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# DIFFERENTIAL FATTY ACID EXPRESSION IN NATIVE, INJURED AND INFECTED HOUSEFLY - MUSCA DOMESTICA L., LARVAE

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**ABSTRACT**

Housefly lays eggs on moist matter their larvae consume dead flesh to survive and grow into pupal stage. Adult houseflies feed and breed on unsanitary areas. Thus at every stage through their survival they are associated with feces, garbage and unsanitary matter. They are carriers of diseases, yet are resistant to microorganisms. As fatty acids play diverse roles in immune cells and are a source of energy, we have studied the fatty acid profiles of housefly Musca domestica larvae to identify their innate immune response. We have used GCMS analysis to identify differential fatty acid expression in three stages, injured, and infected by gram positive and gram negative bacteria to compare with the fatty acid profile of native housefly larvae. Housefly M.domestica larvae has abundant of phosphatidylethanolamine and phosphatidylcholine. In our study, some fatty acids have over expressed, some have under expression and some fatty acids were newly identified.

**Keywords:** Fatty acids / Differential expression / GCMS analysis / Musca domestica / polyunsaturated fatty acids / wax / pheromones.

## INTRODUCTION

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 [22]. They represent metabolic energy changes that are 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 sterol ester 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 [11]; [2]; [8]: [9]. These apply broadly to animal cells and have received considerable attention [13]; [1]; [6]; [27]; [18].

In most of insects, the largest component of fatty acids is associated with triacylglycerol. In other words, the question may be understood as, why does 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 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, and/or infection shows special

fatty acid mobilization in all biochemical regulations. Elongation, desaturate, 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) [26] indicated that the fatty acid compositions of all insect orders were fairly similar in a qualitative way. The profile seems to include about 8 components. Most of them are saturated (mono saturated) fatty acids with two of polyunsaturated derivatives.

In the present study, housefly larvae were subjected to injury and different infections followed by homogenization, lipid isolation, characterization and comparison of the fatty acid profiles.

## MATERIAL AND METHODS

### Insect rearing and challenged:

Third instar housefly maggots were reared in grape juice agar media containing 0.91% grape juice, 1.76% yeast granule, 2.6% sugar and 2.8% agar. Reared maggots were injured with sterile sharp needle and subjected to infection with various strains of bacteria such as Escherichia coli (MG 1655), Staphylococcus aureus (ATCC 8530), Pseudomonos aeruginosa (NCTC 6751) in LB agar petriplates. The experiment of native maggots was carried out by injuring them with sterile needle and leaving overnight without bacterial culture.

### Lipid Extraction and fatty acids analysis:

These sets of infected and native maggots were homogenized with RB buffer containing 2M thiourea, 7M urea, 4% chaps, 1mM EDTA and 20mM tris. HCl. The homogenized tissues were spun at 15,000 RPM for about 20 minutes at 4˚C and the supernatant was collected to carry out isolation of lipids [3] by extracting total lipids from 1 ml of homogenate and added to the 3 ml of mixture of chloroform and methanol ratio of 2:1, this mixture is vortexes for 5 minutes and incubated at room temperature for 3 hours in a closed glass screw capped tube.

This mixture was spun at 5,000 RPM using table top centrifuge for about 10 minutes. The supernatant was collected into a fresh screw cap tube. The total isolated lipid was dried under the stream of dry nitrogen gas and to the dried sample 1ml of methanol was added followed by 50µl of concentrated HCl. The tubes were mixed and capped properly using Teflon coated caps and incubated at 70C for 3 hrs. Methylated fatty acids were then extracted fromthis mixture by adding n-hexane 2ml for 3 times. Pooled hexane extract was dried under the stream of nitrogen.

Dried fatty acid methyl esters were dissolved in 200 µl of n-hexane, examined by GC-MS (Agilent 5972 MSD coupled to 6890 N GC, USA. Column used: DB 225MS). Fatty acids were identified by comparing with authentic standards run under similar conditions.

