CHAPTER-I

INTRODUCTION
1. INTRODUCTION

Insects are highly diversified and successful group of animals occupied every niche on Earth. Until now more than one million species of insects species were identified (Hernandez et al., 2008). At every stage of insect life cycle they are exposed to different microorganisms such as bacteria, virus, fungi, protozoan etc. To combat microbial exposure and infection they have developed effective defense mechanisms (Hoffman, 1995). The innate immune system appeared in the early evolution and conserved throughout the animal kingdom (Hoffmann et al., 1999). Insects rely on innate immunity only as they are deficient of adaptive immune response mechanism like vertebrates (Silverman and Maniatis, 2001).

1.1 Innate Immunity of Insects

Housefly (*Musca Domestica*) are cosmopolitan and are vector for more than 100 human and animal intestinal diseases (Scott and Lettig, 1962; Greenberg, 1965; Keiding, 1986) including bacterial infections such as Salmonellasis, Anthrax Ophthalmia, Shigellasis, Typhoid fever, Tuberculosis, Cholera and Infantile diarrhea, Protozoan infections such as Ringworms, Roundworms, Hookworms and Tapeworm as well as viral and rickettsional infections. Houseflies also transmit eye diseases such as Trachoma and epidemic conjunctivitis, and Infect wounds or skin with disease such as cutaneous diphtheria, mycoses, yaws and leprosy (Keiding, 1986). Considering that houseflies are highly mobile, come into contact with excreta, carcasses, garbage and other septic matter and that they are intimately associated with humans, food and utensils, they are involved in transmission of so many serious and wide spread diseases (Scott and Lettig, 1962; Keiding, 1986). Recently houseflies are found to be vector life threatening antibiotic resistant bacteria, which are ever increasing problems in hospitals and other health care facilities (Sundin, 1996; Graczyk, *et al* 2001; Maisnier-Patin and Anderson, 2004). According to Scott, *et al* (2006), house fly biology is closely linked to microbes, hence, unlike Drosophila and several other Diptera, development of housefly larvae is strictly dependant on a live and active microbial community in a natural developmental habitat. Larvae cannot develop beyond the first instar in sterilized a natural or artificial
substrate/medium. The principle of this symbiosis is unknown although it has been shown that different bacteria support the housefly development to different degrees.

The microorganism invading the general body cavity were countered by both humoral and cellular reaction (Chaves, et al 2008). Antibacterial peptides were a significant part of the humoral immunity effector molecules in housefly (Musca Domestica). After the insect being infected or injured, insect antimicrobial peptides were rapidly synthesized and cleaned by signal peptidases. They become functionally active and then rapidly secrete in to hemolymph (Imler, et al 2005). Innate immunity is the first line of defense against infectious microorganisms. The innate immune system relies on germ line-encoded pattern recognition receptors (PRRs) to recognize pathogen-derived substances (Janeway, 1989). Activation of the innate immune system through these receptors leads to the expression of a vast array of antimicrobial effector molecules that attack microorganisms at many different levels. The innate immune system appeared early in evolution, and the basic mechanisms of pathogen recognition and activation of the response are conserved throughout much of the animal kingdom (Hoffmann, et al 1999)

Immediately after septic injury, the insect fat body (a homologue of mammalian liver (http://www6.ufrgs.br/favet/imunovet/molecular_immunology/gastrointestinal.html - liver) and some blood cells start to produce a battery of potent 20-40 amino acid-long antimicrobial peptides (Hultmark, 1993). These molecules are released into the blood, where they synergistically act to destroy the invading microorganisms. Many induced antimicrobial molecules are apparent in the 2-4 h after infection at a concentration as low as 15 mM (LC$_{50}$). Insects have a family of 12 peptidoglycan recognition proteins (PGRPs) that recognize peptidoglycan, a ubiquitous component of bacterial cell walls. In insects PGRPs activate antimicrobial pathways in the hemolymph and cells, or are peptidoglycan (PGN)-lytic amidases. List of statistically significant differentially expressed proteins identified in hemolymph of Drosophila melanogaster third-instar larvae by 2D-DIGE (http://www6.ufrgs.br/favet/imunovet/molecular_immunology/invitrocellfre.html-2D-DIGE) combined with mass spectroscopy, 25 min or 4 h after LPS challenge and 25 min after sterile challenge (Vierstraets, et al 2004). Insects have a very potent innate immune response that effectively combats a broad spectrum of pathogens. For example, Drosophila
can withstand, and clear, bacterial burdens that, relative to their size, would be lethal to mammals (Guo, et al 2006). Induction of innate immunity in both mammals and insects leads to the activation of similar effector mechanisms, such as stimulation of cell-based phagocytic activity and expression of antimicrobial peptides (Luo, et al 2005). For example, *Drosophila* produces a wide range of potent antimicrobial peptides in response to infection by fungi or bacteria (Hoffmann and Reichhart, 1997). Induction of the antimicrobial peptides is regulated at the level of transcription, and they are expressed primarily in the fat body, the insect liver analog (Silverman & Maniatis, 2001).

Recent studies have revealed striking similarities in the signaling pathways used by humans and flies to activate their innate immune responses. In both cases, infection leads to the activation of Toll-like receptors (TLRs), which in turn initiate intracellular signaling cascades that culminate in the activation of NF-κB/Rel family transcription factors. Among the large number of inducible antimicrobial proteins and peptides, lysozyme is the most ubiquitous antibacterial factor and is widely distributed in vertebrate and invertebrate animals. CG18954 protein is homologous to the mammalian phosphatidylethanolamine-binding protein (PEBP), expressed in a wide range of tissues and originally isolated as a cytosolic 21- to 23-kDa protein from bovine brain. It binds hydrophobic ligands and in particular phosphatidylethanolamine (Schoentgen, et al 1995).

1-lysophosphatidylethanolamine is an antimicrobial compound in the housefly (*Musca Domestica*). Meylaers, et al (2004) observed that a methanolic whole body extract of uninfected last instar larvae of the housefly, (*Musca Domestica*), displayed antifungal and antibacterial activity. On further purification of this extract to a single active fraction using reversed phase high performance liquid chromatography. The pure fraction inhibited growth of the Gram-positive bacteria Bacillus thuringiensis and the yeast Saccharomyces cerevisiae, but not the Gram-negative bacteria *E.coli*. The active compound was determined to have a molecular mass of 451.2 Da (Meylaers, et al 2004). Housefly is an important medical insect which has a highly effective immune defense mechanism and is rarely infected even reared in large-scale, high-density conditions (Sukontason, et al 2000; Moreira, et al 2003; Zhao, 2007; Rahuma, et al 2005; Luo, et al 2005; Liu, et al 2007; Ma, et al 2007). Scholars studied the hemolymph of housefly larvae

