich diets have a significant impact on cuticular hydrocarbon variation in male *D. melanogaster* flies. Eighteen compounds were identified in male flies of *D. melanogaster* reared in all three different diets (normal media/ carbohydrate rich media/protein rich media), though, the concentration of CHC varies in the different diet significantly, which was suggest that male diet has a significant influence on cuticular hydrocarbon in *D. melanogaster* flies which inturn effect the attractiveness of the flies and fitness of *Drosophila melanogaster*.

Keywords: Cuticular hydrocarbon, Chromatogram, Nutrition, Carbohydrate, Tricontane

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INTRODUCTION

Identification of potential mating partners is important for successful mating of an organism in a group, individuals in a population select their partner by identifying the characters that specify high fitness and reproductive potential using insect social messenger systems, according to Andersson 1994, Maynard & Smith et al., 2003. Cuticular Hydrocarbons (CHC) are long-chain lipids synthesized from fatty acids that are accumulated on the cuticle of insects and play an important role in identifying the species, sexual group, dominance, and reproductive

status of the insects (Howard & Blomquist, 2005). Contact sex pheromones are used by many dipterans for species identification and gender recognition⁴, and these substances change with physiological state and age (Trabalon et al., 1988; Pomonis, 1989).

Cuticular hydrocarbons may be used to classify sexual attractiveness in *Drosophila* species, which indicates the reproductive value of potential mates (CHC). In a variety of insects, including *Drosophila*, CHC composition varies depending on genetic content, diet, and environmental conditions (Gosden & Chenoweth, 2011; Savarit &

Ferveur, 2002; Kent et al., 2007), suggesting a connection between main metabolic pathways and CHC biosynthesis. Experiments relating the impact of dietary restriction without malnutrition on lifespan and reproductive outputs have been used to link dietary effects on fitness traits from nematode worms to mammals (Fontana et al., 2010).

Food provides nutrition to organisms for various biological purposes, and the quality and quantity of nutrients play an important role in sexual attractiveness and fitness (Pough, 1989; Sibly, 1991). Also in nature, animals are often subject to changes in food quality, quantity, and availability, causing changes in their stress tolerance, life history characteristics, sexual attractiveness, reproduction, accessory gland protein, sperm traits and male fitness (Djawdan, et al., 1998; Bross et al., 2005; Broughton et al., 2005; Sisodia & Singh, 2010; Reddiex et al., 2013; Rodrigues et al., 2015; Kristensen et al., 2016; Krishnan & Anitha, 2020). These studies have revealed that the apparent effects is due to the percentage of protein to carbohydrate in the diet, comparatively than the caloric content per se (Piper et al., 2011; Fanson et al., 2011; Simpson & Raubenheimer, 2004).

Furthermore, the experimental link demonstrates that various physiological and sex-specific tasks have revealed the need for specific nutrients, as well as the allocation of decisions that guide survival versus reproduction, which are further optimized by diet variation (Maklakov et al., 2008; Vargas et al., 2010; Gosden & Chenoweth, 2011). Thus these studies point out that diet can change dramatically over time, however natural choice is possible to support biological mechanisms that quickly modify allocation determine in reaction to nutrient accessibility as well as mechanisms in those of the opposite gender to estimate such decisions in their possible mates. In a number of nutritional researches, *D. melanogaster* is one of the most commonly used model organisms. The difference in cuticular hydrocarbons in *D. melanogaster* (Meigen) has an effect on mating behavior.

At most stage of copulation time male usually acquire 7,11- heptacosadiene from the mated female, where the female (Jallon & David, 1984) get 7- tricosene from the male

which is mated (Scott, 1986). Flies of *D. melanogaster* they feed on diet containing carbohydrate and protein, however the availability and quantity of carbohydrate and protein in the diet has significant effect on cuticular hydrocarbon of *Drosophila*. Therefore, the present study has been undertaken in *D. melanogaster* to study the male diet related changes on cuticular hydrocarbon.

