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LIPID PROFILE OF HOUSEFLY LARVAE DURING SUMMER SEASON

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Abstract: Houseflies are the most efficient insects which can adjust themselves according to the surrounding atmosphere. Seasonality plays a major role in the distribution of houseflies. Occurrence and abundance is also monitored by the weather conditions, along with the breeding season. Lipids play an important role in protecting these insects in adapting themselves with change in environment. Summer season is considered as adverse season for thermoregulation of body. Lipid profile is a major indicative criterion during the change of season and adaptability of these insects. In the present investigation the experimental design is made in such a manner that lipid profile can be understood in the housefly maggots with the help of advanced molecular technique MALDI-TOF-MS. The results are discussed in the light available literature and impact of change of the season on housefly maggots.

Keywords: *Houseflies, maggots, lipidomics, MALDI-TOF-MS*

I INTRODUCTION

Houseflies are abundant and active during day time and they can fly very effectively and escape from predators easily. The most common housefly is the Musca domestica which is being widely studied by various workers especially entomologist. The houseflies are generally distributed and found everywhere in and around human locality. These insects can breed and survive in various places like vegetable and fruit market, poultry farms, slaughter houses. They survive and feed a lot where animal excreta accumulates. These house flies are seen in garbage and heaps of garden waste also. During night hours they can rest in dark places .Housefly is an important medicinal insect which has high efficient susceptible defense mechanism and is not often infected.

Houseflies are abundant also active during summer season. They lay eggs in decaying organic matter, wet stable manure and even on garbage. The breeding period may last from one two months. The development includes larval stages and they transmit the pathogens, freely feeds on human food and they are adapted for communicating dreadful diseases. They are causative agents for pathogenic diseases like dysentery, thyphoid, cholera etc. Maggot debridment therapy is widely used for controlling chronic wounds with the help of maggot larva [1].

In the present investigation an attempt has been made to know the importance of lipids in coping up the adverse temperature stress in summer season.

II MATERIALS AND METHODS

For studying lipid profile of housefly, Musca domestica following material and methods were used. Insect culture : The third instar of housefly, Musca domestica were reared for obtaining the larval stages with the help of media. For culturing the larva under clean conditions in laboratory 75 gms corn flour 80 gms sugar 24 gms yeast 60 gms malt 10 gms of agar was used.

All of this was mixed and the volume was made up to 1 liter composition of larval food. The following chemicals were added. Propionic acid – 5ml Methyl benzoic acid- 5ml Ortho phosphoric acid- 5ml.All this mixture was dispended at 70 ˚C.

Lipid Extraction (Isolation of total lipids

The oxygen tube was used to semidry the extract. To it 150µL of methanol in semidried pellet was added, Vortexed powerfully and prepared a homogenous cloudy mixture. The

entire pellet was been re-suspended. To it 150µL of MTBE (Methyl tertiary butyl ether) was added and kept on vigorous vortexing for 1hr.Then added 150µL of water and centrifuged at 5000rpm for 5 min. Protein pellet has settled down at underside. MTBE layer was at the top. Without disturbing water layer 400µL of MTBE layer was taken carefully and transferred to another oxygen tube and semi dried in speed vacuum.

Phospholipid Estimation:

Phospholipids were quantifed by ferrous ammonium thiocyanate method,using PC,PE,PS,PG(10-100µg as reference standard. The lipid extract in chloroform was evaporated under a steam of nitrogen and dissolved in 2.0ml of chloroform. Two ml of ammonium ferro thiocyanate was added and vortexed for 1min. Following the phase separation absorbance of chloroform phase was measured at 448nm for PC, 470nmfor PE and 452 nm for PS was measured in Shimadzu 160A spectrophotometer.