## RESULTS

Most of the Diptera are characterized by very high proportion of C16:1 [10]. The enzymology of fatty acid bio-synthesis in the conditions studied so far is capable of producing the particular fatty acids that characterize overall patterns. Hence, we have chosen unusual patterns to argue against a particular representative fatty acid composition of M. domestica. The alteration of whole body profile in response to developmental parameters and possibly with environmental conditions such as temperature or infection would suggest the fatty acid composition as meaning in a physiological context. The physiological process takes place at cellular tissue and organ levels of insect. The fatty acid composition then assumes considerable

significance/importance in these functional levels. The few studies so far recorded have been towards understanding the physiological fatty acid composition that come from specific tissues and not from the whole larvae.

In our study, bio-synthesis of of C16:0, C18:0 and C18:1 fatty acids are synthesized insects. In insects lipogenic enzyme fatty acid synthetase plays central role in lipid bio-synthesis. It has been observed that fatty acid synthetase (FAS) requirement for NADPH for the apparent Michael's constant for these substrates, Acetyl-CoA:7-60µM, Malonyl-CoA:24-123µM, NADPH:9-13 µM. There is a considerable variation in the distribution of chain length components synthesized by FAS in these experiments.

The fatty acid bio-synthesis in native (Graph 1, Table 1 and GC-MS Spectra) C16:0 and C16:1w9c is

considerably high, about 20%, when compared to C18:1 9c, C18:1 11c and C18:1 9,11c whereas when injured and infected with bacteria have drastically reduced to 3%. When injured, 100% over expression of C16:0 has been observed, whereas when infected by gram positive and gram negative bacteria C16:0 has remarkably under expressed. C16:1 w9c has not shown any traces when injured and infected by P. aeruginosa, and under expressed by about 25% when infected by S.aureus. The fatty acid C16:0iso is a newly expressed fatty acid compared to native. It is observed in traces when injured and infected by E.coli and P.aeruginosa but it is found abundantly of 21.45% when infected by S. aureus. Similarly C17:1 anteiso is another newly identified fatty acid when injured and infected by gram positive and gram negative bacteria, traces found when injured and infected by E.coli and P.aeruginosa while it is abundant in S. aureus of 29.18%. C18:0iso is another newly expressed fatty acid when injured and infected by P. aeruginosa, 9.3% and S. aureus, 20.9% but not when infected by E.coli. C18:1 w9c has newly expressed very abundant when injured, 27.32% and when infected by E.coli,19.03% under expressed by about 5% compared to injured, and drastically under expressed by less than 50% when infected by P. aeruginosa and only traces found when infected by S. aureus. C 14:0iso, C15:0iso, C15:0anteiso and C18:0 have newly expressed in all stages of injury and infected by bacteria, but only in traces. C18:1 9c, C18:1 11c and C18:2 9,11c have disappeared in all experimental stages of injury, and infected by bacteria. C14:0 has negligibly over expressed when injured and has under expressed by less than 50% when infected by E.coli and only traces when infected by S. aureus, but interestingly it has raised approximately to its normal levels, 4.42% when infected by P. aeruginosa. These observations are summed up as below.

**Biosynthesis of fatty acids under stress:** C14:0iso, C15:0iso, C15:0anteiso, C16:0iso, C17:1anteiso, C18:0, C18:0iso, C18:1 w9c were found as newly expressed fatty acids which did not exist in native state, out of these C18:1 w9c was found in abundant of 27.32% while others were only in traces. C14:0 did not show any remarkable change, whereas C15:0, C16:1 w9c, C18:1 9c, C18:1 11c and C18:2 9,11c have disappeared. C16:0 has over expressed by more than 100%.

**Biosynthesis of fatty acids when infected by Escherichia coli**: When compared with native housefly larvae, C14:0iso, C15:0iso, C15:0anteiso, C16:0iso, C17:1anteiso, C18:0, C18:1 w9c were found as newly expressed fatty acids which were not existing in native state, out of these C18:1 w9c was abundant of 19.03%, followed by C15:0iso of 5.08%, while others were in traces. C14:0 has declined by 50%, whereas C18:1 9c, C18:1 11c and C18:2 9,11c have disappeared. C16:0 and C16:1 w9c were under expressed.

When compared with injured state, C15:0, C16:1 w9c were newly expressed, C15:0iso was over expressed and C18:1 w9c was under expressed, whereas C18:0iso has disappeared.