MATERIALS AND METHODS

Oregon- K (*Drosophila melanogaster*) flies taken from stock center of *Drosophila*, Zoology department, University of Mysore, were used in the present experiment. Eggs collected from these flies were reared using wheat cream agar media and maintained them at $\pm 22^{\circ}\text{C}$ and 12 h L/D cycles. Experimental flies were obtained by transferring eggs collected from above flies in to separate culture bottles containing control diet (wheat cream agar media), carbohydrate and protein enriched diets and each with hundred eggs. Carbohydrate and protein enriched diet was made by mixing either sucrose or casein with wheat cream agar medium [For carbohydrate enrich diet -sucrose and wheat cream agar medium was mixed in 1:4 ratio. For protein enrich diet -casein and wheat cream agar medium was mixed in a 3:2 ratio]. These experimental flies were maintained them at $\pm 22^{\circ}\text{C}$ and 12 h L/D cycles. Virgin flies and unmated male were collected and this were used for present experiments.

Dietary effects on CHCs

Unmated males which is reared on different diet (Normal media/Carbohydrate rich media/Protein rich media) are collected above were used to study CHCs using GC-MS analysis.

Cuticular hydrocarbons isolation and purification (CHCs)

To Analysis cuticular hydrocarbon of *D. melanogaster*, variation in cuticular hydrocarbon compounds has been studied using Gas chromatography- Mass spectrometry (GC-MS). Male flies reared on different diet (normal media/ carbohydrate rich media/ protein rich media) were separately taken and introduce into supelco vials consisting 30 μl of Hexane and 100ng of tricontane which used as an internal standard. Ten flies from each experimental sample were

introduce into the vials containing the extraction solution for 5 min (3 replicates of flies reared in each media was made separately and each samples were taken in a separate vials). 5 minutes later, the extraction solution are separated and set aside for room temperature for 24 hours. 3 μ l of the solution is used for the analysis finally (Figure 1A).

GC-MS analysis of cuticular hydrocarbons

To analysis the CHC compound of *Drosophila melanogaster* flies, first we had started the temperature profile of the column from 70°C for 60 sec, gradually the experiment

temperature was increased to 20°C per minute and this was done until the temperature goes upto 240°C. Subsequently per minute 4°C was increased until the temperature increases upto 320°C. Totally 36 min are required to do gas chromatography.

Statistical Analysis

One- way ANOVA followed by Tukey's post Hoc test was carried out to analysis the data of cuticular hydrocarbon on *D. melanogaster* male flies. All the above analysis was performed using SPSS 20 version software.

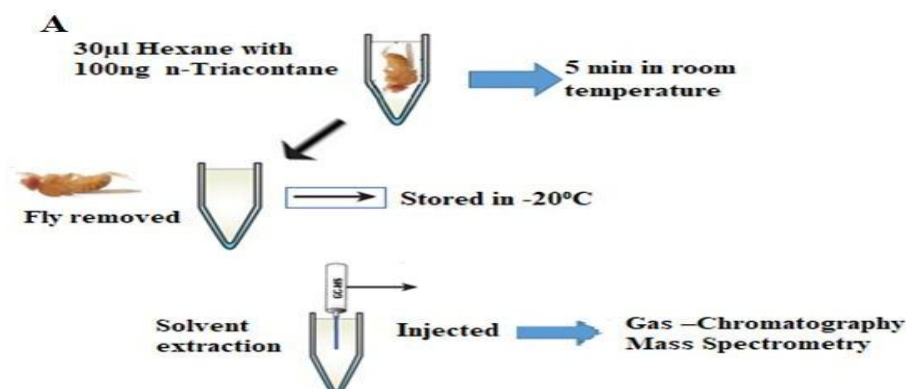


Figure 1: Extraction of CHC from *Drosophila melanogaster*

RESULT AND DISCUSSION

The study was performed using Gas Chromatography-Mass Spectrometry (GC-MS). In *D. melanogaster* experimental flies, the composition of cuticular hydrocarbons with purity greater than 75% was reported. A total of 18 compounds have been identified, the majority of which are alkane and fatty alcohol components (Table. 1). The Retention time (RT) value was used to identify these compounds (Fig. 2, 3). Different peaks in chromatogram represent a cuticular hydrocarbon profile. Compounds having retention time were more volatile as a result they appear first in the chromatograph. (Fig. 2, 3, 4). The following compounds were found

namely: tridecane, undecane, indane, dodecane, naphthalene, hexadecane, pentadecane, penta 2,6,10,14, eicosane, heptadecane, heneicosane, decane 4- methyl, decane 2- methyl, decane, tetracosane, 1-Hexanedecane, tetradecane, 7 tricosane (Table. 1). It was also noticed that all the eighteen compounds were found in male flies reared on normal media, carbohydrate rich media and protein rich media. The analysis was carried out independently on each of the eighteen compounds of male flies grown on various diets. We noticed that, the concentration of the CHC compound varies amongst flies given different medium (normal media/arbohydrate rich media/protein rich media).