Estimation of total lipids:

About 1 mg protein was used to extract the total lipids. 1 ml of the extract was homogenized with 3.75 ml of methanol: chloroform (2:1) and incubated at room temperature for 3 hrs. in a cloded glass screw capped tube. The mixture was spun at 5000 rpm using table-top centrifuge for 10 min and the supernatant was collected in to a fresh screw cap tube. To the pellet added 4.25 ml of methanol: Chloroform: water (2:1:0:8) and incubated for 1 hr at room temperature followed by 10 min spin as described earlier. The supernatants were mixed together and added 2.5 ml of chloroform and 2.5 ml of water , mixed thoroughly, kept at 4˚C for 30 min and then centrifuged at 5000 rpm for 10 min. Upper aqueous phase was removed using a Pasteur pipette and to the lower chloroform phase 5 ml of benzene was added, mixed and evaporated under the steam of dry nitrogen gas. Finally the Nitrogen dried total lipids were estimated using Itachi Spectrophotometer at 525nm.

III RESULTS

Table 1.1 Maldi ToF-MS analyses of lipids identified Summer Season

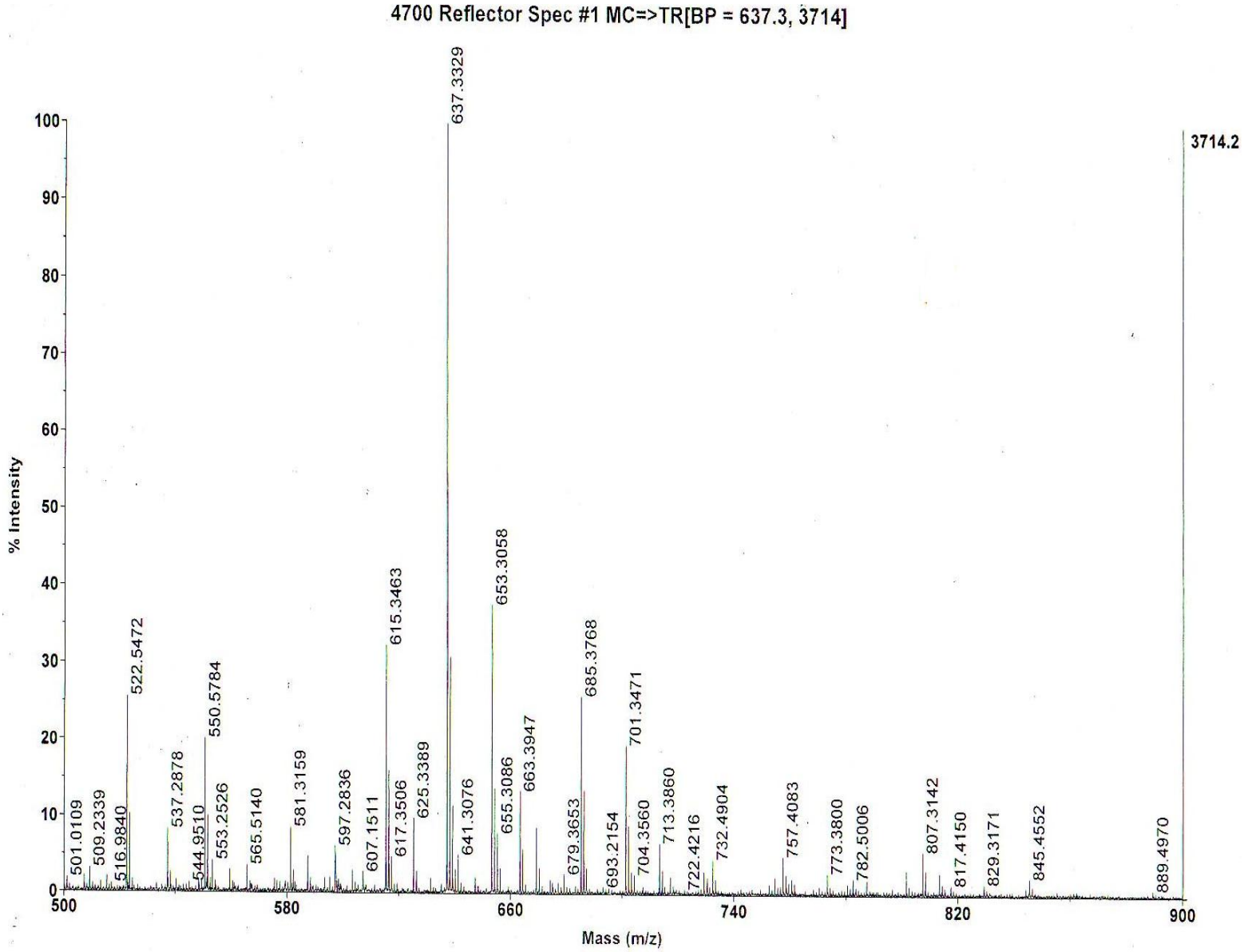
Observed M/Z	Exact M/z	Systematic Name	Formula	Category	Main class
522.5472	522.54	Pentatriacontanoic Acid	$C_{35}H_{70}O_2$	Fatty Acyls [FA]	Fatty Acids and Conjugates [FA01]
550.5784	550.57	Heptatriacontanoic Acid	$C_{37}H_{74}O_2$	Fatty Acyls [FA]	Fatty Acids and Conjugates [FA01]
615.3463	616.11	Quercetin 7-(6"-galloylglucoside)	$C_{28}H_{24}O_{16}$	Polyketide [PK]	Flavonoids [PK12]
637.3329	637.4	1-dodecanoyl-2-tridecanoyl-sn-glycero-3-phosphoserine	$C_{31}H_{60}NO_{10}P$	Glycerophospholipids [GP]	Glycerophosphoserines [GP03]
653.3058	653.19	Hirsutidin 3-O-(6-O-p-coumaroyl) glucoside	$C_{33}H_{33}O_{14}$	Polyketide [PK]	Flavonoids [PK12]
685.3768	685.47	1-dodecanoyl-2-(8Z,11Z,14Z-eicosatrienoyl)-glycero-3-phosphoethanolamine	$C_{37}H_{68}NO_8P$	Glycerophospholipids [GP]	Glycerophosphoethanolamines [GP02]
701.3471	701.43	1-dodecanoyl-2-(6Z,9Z,12Z-octadecatrienoyl)-glycero-3-phosphoserine	$C_{36}H_{64}NO_{10}P$	Glycerophospholipids [GP]	Glycerophosphoserines [GP03]