**Biosynthesis of fatty acids when infected by Pseudomonas aeruginosa**: When compared with native housefly larvae, C14:0iso, C15:0iso, C15:0anteiso, C16:0iso, C17:1anteiso, C18:0, C18:0iso and C18:1 w9c were found as newly expressed fatty acids which were not existing in native state, out of these C18:1 w9c was abundant of 10.39%, followed by C15:0iso of 3%, while others were in traces. C14:0 has not shown any remarkable change. C15:0, C16:1 w9c, C18:1 9c, C18:1 11c and C18:2 9,11c have disappeared and C16:0 of 13.40%, was under expressed by about 30%.

When compared with injured state, C16:0 and C18:1 w9c were under expressed and C18:0iso was over expressed. No under expressed or newly expressed fatty acids were found.

**Biosynthesis of fatty acids when infected by Staphylococcus aureus:** When compared with native housefly larvae, C14:0iso, C15:0iso, C15:0anteiso, C16:0iso, C17:1anteiso, C18:0, C18:0iso and C18:1 w9c were found as newly expressed fatty acids which did not exist in native state, out of these C16:0iso of 21.45%, C17:1anteiso of 29.18%, C18:0iso of 20.90% were abundant followed by C16:1w9c of 4.39%, while others were in traces. C15:0, C16:1 w9c, C18:1 9c, C18:1 11c and C18:2 9,11c have disappeared and C14:0, C16:0, C16:1 w9c, were remarkably under expressed. When compared with injured state, C14:0, C14:0iso, C15:0iso, C15:0anteiso, C16:0, C18:1w9c were under expressed, out of these C16:0 of 3.10% and C18:1w9c of 2.69 were remarkably under expressed as against 48.7% and 27.32% respectively. C16:0iso, C17:1anteiso and C18iso were remarkably over expressed. C16:1w9c of 4.39% was newly expressed.

**Discussion**

Lipidomics in insects is gaining importance in insects, since lipids are used by these winged animals for various needs like protection from the external environment involved as cellular constituents of the membranes, needed in physiological regulation as hormones and are also useful as pheromones during communication, Thompson, 1973 [26] has revived the fatty acid composition from various insects and observed that the insects generally maintain fatty acid pattern during their development. [5] [24] observed that silkmoth Hyalomorpha cercopia convert various fatty acids like acetate to palmitate, stearate, and oleate. According to [12] lipids are needed for the metabolic needs of many insect species and they are found in fat bodies which act as major metabolic center. They further stated the fact that fat bodies present in haemolymph, the functions of which are transportation to the areas where there is high metabolic demand. Total lipid fatty acid and sterol composition of larva and the adults of the Dipteran insect M.domestica was studied by Stefanov et al., 2002 [23], suggested that the variations in lipid profile after treatment with sucrose observed that lipid profile, variation control the permeability of the cell membrane and may be an adaptive response of the organism to the change in environment.

In the present study after giving the various treatments to the 3rd instar housefly maggots, there significant variations were observed in fatty acid composition. When compared with the native maggots, after injury the first experimental group, it was noticed that in C15:0 (Pentadeconoic acid) as well as the same fatty acid in the second experimental group after infecting with pathogenic microbes showed down regulation in S. aureus and P. aeruginosa . This may be due to protective mechanism of the insect larva [14] while studying the innate immunity of higher insects stated that when insects are infected with microorganisms, the internal

metabolism may be divided towards an interconverting pathway which includes defense manipulation, cascades, a series of proteolytic reactions followed by encapsulation of attacked organism and also phagocytosis. This is finally followed by faster production of antimicrobial peptides by fat bodies [16]. Ozlem Cakmak et al; 2008 [20] studied the phospholipid and triglycerol fatty acid profile of Aelia rostrata chief components were C16 and C18 fatty acids. In addition to these components, they have also observed several odd chains like C13:0, C15:0, and C17:0 and few prostaglandin precursors. They noticed substantial differences in fatty acid composition in different developmental stages. They have further observed percentage of linolic acid increased significantly at the expense of palmitic acid from new generation adult as compared to other nymph stages. They have concluded that temperature and developmental stages seems to play an important role in lipid metabolism. In the present study after exposing the maggots of housefly to an unusual environmental/stress, it was interestingly noted that few fatty acids patterns were expressed in the treated groups. The newly appeared fatty acids are C14:0 iso (Myristate); C15:0 iso (Pentadeconoic acid); C15:0 anteiso(Pentadeconoic acid); C16:0 iso (Palmitate); C17:1 ante iso (Margaric acid); C18:0 (Stearate acid); C18:0 iso (Stearate); and C18:1 w9c (Oleate). In fact these newly formed fatty acids were not observed in native condition. It is due to inter conversions of certain fatty acids groups during abrasive conditions or stress conditions resulted in appearance of new fatty acids.