Table 1: shows the profile of cuticular hydrocarbon compounds in male *Drosophila melanogaster* flies reared on various diets based on retention time (RT) (normal media, carbohydrate rich media and protein rich media).

Sl. No.	CHC Name	Mol. Formula	Mol Wt.	RT	Normal media	Carbohydrate-Rich media	Protein-Rich media
1	Tridecane	C13H28	184.37	8.87	+	+	+
2	Undecane	C11H24	156.31	10.28	+	+	+
3	Indane	C9H10	118.176	6.48	+	+	+
4	Dodecane	C12H26	170.33	7.39	+	+	+
5	Napthalene	C10H8	128.1705	8.82	+	+	+
6	Hexadecane	C16H34	226.41	10.28	+	+	+
7	Pentadecane	C15H32	212.42	11.61	+	+	+
8	Penta,2,6,10,14	C19H38	266.5	11.7	+	+	+
9	Eicosane	C20H42	282.547	14.07	+	+	+
10	Heptadecane	C17H36	240.48	15.21	+	+	+
11	Henicosane	C21H44	296.57	20.99	+	+	+
12	Decane 4-methyl	C11H24	156.31	8.87	+	+	+
13	Decane 2-methyl	C11H24	156.31	10.28	+	+	+
14	Decane	C10H50	142.29	8.87	+	+	+
15	Tetracosane	C24H50	338.65	20.99	+	+	+
16	1-Hexadecane	C16H34	226.41	12.78	+	+	+
17	Tetradecane	C14H30	198.39	14.07	+	+	+
18	7-Tricosane	C23H48	324.6	20.9	+	+	+
19	Triacontane	C30H62	422.82	26.2	+	+	+

(RT) Retention time (minutes), (IS) internal standard, (+) present compounds in experimental samples. (-) Absent compounds in experimental samples.

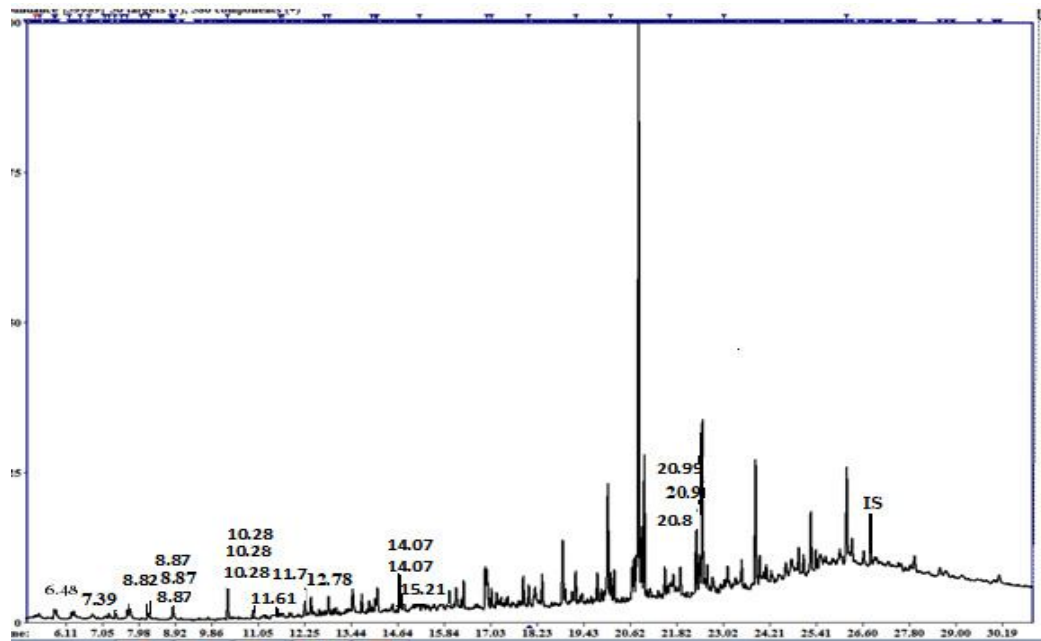


Figure 2: Typical chromatogram showing cuticular hydrocarbon profile of male *D. melanogaster* reared on Normal diet [Triacontane used as an internal standard (IS) (n=3)]

