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DIFFERENTIAL EXPRESSION OF LIPIDS IN HOUSEFLY LARVAE (*MUSCA DOMESTICA*) BY MALDI-TOF-MS DURING WINTER SEASON

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Abstract: ***Houseflies are the most common insects. They are considered as a pest because they spread various diseases. These houseflies are diurnal in habitat and can fly very efficiently and escape from predators easily. They are abundant and very active especially during the rainy season, due to environmental conditions which are conducive for the houseflies' survival and existence. However, during winter season majority of the flies protect themselves from the cold and occupies warmer places. The most commonest housefly is the Musca domestica which is being extensively studied by various workers especially entomologist for their role in the environment. An attempt has been made to understand the lipid profile with the help of lipidomics study of housefly maggots with the help of MALDI-TOF-MS during winter season. The main aim of the study is to know the protective role of lipids during adverse environmental conditions.***

Keywords: *Housefly, Musca domestica, lipid profile, MALDI-TOF-MS*

I INTRODUCTION

Housefly is an important medical insect which has high effective immune defense mechanism and is rarely infected. Insects have extremely effective physico-chemical barrier as first line defense [1]. They develop a host defensive mechanism to counteract microbial infection and they offer a powerful resistance against them [2]. Lipidomics is a field of research which is involved in the analytical techniques and is useful for investigation and integration of cellular lipids from various organs [3]. This branch is commonly related with large scale study of networking of cellular lipids in a given biological system. There are various applications of lipids in the metabolic pathways. If there is an imbalance in metabolism of lipids, it will obstruct various cellular activities. In the present study, an attempt is been made to understand the lipid profile with the help of lipidomic study of housefly maggots with the help of MALDI-TOF during winter season as this season is unfavourable and the houseflies protect themselves under protected areas.

II MATERIALS AND METHODS

For studying lipid profile of housefly, *Musca domestica* following material and methods were adapted. Insect rearing : For getting maggot larva, which is the third instar of housefly, *Musca domestica* were reared for obtaining the larval stages with the help of media. For culturing the larva under sterilized conditions in laboratory 75 gm corn flour, 80 gm sugar 24 gm yeast, 60 gm malt, 10 gm of agar were used.

All of these were mixed and the volume was made up to 1 liter composition of larval food. The following preservatives 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 strongly and prepared a homogenous turbid mixture. The entire pellet was been resuspended. 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 vaccum. Mass Spectroscopy Model : (Shimadzo 160 A).

MALDI-TOF-MS For lipidomics: It is a technique useful in lipid analysis and lipidomics. This is largely due to its fast and easy performance which will get the result and recorded in mass spectra cells. It is a major ionization technique with the help of Mass Spectroscopy and easy to draw conclusion with the help of peaks available at different stages and seasons.

III RESULTS AND DISCUSSION

Following 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 winter season. It was observed that after thorough analysis of MS spectra graph of larval extract – the highest peak was at the intensity (%) reading at 701.2883 which corresponds to main class glycerophosphoserine (GP03).