Wang, et al (2006) observed a 430-bpcDNA encoding the insect antimicrobial peptide defensin was cloned from the housefly, and designated (Musca Domestica) defensin (Mdde). Northern analysis and in situ hybridization identified the corresponding mRNA in the fat body of bacterially challenged houseflies and in the epidermis of the body wall of naive and challenged houseflies. The gram-negative bacterium (E.coli) is a strong inducer of the gene. By RT-PCR, Mdde mRNA was also detected in native and challenged insects. These findings suggest that the defensin gene is constitutively expressed in the epidermis of the housefly body wall. The predicted mature form of Mdde was expressed as a recombinant peptide in E.coli and Pichia pastoris. The recombinant Mdde expressed in Pichia was active against gram-positive and some gram-negative bacteria (Wang, et al 2006). Hoffmann (1995, p4-10) claimed that insects are particularly resistant to microorganisms. Their host-defense system relies on several innate reactions: upon injury, the immediate onset of two proteolytic cascades leading to localized blood clotting and to melanization, the later process involving production of cytotoxic molecules namely reactive oxygen intermediates, the phagocytosis of bacteria, and the encapsulation of larger parasites by blood cells, the induced synthesis by the fat body of a battery of potent antimicrobial peptides/polypeptides which are secreted into the hemolymph, where they act synergistically to kill the invading microorganisms. The insect host defense system shares many of the basic characteristics of the mammalian acute phase response, especially at the level of the coordinate control of gene expression, where similar cis-regulatory and inducible transactivators appear to play key functions. Pricking the larva with a needle causes a severe injury to the animal. Therefore, it is likely that some of the increased proteins have nothing to do with the immune response, but are only part of the stress/injury response (e.g. glutathione S-transferase and actin-57B).

According to Stanley, et al (1991), a single insect produces approximately 10-15 antibiotics, including antiviral peptides such as alloferons which have antiviral and
antitumoral capabilities and antibacterial peptides like cysteine-rich antimicrobial peptides synthesized in the fat body of insects comprising of defensins which selectively kill Gram-positive Bacteria with a 1-min contact with 0.5 μM concentration. They have the lowest net positive charge/mass ratio among the various antibacterial peptide families, impairing permeability of the outer membrane of Gram-negative strains. They contain 3 intrachain disulfide bonds, forming analogues called sapecin, defensin A, defensin, royalisin, tenecin. Cecropins are also from antibacterial peptide family, almost exclusively restricted to the Lepidoptera and Diptera orders, forming amphipathic μ-helices, active against both Gram-negative and Gram-positive Bacteria, with very little hemolytic activity. Stanley, et al. (1991) observed that in fact they mainly act by altering the way bacterial genes are regulated. Proline- and/or glycine-rich peptides are predominantly active against Gram-negative Bacteria. Thanatin, from Podisus maculiventris, is active against both Gram-positive and Gram-negative Bacteria. Moricin is active against both Gram-positive and Gram-negative Bacteria. The larvae were shown to contain all of the C20 polyunsaturated fatty acids necessary for eicosanoid biosynthesis and to be capable of converting radioactive arachidonic acid into several primary prostaglandins. These results strongly suggest that eicosanoids mediate transduction of bacterial infection signals into the complex of cellular and humoral responses that comprise invertebrate immunity (Stanley, et al. 1991).

1.2 Dipterans Immunity

Flies belong to Diptera order, insects with a pair of wings on the mesothorax and a pair of halters, derived from the hind wings, on the mesothorax. It is to note that the presence of a single pair of wings distinguishes true flies from other insects. Diptera is a large order, containing an estimated 240,000 species of mosquitoes, gants, midges and others, wherein only half of these (about 120,000 species) have been identified and described (Wiegmann and Yeates, 1996). It is one of the major insect orders both in terms of ecological and human importance for medical and economic aspects. Dipterans show genetically immune response. AT-rich motif enrichment in 5’ upstream sequences in A. gambiae, Ae. Aegypti and the Drosophila genus immunity genes suggest a particular pattern of nucleosome formation/chromatin organization. The co-occurrence of such motifs
with the NfκB response elements suggests that these sequence signatures may be functionally involved in transcriptional activation during dipteran immune response. AT-rich motif enrichment in regulatory regions in this group of co-regulated genes could represent an evolutionary constrained signature in dipterans and perhaps other distantly species (Hernandez-Romano, et al 2008). Dipterans produce defensins and diptericin, two small antibacterial peptides. Defensins are primarily active against Gram positive bacteria, but various mammalian defensins have been reported to show significant invitro activity against fungi (Ganz, et al 1985; Lehrer, et al 1985) and enveloped viruses (Lehrer, et al 1985; Daher, et al 1986). In the Dipteran P.terranovae, the anti-Gram positive defensins, together with the anti-Gram negative diptericins, account for the bulk of the inducible antibacterial response as evidenced by growth inhibition assays (data from Keppi, et al 1986; Dimarcq, et al 1988; Lambert, et al 1989). Male fruit flies produce andropin, thought to protect seminal fluid from infection, a 28 kDa male accessory gland-derived protein and two ejaculatory duct-derived proteins all with antibacterial activity. Based on its gel mobility and tissue of synthesis, one of the ejaculatory duct proteins is likely to be andropin, a previously-reported 6 kDa antibacterial peptide. (Lung, et al 2001)

1.3 Humoral Immunity of Insects

Tzou, et al (2000) claimed that insect innate immunity is based on the recognition of microbial molecules, such as LPS, peptidoglycans, or b-1,3-glucans, by specific receptors with the subsequent activation of immune effector responses. Non-microbial surfaces (e.g. Sephadex beads) also elicit responses. Proteins that are considered to have a recognition or opsonizing function include GNBP, TEP and PGRP. Recognition leads to activation of cellular and/or humoral effector mechanisms. In their study, Tzou, et al (2000) observed that these include phagocytosis by hemocytes, encapsulation or nodulation of pathogens by hemocytes, activation of proteolytic cascades leading to localized melanization and clotting, and synthesis of a battery of AMPs. The latter process can occur in most insect tissues including the fat body, hemocytes, respiratory system, cuticular and midgut epithelia, Malpighian tubules, and male and female genital tracts (Tzou, et al 2000). Insects possess an open circulatory system that contains the hemolymph (the insect blood), which is pumped by a basic heart called the dorsal vessel. The
hemolymph does not play a role in oxygen transport but is the major site of the resistance during systemic infection. Innate immunity allows insects to protect themselves against a wide range of microbial pathogens that cause infection. Insect innate immune systems comprise cellular and humoral defense responses (Boman, et al 1987; Hoffmann, 1995; Hoffmann, et al 1996; Bulet, et al 1999; Lavine, et al 2002). The humoral defense response takes effect by over-expressing an array of potent antimicrobial proteins and peptides at the time of pathogenic infection (Bulet, et al 1999; Hoffmann and Reichhart, 2002).