Above are the results analysed after subjecting for MALDI- TOF- MS(Graph:1 and Table:1) to understand the lipid profile of housefly Musca domestica maggots, during summer season. In summer seven types of lipid peaks were observed. It was noticed that after thorough analysis of MS spectra graph of larval extract – the highest peak was at the intensity (%) reading at 701.347 which corresponds to main class glycerophosphoserine (GP03). It was also noticed that the preceding peak as per the MS spectra of summer season of larval extract was 685.3768 (%) intensity which corresponds to main class glycerophosphoethanolamine. [GP02]. The next peak detected in MS spectra of summer season of larval extract was 653.3058 (%) intensity which corresponds to the main class flavanoids. (PK 12).During summer, two polyketides (Quercetin-7-6``Galloylglucoside) and Hirsutidin3-O-(6-O-pcoumaroyl) glycoside was observed.

In the present study the expressions of polyketide groups especially summer indicates their role in defense mechanism due to stress conditions from Musca domestica larva.

It was also noticed that during summer season glycerophospholipids (GP) were expressed and also the three types of GP are :1-dodecanoyl-2- tridecanoylic-_glycero-3-phosphoserine.,1-dodecanoyl-2-(8Z,11z,14Z,eicosatrienoyl) glycerol -3- phoshoethanolamine; 1- dodecanoyl - 2-(6Z,9z,12Z,octatrienoyl) glycerol-3-phoshoserine.