Graph 1. Maldi TOF-MS analyses of lipids identified Winter Season

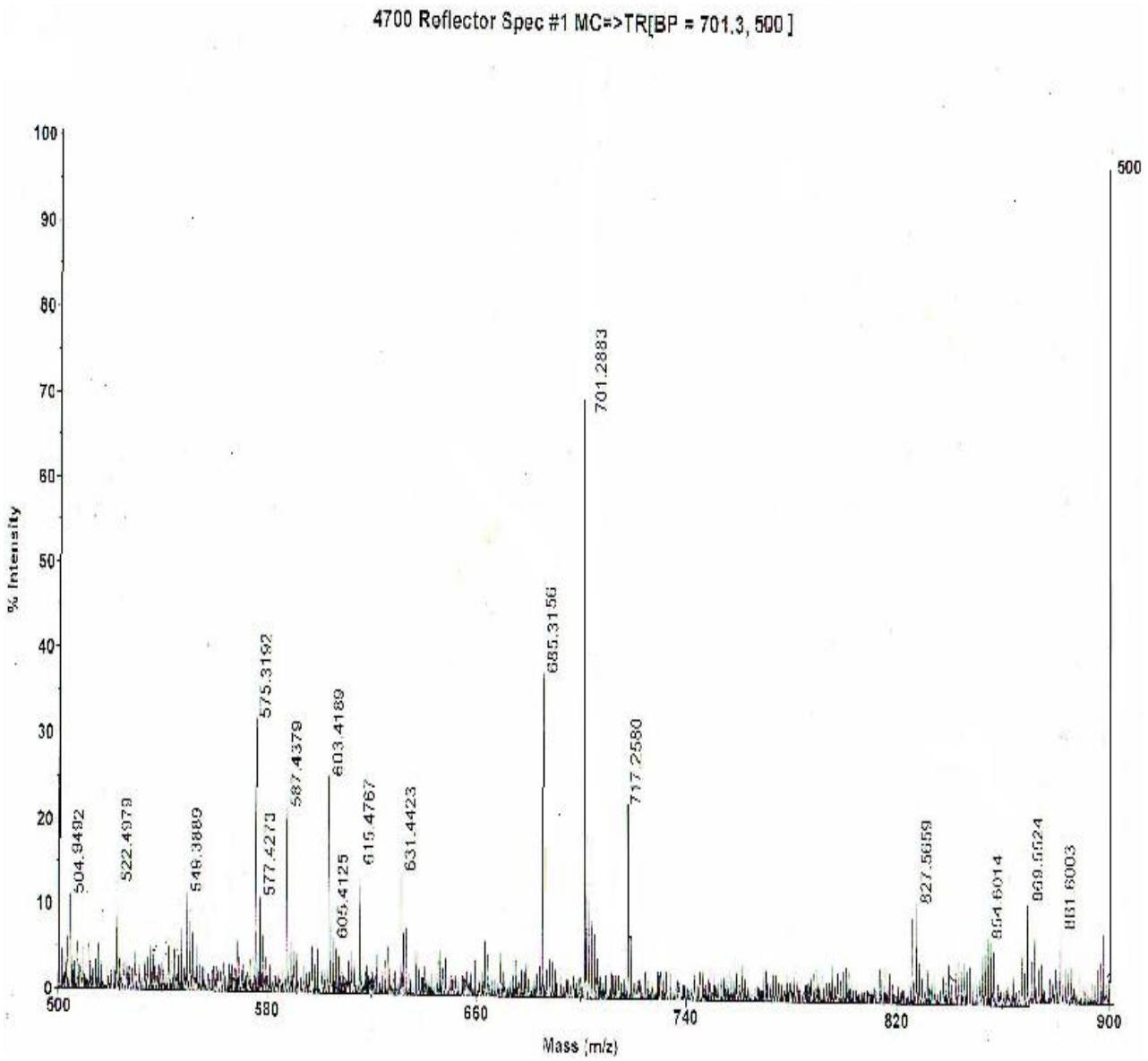

Table. 1 Maldi TOF-MS analyses of lipids identified Winter Season

Observed M/Z	Exact M/z	Systematic Name	Formula	Category	Main class
575.3949	575.4	1-(13Z,16Z-docosadienoyl)-glycero-3-phosphocholine	C30H58NO7P	Glycerophospholipids [GP]	Glycerophosphocholines [GP01]
587.4379	586.46	1-dodecanoyl-2-(7Z,10Z,13Z,16Z,19Z-docosapentaenoyl)-sn-glycerol	C37H62O5	Glycerolipids [GL]	Diradylglycerols [GL02]
603.4189	603.10	5-Carboxypyranocyanidin 3-O-(6"-O-malonyl-β-glucopyranoside)	C27H23O16	Polyketide [PK]	Flavonoids [PK12]
685.3192	685.47	1-dodecanoyl-2-(8Z,11Z,14Z-eicosatrienoyl)-glycero-3-phosphoethanolamine	C37H68NO8P	Glycerophospholipids [GP]	Glycerophosphoethanolamines [GP02]
701.2883	701.43	1-dodecanoyl-2-(6Z,9Z,12Z-octadecatrienoyl)-glycero-3-phosphoserine	C36H64NO10P	Glycerophospholipids [GP]	Glycerophosphoserines [GP03]
717.2580	717.46	1-tridecanoyl-2-(9Z,12Z-octadecadienoyl)-glycero-3-phosphoserine	C37H68NO10P	Glycerophospholipids [GP]	Glycerophosphoserines [GP03]

It was also noticed that the preceeding peak as per the MS spectra of winter season of larval extract was 685.3192 (%) intensity which corresponds to main class glycerophosphoethanolamine. [GP02].