1.4 Innate and Humoral Immunity of Housefly (*Musca Domestica*)

The proteomic study of the housefly will shed light on the immune defense system of this important species and provide valuable information about how it is able to flourish, while living in innate contact with such a multitude of pathogens. Housefly larvae development depended on active microbial community in a natural developmental habitat. Larvae cannot develop beyond 1\textsuperscript{st} instar in sterile condition, this principle of symbiosis is unknown although it has been shown that various bacteria support the housefly development to different degree. It is clear that housefly biology is closely linked to microbes. Many studies have shown that innate immunity as the role of antimicrobial peptides including cecropin, attacin, drosomycin and defensin has become the primary line of defense against the infection the infection of pathogens and parasites in insects (Boman, 1998; Naitza, et al 2004). Wang, et al (2009) expanded on the theory of antimicrobial peptides in dipteran insects has been extensively investigated, three types of AMP have been described in (*Musca Domestica*). AMPs are essential effector molecules for the maintenance of humoral immunity. The regulation of which is influenced with change in the environment. It is essential to determine if the expression of the AMPs and some protein is followed rapidly after challenge, if the expression of different AMPs and protein existed during temporal differences, if these different AMPs in some sense cooperate. Houseflies are resistant to micro organism. In their study, Wang, et al (2009) observed that their host defense system relies several innate reaction upon injury and infection. The two immediate on set of proteolytic cascades leading to blood clotting and melanization. The later process involving production of cytotoxic molecules, the phagocytosis of
bacteria, the induced synthesis by the fat body of a battery of potent antimicrobial peptides which are secreted into hemolymph where they act synergistically to kill the invading microorganisms.

Northern analysis and in situ hybridization identified the corresponding mRNA in the fat body of bacterially challenged houseflies and in the epidemics of body wall of native and challenged housefly. Immediately after injury, the insect fat body and some blood cells start to produce a battery of potent 20-40 amino acid, long antimicrobial peptides. Jiang (1999) claimed that the housefly (Musca Domestica) larvae are used clinically to cure malnutritional stagnation decubital necrosis, osteomyelitis, ecthyma, and lip boil since the Ming/ung Dynasty (1386 Domini) and also are used to treat coma and gastric cancer when combined with other drugs. Housefly larvae are used in traditional Chinese medicine and they are commonly assumed to be safe for medicinal use.

1.5 Insect physiology

Most of the insects hatch from eggs and the life cycles of insects vary. The structure, habit and habitat varies from adult to immature stages of larva and pupa, while pupal stage is passive and complete metamorphosis. Some insects undergo incomplete metamorphosis without having a pupal stage and adults develop through a series of nymphal stages (McGraw-Hill, 2007). The most diverse insect groups appear to have coevolved with flowering plants. Female house fly lays eggs periodically, following a cycle of egg maturation and oviposition. The eggs are laid on moist organic matter that serves as growth medium for the larvae. Development sites for larval house flies in confined cattle systems include silage mounds and indoor bedding material (Meyer and Shultz, 1990; Lysyk, 1993). Manure can support development of house fly larvae (Meyer and Shultz, 1990) largely because this is rich in bacteria that larvae use as a food source. House fly larvae failed to develop on an artificial growth medium sterilized immediately after preparation, but reached the adult stage on the same medium incubated for two days prior to sterilization (Greenberg, 1954). This indicates larvae need bacterial growth to develop properly but can develop on lysed bacteria. House fly larvae reared on agar-based diet did not grow on sterile media, but completed development when selected bacterial strains were present (Schmidtmann and Martin, 1992; Watson, et al 1993; Lysyk, et al
As the housefly larvae (maggots) live and develop consuming dead flesh, they were formerly used to treat wounds to prevent or stop gangrene. Even in modern era, this treatment is in usage in some hospitals. Adult insects, such as crickets, and insect larvae of various kinds are commonly used as fishing bait. The transmission of pathogens is of two kinds, biological or mechanical. In biological transmission close association with their arthropod vector and infection of the arthropod is a necessary step in the completion of the pathogen lifecycle. In mechanical transmission, the vector essentially transports the pathogen. The pathogen may be carried externally on the body surface or the mouth parts of the fly and then transmitted through regurgitation or defecation (Dipeolu, 1982; Glass et al 1982; Sasaki, et al 2000). Flies are good indicators of the microorganisms present in the environment. Houseflies trapped within a hospital had higher bacterial counts and carried more pathogens than flies captured in a residential area (Fotedar, et al 1992). Attenuated polioviruses used for vaccination were isolated from houseflies; demonstrating humans may actually be responsible for contaminating flies (Greenberg, 1973). The association of houseflies with facies is an important issue in disease transmission. The house fly was implicated in the transmission of numerous enteric pathogens such as *Salmonella* spp. (Greenberg, 1964), *Shigella* spp. (Lindsay and Scudder, 1956; Levine and Levine, 1991), and *E. coli* (Moriya, et al 1999). House flies may also be important carriers of *Vibrio cholerae* during outbreaks (Fotedar, 2001). The housefly, known as common housefly, (*Musca Domestica*), belongs to the Brachycera suborder. The most common of all domestic flies accounting for about 90% of all flies in human habitations, and indeed one of the most widely distributed insects, housefly is found all over the world and is considered a pest that can carry serious diseases. Our interest is to investigate biochemical changes and biological activity and innate immunity of housefly, (*Musca Domestica*) which belongs to Diptera.

### 1.6 Significance of Maggots

Maggots are exposed to more than 100 pathogenic microorganisms. When compared to egg, pupa and adult fly, the maggots possess more resistance to the microorganisms. One of the significance of traditional Chinese medicine is the use of insect larvae, insect and other arthropods and their products as drugs, based on the work
carried out is “Anti bacterial activity and in vitro tumour activity of the extract of the larvae of the housefly (Musca Domestica)” (Hou, et al, 2007). The housefly thrives in a virtual sea of animal pathogens. Sequencing of the housefly genome will shed light on the immune defense systems of this important species, and provide valuable information about how it is able to flourish, while living in intimate contact with such a multitude of pathogens. Comparison with the innate immune systems of Musca with Drosophila (and Anopheles), which face different ecological pressures and pathogens, will be informative, just as the Drosophila-Anopheles comparison has been (Christophides, et al 2002). The relatively close relationship to Drosophila has already greatly expedited this analysis, as over 30 individual genes in innate immunity have been sequenced in Musca. The advantage of a genome sequence is that it will allow discovery of genes unique to Musca, and regulatory systems that allow it to survive in a far more septic environment.

1.7 Maggot Debridiment Therapy

The infestation of wounds by certain species of fly larvae (maggots) has been recognized to debride, to enhance healing, and to decrease the mortality associated with the underlying injury. During World War 1, an observation on the benefits of maggot-infested wounds was done for the first time. During 1940, the popularity of maggot therapy decreased. But when conventional medical and surgical therapy failed to control a serious tissue infection, the value of maggot therapy had to be acknowledged. Maggot therapy is essentially by controlling the wound, and thus the benefits outweigh the risks. The 3 distinct but simultaneous effects upon host wound are debridiment, disinfection and tissue growth. The maggots secrete their proteolytic digestive enzymes that selectively dissolve necrotic tissue. Multiple mechanisms appear to play a role in maggot-induced wound healing, such as debrid, disinfect wounds, promote wound healing, tissue oxygenation (Wollina, et al 2002).