Maldi ToF-MS spectra of summer season larval extract



From larval extract of Musca domestica the lipidomic analysis revealed two fatty acids and conjugates namely pentatriacontanoic acid and heptatriacontanoic acid, expressions of major lipid components gives overall protection to the larval maggots of housefly, Musca domestica.

The major composition of the total lipids is phospholipids. These lipids play important role in immunity as they act as signaling molecules. They show major component of two

Phospholipid serine (PS), one Phosphatydyl ethanolamine (PE) and one Polyketide (PK).In summer season the significant change in lipid expression is being observed. The differential expression of lipids in summer is due to climatic change. In this season the temperature is above 40˚C ± 2, the humidity is very less, hence lipids like PS of m/z 637.3, PK of m/z 6153.2 and FA of m/z 522.1 and 550.3 were expressed. The differential lipid expression may be for the purpose to combat increased temperature and stress.

IV DISCUSSION

The lipidomic data indicates that about 19-20% of housefly larvae contains neutral lipids, and in these lipids the major groups are phospholipids. Insects are exposed to various pathogenic organism, but they successfully live without major infection. They have got biological defense mechanism against various pathogen and parasites. There are various components involved in protecting the insects by inducing the synthesis of antimicrobial peptides, proteins and fat bodies. However the lipids are also playing a major role in their defense mechanism in insects. Dietary fats derived from the biological sources plays an important role in the immune function [1 and 7]. The polyketides which were expressed during summer season are natural products present in prokaryotic and eukaryotic individuals mainly from bacteria.The polyketides have significant role in pharmacological activities and in neurological behavior and in few occasions involved in anti-cancerous agents. Teuscher and Lindequest [2], Rein and Borrome [3], Laurant *et., al* [4], while studying the insect chemical defense stated that the polyketides are major components utilized for body protective mechanism. In the present study there are two polyketides expressed and noticed as flavanoids (PK-12) which have chemical formula C28H24O16.Our results are in correspondence with above cited work. Bhikshapati *et al.,* [5] studied the differential fatty acid expression in native, injured and infected housefly larva. They found that the housefly larva have abundant phosphotidylethanolamine and phosphotidylcholine.

Joseph Vitale and Selwynbroytmen [6], while studying the role of lipids in relation to the immune function stated that the dietary lipid plays a role in modulating immune functions. Norris and Denis [7], studied dietary fish oil omega-3 fatty acids role in eliciting cardio protective and anti inflammatory effects. Kihara *et al.,* [8], during the study of therapeutic effects on multisclerosis by using mPEGS-1-PGE-2 stated that targeted lipidomic study can be useful in a major way for the treatment of multiple sclerosis. Zhen zhang *et al*.,[1], studied fatty acid extracts from *Lucelia sereata* in promoting cutaneous wound healing. They concluded that the extracts can be used as a powerful angiogenic activity in controlling the healing. It has been reported that polyketides are mainly for defensive mechanism Laurant *et al*. [4].

The Glycerophospholipids are very important components in the bilayer of lipids in the cell membrane. They are involved in metabolism, signal pathway and also keeps the animals survive in assistance in immune system. Lysyk [9], studied a detail account of impact of temperature, food and feeding on longitivity of housefly *Musca domestica.* According to the researcher temperature has an impact on the longitivity which was influenced along with diet provided to the houseflies. The author further stated that temperature is not solely depending on itself ,but with the combination of different diet including sucrose as major impact on longitivity of the flies. Schou *et al.* [10], have studied the influence of temperature and population density in response to their impact on locomotory activity of houseflies. They further studied that rhythms play a major role in locomotory activity which is also influenced by the temperature. In their observation, they noticed that the activity was more during day time up to 30ºC. However in both the sexes the activity of the locomotion increased even up to 35˚C.Hence they further stated that male houseflies are more active as compared to the females and as night time progresses the locomotory activity diminishes.

Over all from our results it can be concluded that the houseflies can adapt very efficiently during summer season by mobilizing the internal lipid profile to cope up with the change in high temperature in the environment and adapt to the surrounding atmosphere efficiently.

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