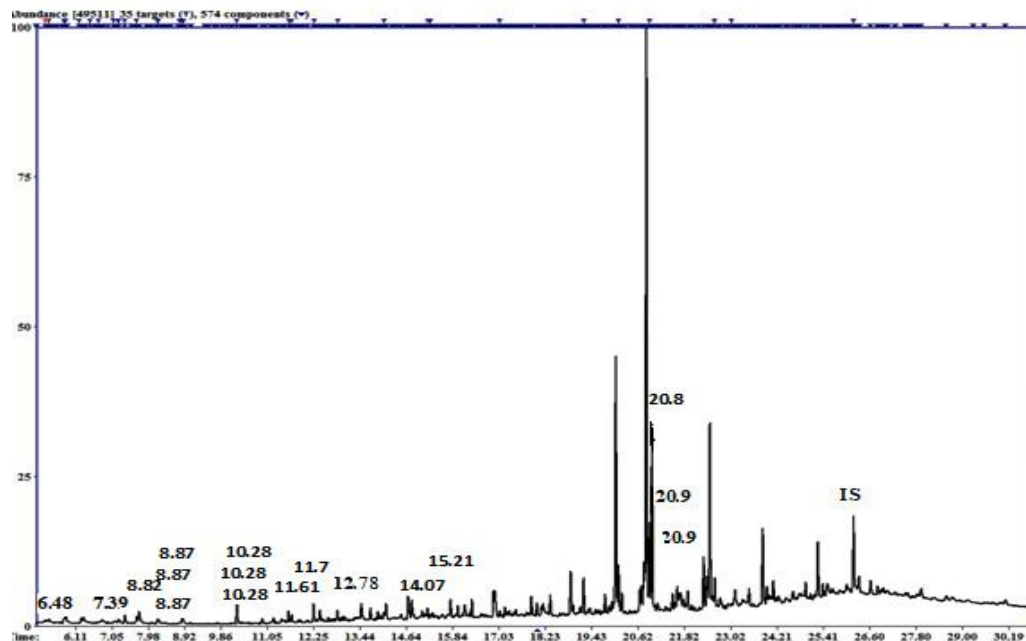


Figure 3: Typical chromatogram showing cuticular hydrocarbon profile of male *D. melanogaster* reared on Carbohydrate- rich diet [Triacontane used as an internal standard (IS) (n=3)]

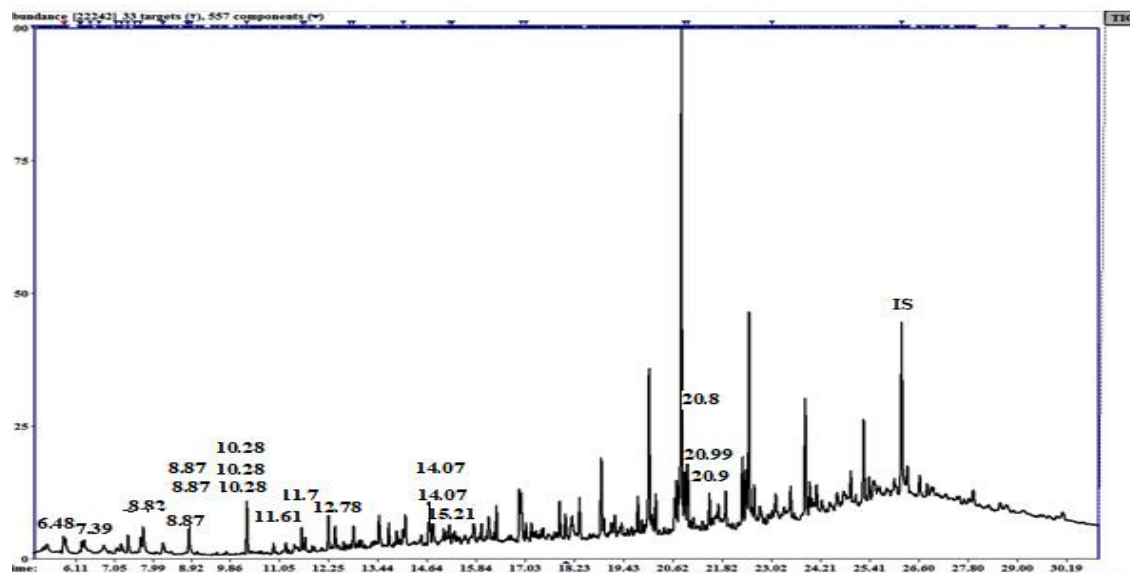


Figure 4: Typical chromatogram showing cuticular hydrocarbon profile of male *D. melanogaster* reared on protein- rich diet [Triacontane used as an internal standard (IS) (n=3)]