1.8 Maggot Therapy in Infections

Hou, et al (2007) studied that maggots kill microbial agents infecting wounds and attempt to isolate antibacterial factors including anti-Methicillin Resistant Staphylococcus Aureus (anti MRSA) agents, from maggot secretions. The excretion of a waste product,
ammonia, by Phaenicia sericata was believed to be responsible for combating bacterial infections, since ammonia increases wound pH, resulting in alkaline conditions unfavorable to many bacterial species. In their study, Hou, et al (2007) observed that these larvae carry in their midgut a commensal, proteus mirabilis which produce agents such as phenylacetic acid (PAA) and phenylacetaldehyde (PAL), with antibacterial properties. While maggots combat wound infection, the larvae ingest wound bacteria, which are killed while passing through the maggot’s digestive tract. Maggots secrete potent bactericide. Significant antibacterial activity of 05.-10 kDa and the <500 Da ultrafiltration fractions was demonstrated against S.aureus, and the >10 kDa fraction was free of antibacterial activity. Maggot therapy characterizes in rapidity of healing and appearance of healthy new extracellular matrix (ECM) (granulation tissue). The reports show the antimicrobial activity, in vitro anti-tumor activity of the extract of housefly larvae (Hou, et al 2007). This report suggests that extract of native larvae and extract of inoculated larvae, the later showed higher antibacterial activity and in vitro anti-tumor activity than the extract of native larvae, which indicated the same principle involved in the use of larvae infected with silk moth fungus and cordicepe fungus in traditional Chinese medicine. When infected with microorganisms, insects activate three interconnected reactions such as proteolytic cascades, cellular defense reaction and the rapid and transient synthesis of an array of antimicrobial peptides by the fat body (Holfmann, et al 1996).

1.9 Biological studies

In order to study the biological significance of housefly larvae, the housefly larvae of unchallenged, injured and infected were selected and homogenated to get extracts. investigation was carried out to study the differential expression of proteins/peptides lipids, fatty acids using MALDI TOF/MS and GCMS These extracts were used for biological activity studies such as antibacterial activity, hemolytic activity and anti-tumour activity.

1.10 Proteomics and Lipidomics

For the analysis of lipids and proteins, MALDI-TOF MS techniques have been used. Lipidomics and Proteomics quantitatively describe lipids and proteins along with their functions. Lipidomics must quantitatively describe all lipids and their functions at the
cellular level (van Meer, 2005). Lipid and protein amounts in the range of a few nanograms are sufficient for MS analysis. The initial reports on matrix-assisted laser desorption/ionization (MALDI) analysis of intact glycerophospholipid and sphingolipid species (Harvey, 1995; Mart, et al 1995; Harvey, 1995) did not enjoy initial rise in popularity. MALDI-MS of lipids has received increased attention in recent years (Huang, et al 2006; Jackson, et al 2008; Schiller, et al 2006; Ma, et al 2007) largely due to its ability to sample directly from tissue (Caprioli, et al 1997). Attention has mostly been focused on the application of MALDI to glycerophospholipid analysis, although this technique has also been applied to other lipid classes, including sphingolipids (Caprioli, et al 1997) triacylglycerols (Guittard, et al 1999), diacylglycerols (Asbury, et al 1999), cholesterol and cholesteryl esters (Benard, et al 1999), wax esters (Schiller, et al 2001) and even free fatty acids (Vrkoslav, et al 2009; Ayorinde, et al 2000).

1.11 Proteins

Tzou, et al (2000) studied that some proteins have known to be or likely functions in insect immunity. Insect innate immunity is based on the recognition of microbial molecules, such as LPS, peptidoglycans, or B-1,3-glucans, by specific receptors with the subsequent activation of immune effector responses. Non-microbial surfaces (e.g. sephadex beads) also elicit responses. Proteins that are considered to have a recognition or opsonizing function include GNBP, TEP and PGRP. Recognition leads to activation of cellular and/or humoral effector mechanisms. These include phagocytosis by hemocytes, encapsulation or nodulation of pathogens by hemocytes, activation of proteolytic cascades leading to localized melanization and hemolymph clotting, and synthesis of a battery of AMPs. The latter process can occur in most insect tissues including the fat body, hemocytes, respiratory system, cuticular and midgut epithelia, Malpighian tubules, and male and female genital tracts (Tzou, et al 2000). Innate immunity refers to the first-line host defense against the early phases of microbial infection and is an evolutionarily ancient defense mechanism. Quite recently this first-line defence received renewed attention. Insects and vertebrates display considerable overlap in the intracellular signaling pathways that regulate innate immune responses (Salzet, 2001; Giot, et al 2003; Hultmark, 2003) and in some of the effector mechanisms used against microbes like phagocytosis, fluid
lysozymes. Thus, discoveries made through research in the fruit fly, *Drosophila melanogaster* may be applicable to innate immunity in humans (Fallon, *et al* 2001). The study of innate immunity in insects has also garnered increasing attention because of the role of many insects in transmission of human disease agents. Understanding how the insect immune system interacts with pathogens may contribute to development of new strategies to block transmission of disease agents (Christophides, 2005). For example, cecropin is a protein originally identified for its antibacterial activity in lepidopteran insects but eventually shown to reduce malaria parasite development (Gwadz, *et al* 1989). As a result, transgenic mosquitoes were developed to overexpress cecropin in the midgut, resulting in significant decreases in the number of developing malaria parasites following infection (Kim, *et al* 2004). The sequencing of the genomes coupled with EST projects for two dipteran species, *D. melanogaster* and the African malaria mosquito, *Anopheles gambiae*, provided new opportunities for studying immunity. New genes with candidate immune functions were quickly identified (Christophides, *et al* 2002) and microarrays were applied to survey transcriptome changes after bacterial, fungal or parasite infections (DeGregorio, *et al* 2001; Irving, *et al* 2001; Dimopoulos, *et al* 2002; Roxstrom-Lindquist, *et al* 2004). These studies have been fruitful in identifying a set of genes that can be tested for functional involvement in insect immune responses. Insects respond to microbial infection by the rapid and transient expression of several genes encoding antibacterial peptides. The two-dimensional differential gel electrophoresis, combined with mass spectrometry can be used to study the immune response of housefly larvae at the protein level.