Insects are susceptible to pathogens /stress and can show resistance and try to resist the infection by modifying their internal physiology, morphology, molecular, genetic mechanism etc. [19]

Stanley and Samuelson et al., (1988) [22] stated fatty acids can act as precursors in biosynthesis of waxes and also act as component of defensive secretions. In the present investigation also few newly formed fatty acids were obtained in the treated groups which were not noticed in the native condition.

A significant change in fatty acid profile was also observed with native maggots after injury. C15:0 was observed up-regulated in E.coli infected group significant down regulation in injury or infected with P. aeruginosa and S. aureus.

After exposing the maggots to an unusual environment/stress, it was interesting to note that few new fatty acid patterns were expressed in the treated groups. The fatty acids like C14:0 iso, C15:0 iso; C15:0 ante iso; C16:0 iso; C17:1 ante iso; C18:0; C18:0 iso and C18:1w9c were not expressed in native condition.

Bridges., 1971,[4] While studying the incorporation of fatty acids into lipids of housefly Musca domestica observed C14, C16 and C18 fatty acids in the phospholipids and triglyceride fractions extracted from housefly larvae reared under aseptic condition under fat free diet. Savoboda et. al. 1991 [25] found out that dealkaylation at C24 is the most common metabolic pathway for the conversion of C28 & C29 dietary sterols in cholesterol.

In the present study fatty acids C18:1w9c was observed in the native but showed regulatory trend in challenged groups, C18:1 9c, C18:1 11C and C18:2 9,11 C were observed in native condition but were not expressed in all the challenged groups. It has been studied by [7] that some members of Orthoptera revealed upon infection fatty acid deficiency by a markedly retarded nymph growth and emergence of deformed adult. With an exception of mosquitoes, PUFA (Poly Unsaturated Fatty Acid) have not been found to be essential for any of Diptera species was found by Farlane in 1983 [17]. These changes in phospholipid fatty acids probably contributed to losing and maintaining the homeoviscosity of the cellular membranes respectively reported by Shin et al 2010 [21].

Considerable variations of fatty acids of challenged insects may having high rigidity and viscosity of cell membrane. So that, they may protect themselves and immune response is mounted against pathogenic and non pathogenic infection.

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**Figure**

Spectra 1- Control (native),Spectra 2- Injured, Spectra 3- E-coli infected, Spectra 4- Staph aureus and Spectra 5- Pseudomonos aeruginosa