In Table 1 shows that all the eighteen compounds were found in all flies reared in three diet (normal media, carbohydrate rich media, protein rich media), In Table 2 shows that mean values of the compounds tridecane, undecane, indane, dodecane, hexadecane, pentadecane, penta 2,6,10,14, eicosane, heptadecane, henicosaene, decane 4- methyl, decane 2- methyl, 7- tricosane shows

significantly higher in male flies reared on protein rich diet when compare to carbohydrate rich diet/normal diet, naphthalene and decane compounds shows more or less same concentration in male flies reared on protein rich diet/carbohydrate rich diet. Compound tetracosane and 1-Hexadecane shows higher concentration in male flies fed in Carbohydrate rich diet when

compare to Normal/Protein rich media. The mean value of tetradecane compound shows same in three diet (Normal

media/Carbohydrate rich media/Protein rich media).

Table 2: Cuticular hydrocarbons (CHCs) concentrations in male *D. melanogaster* reared in Normal (NM), Carbohydrate Rich (CR), and Protein Rich (PR) media (n=3).

Compounds	Df	Normal Media	Carbohydrate-rich Media	Protein-rich Media	F- Value
1. Tridecane	9	0.222±0.00057 ^a	0.282±0.00057 ^b	0.392±0.00088 ^c	15503.154
Undecane	9	0.281±0.00033 ^a	0.282±0.00057 ^a	0.393±0.00012 ^b	6601.647
Indane	9	0.248±0.00057 ^a	0.317±0.00033 ^b	0.452±0.00120 ^c	17197.118
Dodecane	9	0.282±0.00088 ^a	0.317±0.00033 ^b	0.452±0.00057 ^c	7076.727
Napthalene	9	0.368±0.00057 ^a	0.397±0.00057 ^b	0.392±0.00033 ^b	935.286
Hexadecane	9	0.394±0.00057 ^a	0.393±0.00057 ^a	0.812±0.00057 ^b	175143.000
Pentadecane	9	0.386±0.00033 ^a	0.383±0.00152 ^a	0.584±0.00088 ^b	12375.034
Penta 2,6,10,14	9	0.467±0.00088 ^a	0.465±0.00152 ^a	0.526±0.00088 ^b	1544.143
Eicosane	9	0.572±0.00088 ^a	0.573±0.00088 ^a	0.812±0.00088 ^b	12556.415
Heptadecane	9	0.586±0.00145 ^b	0.162±0.00033 ^a	0.627±0.00066 ^c	74517.542
Henicosane	9	0.803±0.00057 ^b	0.735±0.00202 ^a	0.835±0.00200 ^c	919.908
Decane 4- methyl	9	0.234±0.00152 ^a	0.333±0.00120 ^b	0.336±0.00057 ^b	2474.135
Decane 2- Methyl	9	0.282±0.00057 ^a	0.392±0.00088 ^b	0.445±0.00173 ^c	5053.973
Decane	9	0.282±0.00120 ^a	0.336±0.00033 ^c	0.317±0.00152 ^b	697.457
Tetracosane	9	0.666±0.00120 ^a	0.802±0.00088 ^c	0.785±0.00321 ^b	2907.000
1-Hexadecane	9	0.487±0.00057 ^a	0.794±0.00321 ^c	0.493±0.00057 ^b	25211.727
Tetradecane	9	0.496±0.00288 ^a	0.494±0.00100 ^a	0.493±0.00057 ^a	1.966
7- Tricosane	9	0.462±0.00120 ^a	0.462±0.00057 ^a	0.716±0.00208 ^b	10529.527

*Significant at p<0.001, nanogram/ microliter (ng/μl)

CHCs were first used in insects to shield them from water loss. Natural selection and sexual selection are two additional factors that influence CHC profiles. Natural and sexual selection also play a role in the evolution of cuticular hydrocarbons in *D. melanogaster*, according to experimental evidence (Simpson et al., 2004; Raubenheimer et al., 1993). In the present study, eighteen cuticular hydrocarbons compounds have been noticed in male flies of *Drosophila melanogaster* reared on normal media, carbohydrate rich media, protein rich media (Table 1), In table 2 shows that the concentration of same compounds of CHC gets vary when flies reared on different diet (normal media, carbohydrate media and protein rich media) so these variation among different diet suggest that male diet have significantly influence on cuticular hydrocarbon on *Drosophila melanogaster*.