Hultmark, (1993) studied that insects are particularly resistant to microbial infections, although they do not have an acquired immune system that is capable of specifically recognizing and selectively eliminating foreign microorganisms and molecules those are foreign antigens. The defense system of insects consists of different innate reactions. Innate immunity is based on the recognition of microbial molecules, such as lipopolysaccharides (LPS) and peptidoglycans, by specific receptors and the subsequent activation of the cellular response, which includes phagocytosis and encapsulation, and the humoral response. Immediately after septic injury, the insect fat body, a homologue of the
mammalian liver and some blood cells start to produce a battery of potent antimicrobial ad
antifungal peptides (Hultmark, 1993). These molecules are released into the blood, where
they synergistically act to destroy the invading microorganisms. Many induced
antimicrobial molecules are apparent in the hemolymph only 4 hours after infection. The
order Diptera shows genetically immune response at rich-motif archid in five upstream
sequence in a A. Gambiae, A. Egypti, and Drosophila Melangeaster. Dipterans produce
defensins and diptiricin and two small anti-bacterial peptides. In dipterans larvae oral
region, Metchnikowin and defensin, at salivary glands drosomycin, at proventricular and
midgut deptiricin, and attacin, in fat body all AMP cuticle cecropin, in malphygian tubules
metchnikowin, defensin, and cecropin, and in trachea droomycin, drosocin are produced
(review by Hoffmann, 2003). Six distinct Bombyx inducible antimicrobial peptide
families, Cecropins, Attacins and Lysozyme, Lebocin, Moricins and Hemocytin were also
identified (review by Ponnuvel & Yamakawa, 2002). An interesting fact is that in more
than one million described insect species todate, only 170 antimicrobial
peptides/polypeptides from various insect species have been clearly characterized (Bulet,

1.12 Differential Proteins after Immune Challenge

The data of the 4-h experiment suggest changes at the gene expression level for
the identified differential proteins (Irving, et al 2001). Microarray studies (Lissemore, et
al 1990) did not indicate changes in mRNA quantities for these proteins and lack of this
change could be explained by the fact that these studies were performed on adults instead
of larvae. Insect innate immunity is both a model for vertebrate immunity as well as a key
system that impacts medically important pathogens that are transmitted by insects. The
term “proteome” came into existence in 1994 and defined as the entire protein complement
expressed by a sample. Proteomics encompasses a broad set of disciplines aimed at
understanding and monitoring proteins. This includes work correlating genetic sequence
with three-dimensional protein structure and 3D structure with protein function,
development of protein separation and protein profiling techniques, and investigation of
protein-protein interactions. Recent studies of insect immunity concentrate on
“profiling/expression” and “functional” proteomics. Profiling or expression proteomics
focuses on the description of the whole proteome in a given tissue, body fluid, cell type, or organelle, and differential measurement of protein expression levels in samples collected under different conditions (Choudhary and Grant, 2004). Functional proteomics includes research approaches that directly analyze a subset of proteins, such as a family of sequence- or function-related proteins (Kocks, et al 2003), as well as those that characterize the protein’s biological functions, protein protein or protein-DNA/RNA interactions, or posttranslational modifications (Cai, et al 2004). Proteomics is a tool for detecting changes in protein expression and modification in whole organisms and in specific cells, tissues, and fluids. This review will focus on the methods and applications of proteomics to insect immunity. The tools of proteomics have been developing over the past three decades, but it was not until mass spectrometry began to be used for the identification of proteins in complex mixtures that the field really started to take off (Karas and Hillenkamp, 1988; Fenn, et al 1989). Recent developments in proteomics and protein identification techniques combined with the completion of genome sequences for Anopheles gambiae and Drosophila melanogaster provided the tools for examining insect immunity at a new level of molecular detail. Application of proteomics to insect immunity resulted in predictions of new roles in immunity for proteins already known in other contexts of ferritin, transferrin, Chi-lectins and helped to target specific members of multi-gene families that respond to different pathogens such as serine proteases, thioester proteins. In addition, proteomics studies verify that post-translational modifications play a key role in insect immunity since many of the identified proteins are modified in some way. These studies complement recent work on insect transcriptomes and provide new directions for further investigation of innate immunity. (L Shi, 2006).

There are significant and methodological differences in proteomics studies, including types of challenge agents like single species or mixes of living bacteria, LPS, bacterial lysates, yeast, filamentous fungi, microsporidia, picorna-like virus, Sephadex beads as well as the method of introduction of the agent like feeding, external exposure, injection, the length of incubation time after exposure, the tissue examined in hemolymph, hemocyte-like cells in culture, fat body, midgut, thorax, whole insect larvae, the method of tissue collection, and the stage of the insect whether larvae or adult. Standardizing these
aspects would provide better ability to compare innate immunity across taxonomic groups. Nevertheless, taken together these studies identify some patterns in proteins that are affected by immune challenges and provide a framework for future investigations. The tool of Proteomics have been developed over the past three decades. All proteomic technologies relay on the abilities to separate a complex mixture, so that individual proteins are more easily processed with other techniques. 2-DE Gel Electrophoresis is still more widely used in protein separation technology for insect immunity (O’Farrell, 1975). In this approach, the proteins are separated in the first dimension by isoelectric focusing, using immobilized pH gradient strips. Then these proteins are again separated on Polyacrylamide Gel Electrophoresis along with the molecular weight standards resulting in 2DE display of proteins are large in number of about 3000 to 10000 proteins can be visually separated and these spots exhibiting changes between the treatments can then be singled out for the further exploration followed by specific spot digestion and protein identification on MALDI-TOF MS.

1.13 LIPIDS

Insect Lipids

Gilbert and Chino, (1974) observed that many insect species are almost completely dependent on lipids for their metabolic needs, although this is usually a function of developmental stage. The primary storage organ is the fat body, which can constitute 50% of the fresh weight of the insect and also acts as the major metabolic center (analogous to the vertebrate adipose tissue and liver). Although neutral lipids are stored as triglycerides, in times of need they appear to be endergonically released as diglycerides in the majority of insects thus far studied (particularly silkmoths and locusts). Gilbert and Chino, (1974) studied that diglycerides constitute the largest neutral lipid fraction in the hemolymph of silkmoths, locusts, cockroaches, bugs, etc. In the hemolymph the diglyceride is found as a constituent of specific lipoproteins, and one specific lipoprotein class (lipoproteinI; high density lipoprotein) appears to be necessary for the transport of diglyceride from the fat body cell into the hemolymph. This particular lipoprotein is also involved in the transport of cholesterol from the gut into the hemolymph. Thus, lipoproteinI appears to be the major neutral lipid and sterol transport agent in the insects studied and, in addition, plays a
regulatory role in the release of both diglycerides and sterols, lipoprotein II (very high density lipoprotein) may be important in providing protein and lipid to the insect ovary during cogenesis. Ecdysone, the polyhydroxy steroidal insect molting hormone, is probably carried "free" in the hemolymph, although reports exist of specific binding proteins in some species. The other major insect growth hormone, juvenile hormone, is transported by lipoproteins in silkmoths and locusts and by a lower molecular weight protein in the tobacco hornworm (Gilbert and Chino, 1974). Insect lipoproteins are less diverse but more versatile and efficient than their mammalian analogs. Insects, however, appear to rely on a single type of lipoprotein (lipophorin) for most lipid transport. Insect lipophorin seems to be composed of a basic matrix containing two apolipoproteins and a complement of mostly polar lipids, to which an additional apolipoprotein and more lipids can be added as special needs demand. Lipophorin generally functions as a reusable shuttle for lipids, and does not appear to be taken up or degraded during its functioning.