Fig:1

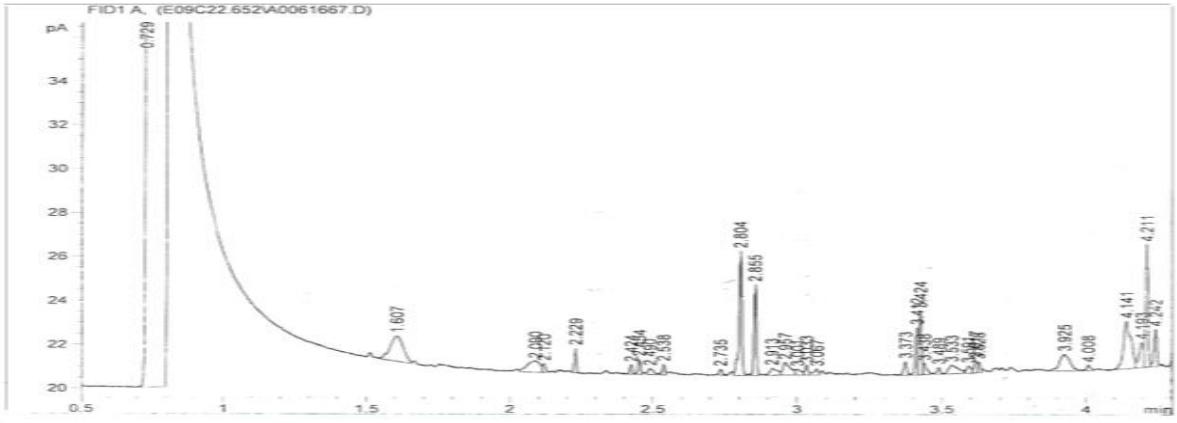



Fig:2

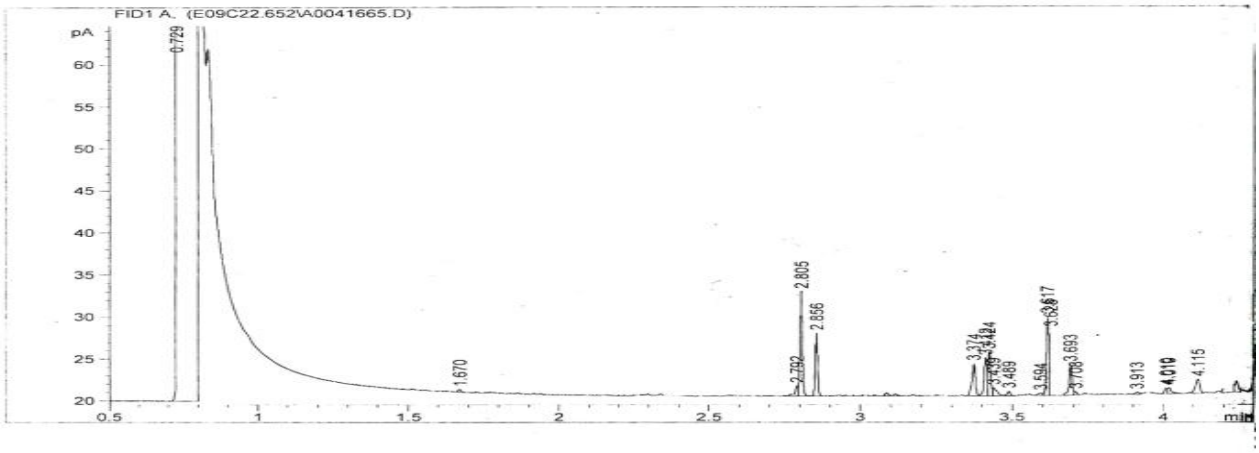


Fig:3

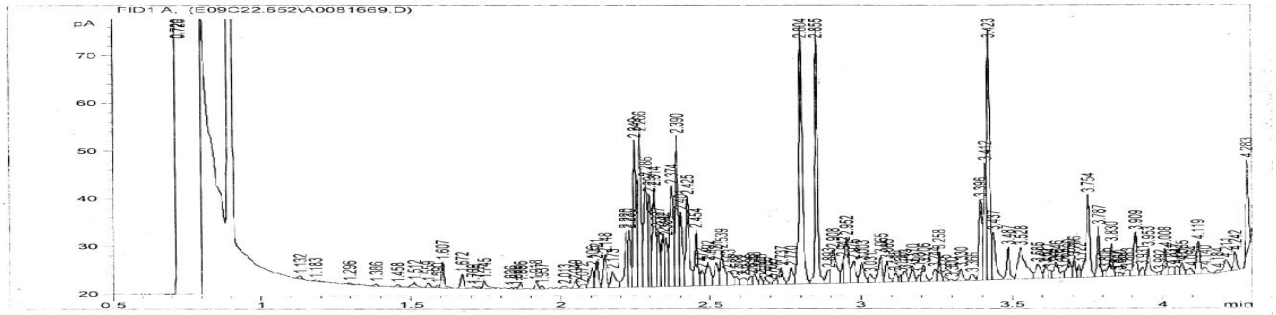



Fig:4

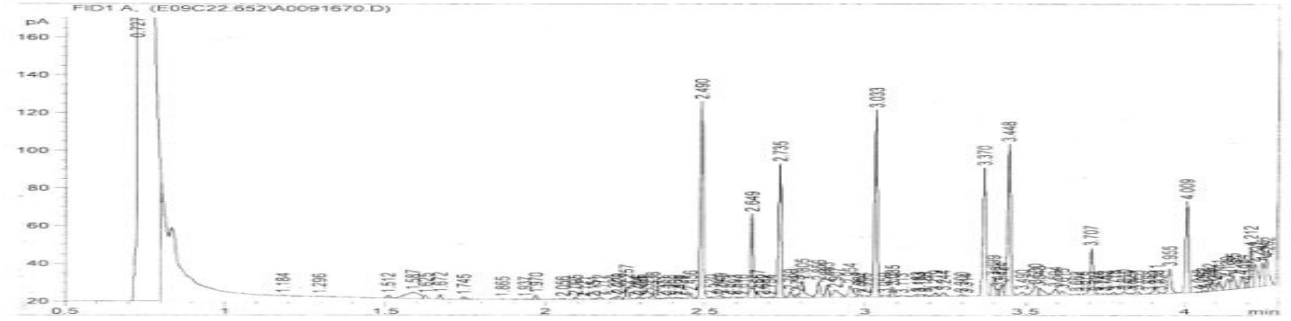
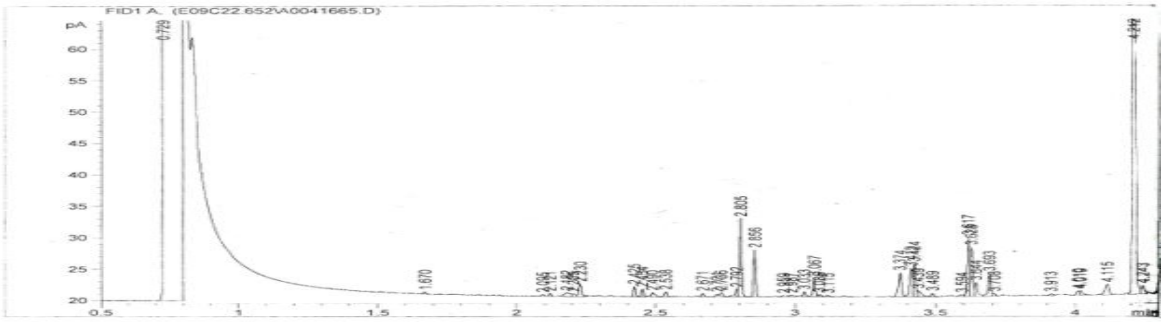



Fig:5




**Table**

**Table 1: Fatty acids of housefly Musca domestica larvae expressed as percentage of fatty acids**

| State | C 14:0 | C14:0iso | C15:0 | C15:0iso | C15:0anteiso | C16:0 | C16:0iso | C16:1w9c | C17:1anteiso | C18:0 | C18:0iso | C18:1w9c | C18:1 9c | C18:1 11c | C18:2 9,11c |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Native | 4.98 | 0 | 7.41 | 0 | 0 | 20.71 | 0 | 21.79 | 0 | 0 | 0 | 0 | 3.75 | 6.82 | 4.1 |
| Injured | 5.00 | 2.53 | 0 | 2.46 | 3.59 | 48.70 | 1.76 | 0 | 2.37 | 1.94 | 3.97 | 27.32 | 0 | 0 | 0 |
| *E.coli* infected | 2.38 | 1.41 | 6.9 | 5.08 | 3.03 | 11.29 | 1.06 | 14.26 (w7c, w 6c) | 0.62 | 1.65 | 0 | 19.03 | 0 | 0 | 0 |
| *Pseudomonas. A* infected | 4.42 | 0.94 | 0 | 3.00 | 2.27 | 13.40 | 1.58 | 0 | 1.75 | 1.19 | 9.30 | 10.39 | 0 | 0 | 0 |
| *S. aereus* infected | 0.60 | 0.10 | 0 | 0.24 | 0.89 | 3.10 | 21.5 | 4.39 | 29.18 | 1.04 | 20.90 | 2.69 | 0 | 0 | 0 |