Where nutritional limitation without malnutrition affects the lifetime and reproductive outputs across a large variety of

species, from nematode worms to mammals, diet is considered the most important primary environmental factor that regulates fitness (Fontana, 2010). According to new evidence, the experimental results are caused by diet composition, such as the protein-to-carbohydrate ratio, rather than caloric intake per se (Piper et al., 2011; Fanson & Taylor, 2011; Simpson et al., 2004). A number of studies have been done in *Drosophila melanogaster* using gas chromatography-mass spectrometry (GC/MS) that show sexual dimorphism in CHC and preserve their role as pheromonal compounds (Coyne & Oyama, 1995; Fervour et al., 1997).

Drosophila's main food is protein and carbohydrates, which they get from fruits. The nutritional benefits that they get vary depending on which fruits they eat. These findings indicate that cuticular hydrocarbons are more susceptible to environmental factors like food composition. Just a few CHC compounds have higher concentrations in males reared on a normal or carbohydrate

diet, but the majority of CHC compounds have higher concentrations in males reared on a protein-rich diet. This is because raising protein in food stimulates the TOR pathway, also known as the nutrients-sensing pathway. The TOR pathway has been implicated in the coordination of nutritional status and growth (Colombani et al., 2003). As a result, it's possible that the male diet has a significant influence on the variation of CHC concentration in *Drosophila* male flies. This means that, although the CHC profile is genetically determined, concentrations of CHC vary in response to environmental factors including diet treatment. This confirms that, previous research that showed a dietary impact on cuticular hydrocarbons, were found to have a dietary effect. While it is generally assumed, increased dietary sugar is contemplated to promote IIS, Furthermore, increasing dietary protein is thought to activate the TOR pathway (Avruch et al., 2009), there is evidence that these two primary nutrient-sensing mechanisms interact (Radimerski et al., 2009; Lizcano et al., 2003). When the activity of both pathways is altered, it's impossible to predict the phenotype that will result.

In *Drosophila*, studies have revealed that the profile of CHCs varies within and among populations of the same species, in *Drosophila*, dietary yeast and sugar have opposite effects on a variety of physiological traits like fecundity, triglyceride levels, sleep, and activity habits, while having similar effects on others like longevity (Catterson et al., 2010; Skorupa et al., 2008). Although 7-tricosene (7-T), the male principal CH, is the most effective pheromone for preventing or reducing male homosexual courtship (Ferveur & Sureau, 1996; Ferveur, 2005), its function in female mating behavior is unknown. Studies have been conducted based on a comparison of *D. melanogaster*. It was difficult to determine the identity of the substance(s) involved because *D. melanogaster* males differed not only in their hydrocarbon profile but also in their genetic history (Averhoff et al., 1974; Jallon & David, 1984; Scott, 1994; Cobb & Ferveur, 1996; Ferveur & Sureau, 1996; Coyne & Oyama, 1995). The main *Drosophila* male cuticular pheromone (7-tricosene) can change female receptivity and mating behaviour, according to Micheline (Micchelli & Perrimon, 2006). Females who mate with a

caring male produce more 7-Tricosene, which acts as a stimulant for females. This finding should help us to better understand the implication of pheromone communication in sexual selection and isolation. In our result the protein fed male flies of *D. melanogaster* shows increased level of 7-tricosene compound when compared to carbohydrate rich media/normal media. So this tells us that diet shows variation in CHC (7-Tricosene) which is interlinked to mating behaviour. Thus present study clearly suggests that availability and quantity of nutrients in the food has significant influence on cuticular hydrocarbon variation which in turn affects sexual attractiveness of the flies and fitness in *D. melanogaster*.

CONCLUSION

Thus, a study in male *D. melanogaster* implies that the availability and quantity of nutrients in the food which was fed by the flies, has a significant influence on cuticular hydrocarbon variation. Male diet is an important factor in bringing about variation in CHC profile in *D. melanogaster*.

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