Lipophorin moves digested fat from the gut to tissues for cell membrane construction, to muscle for combustion, or to or from storage sites (Shapiro, et al 1988). It has additional functions in the distribution of hydrocarbons, cholesterol, and carotenoids, and seems to be involved in the distribution of hydrophobic xenobiotics. In some insects, lipophorin appears to be intimately involved in clotting reactions. While lipophorin appears to be common to all insects so far examined, other specialized lipoproteins have also been identified. Foremost among these is the egg yolk protein precursor, vitellogenin which is a VHDL nomenclature used. Lipoproteins are named according to their density class. The larval forms and those found in resting adults are of the high-density class, and are called high density lipophorin or HDLp. The forms that carry large amounts of lipid from the fat body of the adult to flight muscle are of low density and are called low-density lipophorin or LDLp. Lipid transport in insect hemolymph revealed that phospholipids and diacylglycerols are major lipid components. This fact sets the insects apart from mammals, which have high levels of triacylglycerols in the blood while diacylglycerols are minor components. Diacylglycerols are released from the fat body in vitro and associate with protein components of the hemolymph. (Chino, et al 1969) isolated two diacylglycerol rich lipoproteins from Philosamia Cynthia. One of these, a VHDL, was subsequently shown to
be female specific and identical to vitellogenin (Chino, 1976). The other lipoprotein, a HDL, was later given the name lipophorin in recognition of its function as a reusable lipid shuttle vehicle (Chino, et al 1981; Shapiro, et al 1988).

Lipids are a broad group of naturally occurring molecules which includes fats, waxes, sterols, fat-soluble vitamins (such as vitamins A, D, E and K), monoglycerides, diglycerides, phospholipids and others. The main biological functions of lipids include energy storage, as structural components of cell membranes, and as important signaling molecules. In a recent study employing state-of-the-art MS technologies, Ejsing, et al (2009) identified 342 distinct molecular lipids (and quantified 250 of these) in extracts from yeast. Although this number is impressive, it may ultimately reflect only a fraction of the full lipid diversity in these organisms. Lipids may be broadly defined as hydrophobic or amphiphilic small molecules; the amphiphilic nature of some lipids allows them to form structures such as vesicles, liposomes or membranes in an aqueous environment. Biological lipids originate entirely or in part from two distinct types of biochemical subunits or "building blocks": ketoacyl and isoprene groups (Fahy, et al 2005). Lipids also encompass molecules such as fatty acids and their derivatives (including tri-, di-, and monoglycerides and phospholipids), as well as other sterol-containing metabolites such as cholesterol (Michelle, et al 1993).

During the course of insect evolution, lipids have assumed a significant role in facilitating several important morphogenetic and physiological strategies. Sterol glycerides provide a convenient reservoir of metabolic energy for periods of prolonged energy demand. While cuticular lipids are of profound importance in enabling terrestrial insects to resist dessication. Lipids are organic molecules that are insoluble in water. Lipids are neutral fats. Phospholipids and cholesterol include fats, waxes, sterols, fat soluble vitamins A, D, E & K, monoglycerides, diglycerides, phospholipids.

For a very long time, lipids were considered to be intractable and uninteresting oily materials with two main functions – to serve as a source of energy and as the building blocks of membranes. They were not considered to be appropriate candidates for such important molecular tasks as intracellular signaling or local hormonal regulation. In 1929,
George and Mildred Burr demonstrated that linoleic acid was an essential dietary constituent. Bergström, Samuelsson and others in 1964 have discovered that the essential fatty acid arachidonate was the biosynthetic precursor of the prostaglandins with their effects on inflammation and other disease states, the scientific world in general began to realize that lipids were much more interesting than they had previously thought. Lipids are fundamental constituents of all cellular organisms. Lipids diversify in chemical structures that share the common feature of solubility in organic solvents. The discovery of the first biologically active phospholipid, platelet-activating factor has lead for further study. Later, every individual lipid class has been found to have some unique biological role that is distinct from its function as a source of energy or as a simple construction unit of a membrane. The lipids in membranes function in the trafficking of cellular constituents, the regulation of the activities of membrane proteins and signaling was recognized. Lipids are excellent candidates for signaling purposes. Some key lipids are briefly summarized for a general overview.

1.14 Tri-, Di- and Monoacylglycerols

Virtually all the natural fats and oils of commerce consist of triacylglycerols. Triacylglycerols are the storage lipid in animal and plant cells, where they occur as discrete droplets surrounded by a protective monolayer of phospholipids and functional hydrophobic proteins. The other functions of triacylglycerol depots include that subcutaneous depots serve as insulation against cold in many terrestrial animals, as is in the pig, which is surrounded by a layer of fat, and as it is for marine mammals. These lipid depots are less dense than water and so aid buoyancy with the result that less energy is expended in swimming. Triacylglycerols together with the structurally related glyceryl ether diesters and wax esters are the main components of the sonar lens used in echolocation by dolphins and some whales.

(http://lipidlibrary.aocs.org/lipids/whatdo/index.htm)

1.15 Sterols

Cholesterol is a sterol and an ubiquitous component of all animal tissues, much of it is located in the membranes. It occurs in the free form and esterified to long-chain fatty
acids (cholesterol esters) in animal tissues, including the plasma lipoproteins. It is generally believed that the main function of cholesterol is to modulate the fluidity of membranes by interacting with their complex lipid components, specifically the phospholipids such as phosphatidylcholine and sphingomyelin, increasing the degree of order by promoting a 'liquid-ordered phase'. There is also considerable evidence for more intimate protein-cholesterol interactions that may regulate the activities of certain membrane proteins (http://lipidlibrary.aocs.org/lipids/whatdo/index.htm)

1.16 Glycerophospholipids

Phospholipids play multiple roles in cells in addition to establishing permeability barriers, such as providing a matrix for the assembly and function of a wide variety of enzymes, participating in the synthesis of macromolecules, and acting as molecular signals to influence metabolic events. Specific anionic lipids like phosphatidylinositol and its phosphorylated derivatives, which are concentrated on the cytoplasmic leaflet of membranes, exert a control on the properties of the membrane–cytosol interface and consequently on many aspects of membrane trafficking, including vacuole formation transport and fusion. These specific lipids are associated with particular organelles in combination with other signaling molecules they can recruit effector proteins with appropriate functions for each cellular compartment. (http://lipidlibrary.aocs.org/lipids/whatdo/index.htm)