The next peak detected in MS spectra of winter season 0f larval extract was 603.41(%) intensity which corresponds to the main class flavanoids. (PK 12).

It was also identified through MS spectra graph of housefly larval extract the next peak observed was 587.43 (%) intensity which corresponds to the main class Diradylglycerols [GL02]

The lowest peak seen through MS spectra graph of housefly larval extract was 575. 39 (%)intensity which corresponds to the main class Glycerophosphocholines [GP01].

Lipids are concentrated reserves in the body of insects and is a reserved food. It is composed of glycerides and fatty acids. Insects are able to convert carbohydrates into lipids and even some insects produce the lipids and store them in fat tissue [4]. Lipids comprises glycerides, which are bulk in form. Phospolipids are polyfunctional units. Sphingolipids are compounds of sphingosine. [5], stated that in most of the insects triglycerides are found to comprise various compounds as they are considered in the form of energy storages. The author further noticed that most of the insects composed of larger quantity of choline phosphoglycerides which they correlated to those present in some vertebrates. Fatty acids are synthesized during different stages of development which reflects the environmental parameters such as injury or infection [1]. These authors further stated that fatty acid plays diverse role in immune cells and they identified the innate immune response from, housefly *Musca domestica*.

These activities are generally observed during different seasons. Therefore, seasonality plays a major role in all different activities of insects. During winter season, these insects usually don't hibernate but enter a state of diapause. This reduces their activity of development as well as appetite up to the stage at which temperature rises considerably and they become active again (http..//bbc.com/earth/story/20150204-flies-can-be-active-all-year). Rohlf *et al.* [6], stated that glycerophosphocholine and phosphocholines are the prime choline components in higher vertebrates.In the present investigation the peak main class lipds was glycerophosphoserene. During winter, one Polyketide group identified was 5-carboxypyrinocyanidin.

The polyketides are widely spread original products present in prokaryotic and eukaryotic organisms because these polyketides have pharmacological significance and neurotoxic effect [6]. It is also reported that Polyketides can

also act in defense mechanism of the animal. In the present study appearance of Polyketides in winter season reflects its defence mechanism in winter season, as these houseflies prefer to hide out in avoidance of decreased temperature in surrounding environment. It was noticed that during winter different types of glycerphospholipids were observed namely
GP-1:glycerophopsphocholine
GP2:glycerophosphoenthanolamine,
GP3:glycerophosphoserine.

The role of glycerophospholipids in insects is- they are involved in metabolism, signaling mechanism and help the insects to resist in adverse conditions by helping their immune system. During winter season one glycerolipids (GL-02) was noticed. They are the important component of biolayers in the cell membrane which act as a barrier to passage of molecules and ions in and out of the cell membrane.

Lipids play a vital role in insect metabolism. They are involved in embryogenesis, metamorphosis and also in flight mechanism in insects. They even store 50 percent of its total body weight in their body. Lipoproteins are considered as major neutral lipids and sterol transport agents in insects. The ecdysone which is important insect hormone is generally carried through haemolymph [5-6]. Lipids play an important role in insect architecture and the intracellular membranes. Glycerides are found in large quantity in animals.

Phospholipids eg- Phosphotydyl choline which are compounds of lecithin. Etanolamine phosphotide serine phosphate, Inositol phosphtide and phosphoglycerol phosphotides are also major components of conjugated lipids.There are some references that the etanolamine phosphatide is being used as synonym for cephalin which is a mixture of phosphatides [5].

In the present study an attempt has been made to understand the role of lipids in larvae of *Musca domestica* with special reference to winter season and made an approach in elaborating the molecular interventions. This revealed that glycerophospho serine (717.2580—m/z)is a L serine (amino acid) derivative consisting of L-glycerophospho group attached to the side chain hydro-oxy function. Phosphotydyl serine is a main component of phospholipids and has the function in biological functions. Glycerophosphoetenolamines usually is involved in the membrane phospholipid metabolism at the time of embryonic development of brain and also involved in ageing process [5-6]. Glycerophosphocholine and phosphocholine are major choline metabolites in rat milk.

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