1.17 Phosphatidylcholine

Phosphatidylcholine is an integral component of the lipoproteins in plasma. It serves as a source of diacylglycerols with a signalling function, while the plasmalogen form especially provides arachidonate for eicosanoid production. In addition, phosphatidylcholine is the biosynthetic precursor of sphingomyelin and many other signalling molecules and thus has an influence on innumerable metabolic pathways. (http://lipidlibrary.aocs.org/lipids/whatdo/index.htm)

1.18 Phosphatidylethanolamine

Phosphatidylethanolamine is a major component of membranes, especially in bacteria, with distinctive physical properties because of its small head group and hydrogen
bonding capacity. In the bacterium *E.coli*, it supports active transport by the lactose permease, and other transport systems may require or be stimulated by it. In animal and plants, it acts as a 'chaperone' during the assembly of membrane proteins to guide the folding path for the proteins and to aid in the transition from the cytoplasmic to the membrane environment. (http://lipidlibrary.aocs.org/lipids/whatdo/index.htm)

1.19 Phosphatidylserine

Phosphatidylserine another acid lipid contributes substantially to non-specific electrostatic interactions in the inner leaflet of membranes. This normal distribution is disturbed during platelet activation and in the process of cellular apoptosis when the lipid is transferred from the inner to the outer leaflet of the plasma membrane and acts as a signal to scavenger cells. Phosphatidylserine chelates with calcium to act as the foundation for bone growth. It is also an essential cofactor for the activation of many enzymes, including protein kinase C, which is a key enzyme in signal transduction. (http://lipidlibrary.aocs.org/lipids/whatdo/index.htm)

1.20 Glycosyldiacylglycerol

Glycosyldiacylglycerol have also been found in animal tissues, though usually in rather small amounts, and their role in mammalian membranes is poorly understood. Mono and digalactosyldiacylglycerols and sulfoquinovosyldiacylglycerol are important components of membranes of chloroplasts and related organelles, and are the most abundant lipids in all photosynthetic tissues, including those of higher plants, algae and certain bacteria. When phosphorus is limiting, they may substitute in part for phospholipids. Monogalactosyldiacylglycerols have ability to form inverted micelles important for membrane structure and for interactions with specific proteins. While many different functions have been ascribed to these lipids, it is clear that their primary importance is in their interactions with the photosynthetic apparatus. (http://lipidlibrary.aocs.org/lipids/whatdo/index.htm)

1.21 Sphingolipids

Sphingolipids have an immense range of functions in tissues that are quite distinct from those of the complex glycerolipids. Sphingomyeline
(http://lipidlibrary.aocs.org/Lipids/sph/index.htm) has structural similarities to phosphate dylcholine, but has very different physical and biological properties, while the complex oligoglycosylceramides and gangliosides have no true parallels among the glycerolipids. (http://lipidlibrary.aocs.org/lipids/whatdo/index.htm)

## 1.22 Phospholipids (SM, PC, PE)

Phosphatidylcholine (PC), sphingomyelin (SM) and phosphatidylethanolamine (PE) are easily detectable by MALDI-TOF MS although PE is less sensitively detected in comparison to PC and SM (Petkovic, *et al* 2001). Both, PC and SM possess the choline head group – i.e. a quaternary ammonia group – that leads to a high yield of positive ions (Schiller, *et al* 1999). PC was so far most frequently characterized by MALDI-TOF MS (Schiller and Arnold, 2000; Schiller, *et al* 1999; Asbury, *et al* 1999; Zabrouskov, *et al* 2001; Harvey, 1995) and was one of the first PL studied by MALDI-TOF MS (Marto, 1995). For instance, Harvey investigated in 1995 the use of which matrix compound provides the very best MALDI-TOF mass spectrum of PC. Although a large variety of different compounds was tested, the result was that sinapinic acid, $\infty$-cyano-4-hydroxycinnamic acid and DHB gave the best results. (Schiller, *et al* 2004)

Low attention that MALDI-TOF MS has experienced in the field of lipid analysis is also surprising, if one considers the high reproducibility of this method that is caused by even co-crystallization of the analyte and the matrix. Since both, the lipid and the matrix, are readily soluble in organic solvents, all manipulations can be performed in a single organic phase. This results in extremely homogeneous matrix/analyte co-crystals and an excellent reproducibility in comparison to more polar molecules that require solvent mixtures. MALDI-TOF MS is a fast and convenient method: one sample can be analyzed in less than one minute. MALDI-TOF targets (sample plates) that resemble common microtiter plates and allow the simultaneous (even often automated) analysis of many samples are available from the majority of suppliers (Schiller and Arnold, 2000). MALDI-TOF mass spectrometers are nowadays widespread and available at comparably low prices. Such devices can be used for a number of analytical problems and for a variety of biomolecules ranging from e.g. lipids to proteins and oligosaccharides (Christie, 2003). There is no need to buy an additional device exclusively for lipid analysis. MALDI’s
advantages include its tolerance of impurities, such as buffer salts, and its suitability to the analysis of large sample arrays that arise in biological analysis. An important factor in obtaining reproducible MALDI spectra of lipids is the choice of matrix, the list of matrices used is rapidly expanding (Fuchs and Schiller, 2009; Schiller, et al 2004). The developments in MS-based lipidomics have highlighted the need for appropriate computational bioinformatics approaches. Of particular note is the approach of LIPID MAPS (open access, web-based search engine – http://www.lipidmaps.org) (Fahy, et al 2005; Fahy, et al 2007; Fahy, et al 2009). The coupling of MALDI with IM-MS shows significant promise in separating different biomolecular classes i.e. lipids and peptides in the time domain, which simplifies the analysis of the complex mixture of ions formed from a single laser shot (McLean, et al 2007; Blanksby and Mitchell, 2010).

### 1.23 Complex Lipids in Membranes

Cellular membranes are semi-permeable barriers that enclose and define the cell and its organelles. They control the transport of materials, including signaling molecules, and indeed many reactions occur within membranes, including energy production and biosynthesis of cellular components. In addition, they can deform to enable budding, fission and fusion. It is evident that the specific lipid compositions of membranes have evolved to provide a barrier to the diffusion of ionic solutes and other molecules into cellular compartments where they may not be required. Cellular membranes are the first site for receipt of extracellular signals, they recruit and activate effector molecules, and they are the launch pad for activated effector molecules throughout the cell.

### 1.24 Fatty Acids

Fatty acids are compounds of basic significance in Biology. They play major role in metabolic energy storage, cell and bio-membrane structure and regulate the physiology in most of the organism (Stanley, et al 1988). They represent metabolic energy changes i.e. resulted under a variety of circumstances that have different ecological as well as physiological conditions. Lipid based energy reserves are used to meet the energy requirement of developing eggs of insects that hibernate at one or another stage of development and of locomotory activities. They are also associated with the phospholipids.
and sterolester components of cellular and sub-cellular biomembranes. In most of the insects, triacylglycerol is found to comprise the major compound. The fatty acids serve as compact form of energy storage (Fast, 1970; Downer, 1978; Downer, 1985). These apply broadly to animal cells and have received considerable attention (Gilbert, 1967; Bergsson, et al 1998; Guarnieri and Johnson, 1970; Kabara, et al 1972; Mead, et al 1986). In most of insects, the largest component of fatty acids is associated with triacylglycerol. It is to be noticed that any given insect have a particular fatty acid pattern. Fatty acid composition may be an instantaneous observation of the continuing dynamic process. Certain fatty acids are carried over from the mother by conservation within the egg. Once the feeding begins dietary fatty acids can be absorbed and incorporated without modification into the body tissue. These are synthesized from sugars and certain amino acids and also modified by number of enzyme systems. Fatty acids are one of the defining constituents of lipids and are in large part responsible for the distinctive physical and metabolic properties of the latter. However, they are also important in non-esterified form, i.e. as free (unesterified) fatty acids. They are released from triacylglycerols during fasting to provide a source of energy and of structural components for cells, where they are of course of vital importance. However, it has become evident that there are a number of more dynamic functions of fatty acids, which are attracting great interest. It has long been known that linoleic and linolenic acids are essential fatty acids, in that they cannot be synthesised by animals and must come from plants via the diet. Triacylglycerols are the primary storage form of long-chain fatty acids for energy and structural purposes, and free acids can be mobilized quickly when required for transport in an appropriate form to the heart, liver and other tissues where they can be oxidized. Polyunsaturated fatty acids are important constituents of the phospholipids, where they appear to confer distinctive properties to the membranes, in particular by decreasing their rigidity. The presence of saturated and monoenoic acids ensure that there is a correct balance between rigidity and flexibility.

The essential fatty acids, linoleic and linolenic acids and their longer-chain polyunsaturated metabolites, such as arachidonic acid, can be found in most lipid classes, but they are also the precursors of many different types of eicosanoids, including the hydroxyeicosatetraenes, prostanoids (prostaglandins, thromboxanes and prostacyclins),
leukotrienes (and lipoxins) and resolvins, isoprostanes, which are formed by non-enzymic means. The eicosanoids are highly potent at nanomolar concentrations in the regulation of innumerable biological activities, especially in relation to inflammatory responses, pain and fever. Fatty acids are also the biosynthetic precursors of many insect pheromones and of secondary metabolites in plants. Within cells, fatty acids can act to amplify or otherwise modify signals to influence the activities of such enzymes as protein kinases, phospholipases, and many more. They are involved in regulating gene expression, mainly targeting genes that encode proteins with roles in fatty acid transport or metabolism via effects on transcription factors, i.e. peroxisome proliferator-activated receptors (PPARs) in the nuclei of cells. Such effects can be highly specific to particular fatty acids. Thus, unesterified arachidonic acid may have some biological importance *per se* as part of the mechanism by which apoptosis (programmed cell death) is regulated.

**C₁₂ (lauric acid)** is the most inhibitory saturated fatty acid against gram-positive organisms. **Monoenoic acid** (C₁₈:₁) was more inhibitory than saturated fatty acid, but was less active than dienoic derivatives (C₁₈:₂). Other unsaturated compounds were less active than C₁₈:₂. (Alfin-Slater and Aftergood, 1971). The high levels of activity of lauric acid (C₁₂:₀), capric acid (C₁₀:₀), and particularly that of monocaprin (C₁₀:₀) are notable and suggest that these lipids have specific anti-chlamydial effects (Bailey, 1975).

Fatty acids have diverse roles in all cells. They are important as a source of energy, as structural components of cell membranes, as signaling molecules and as precursors for the synthesis of eicosanoids. Recent research has suggested that the organization of fatty acids into distinct cellular pools has a particularly important role in cells of the immune system and that forms of lipid trafficking exist, the nature and regulation of cellular lipid pools in the immune system, their delivery of fatty acids or fatty acid derivatives to specific locations and their potential role in health and disease. Fatty acids act as gatekeepers of immune cell regulation in the sense that their location and organization within cellular lipids have a direct influence on the behavior of several proteins involved in immune cell activation. It is proposed that modification of the fatty acid composition of lipid domains, and in particular, replacement of n-6 PUFA by n-3 PUFA, could alter immune cell activation. Although the composition of lipid domains within cells of the
immune system can be modified through diet, the functional implications of such modifications are unclear (Bligh and Dyer, 1959). Within the immune system, different fatty acids act by differentially influencing cell membrane structure and function, cell signaling and gene expression, and patterns of lipid mediator production (Dadd, 1983).

Fatty acids are subject to many enzymatic processes. They are synthesized from acetate unit and oxidized in energy production. Regulation of fatty acid metabolism taken in a very broad sense represents frontier in studies of insect lipid biochemistry. Fatty acid synthesis during various stages of environment parameters such as injury, heat, and/or infection shows special fatty acid mobilization in all biochemical regulations. Elongation, desaturated, chain shortened incorporated into various lipid moieties. This structural alteration may simultaneously modify fatty acids and carry them from one area of biological significance into another. The elongation of fatty acids in formation of waxes, the reduction to alcohol, the chain shortened by single carbon form to hydrocarbon form, introduction of a second double bond in denovo biosynthesis of polyunsaturated fatty acids and formation of pheromone components from fatty acids. Thompson (1973) indicated that the fatty acid compositions of all insect orders were fairly similar in a qualitative way. The profile seem to include about 8 components. Most of them are saturated (mono saturated) fatty acids with two of polyunsaturated derivatives. Fatty Acid Identification has been carried out by GC-MS analysis.

1.25 Objectives of the study

Various studies conducted on Diptera, mostly Drosophila Melangoaster, inspired me to carry out the study of housefly larvae extract of native, injury and infected with micro organism. Housefly Larvae (HOUSEFLY LARVAE) were selected as a first candidate in this experiment. HF pupae were not selected as our first candidate, due to the reason, that there is a loss of biomass in pupal development. Pupae are about half weight of mature maggots and the larger amount of chitinous exoskeleton in the adult may reduce nutrient availability (Zhang, 2005). Another reason for selecting larvae is that Housefly Larvae are exposed to more than 100 types of pathogens.
In this study, I have focused on antimicrobial and anti-tumor activity of housefly larvae extracts. My concerns have been, what could be the diversity of antimicrobial and anti-tumor lipids, fatty acids, proteins/peptides in the housefly larvae, and what is the approach that I should take to identify them in various challenged conditions. I have taken to test the antimicrobial activity against gram positive and gram negative bacteria, of injured and bacterial challenged and unchallenged housefly larvae extract. I have studied antimicrobial activity, anti tumor activity, Hemolytic activity, lipid isolation and characterization on MALDI TOF/MS, Fatty acid analysis on GCMS and Protein isolation and identification on MALDI TOF/MS.