PHYSIOLOGICAL STUDIES ON THE BIOTHERAPEUTIC USES OF LARVAE OF *LUCILIA SERICATA* ON PIG SKIN MODEL AND THE LARVAE OF *SCARABAEUS SACER*

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A thesis in the Department of HUMAN PHYSIOLOGY, Faculty of Medical Sciences, Submitted to the School of Postgraduate Studies, University of Jos, in partial fulfillment of the requirements for the award of the degree of DOCTOR OF PHILOSOPHY in HUMAN PHYSIOLOGY of the

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CERTIFICATION

This is to certify that the research work for this thesis and subsequent preparation of this thesis by Ahmed Mohammed Sabo (PGMS/UJ/0037/04) were carried out under my supervision.

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DECLARATION

I hereby declare that this work is the product of my own research efforts: undertaken under the supervision of Professor S.O. Odeh and has not been presented elsewhere for the award of a degree or certificate. All sources have been duly distinguished and appropriately acknowledged.

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DEDICATION

This work is dedicated to the following:

Mallam Sabo Muhammad Mustapha Ahmed Maigoro (Father)

Fatima Aliyu (Mother)

Binta H.Ahmed sabo (Wife)

Fatima, Zainab and Bilal Ahmed Sabo (Children)

TABLE OF CONTENTS

TITLE	PAGE
CERTIFICATION - - - - - -	ii
DECLARATION - - - - - -	iii
ACKNOWLEDGEMENTS - - - - -	iv
DEDICATION - - - - - -	v
TABLE OF CONTENTS - - - - -	vi
LIST OF TABLES - - - - - -	x
LIST OF FIGURES - - - - - -	xii
LIST OF PLATES - - - - - -	xiv
ABSTRACT - - - - - - -	xiv

CHAPTER ONE
INTRODUCTION

1.1	BACKGROUND TO THE STUDY - - -	1
1.2	STATEMENT OF THE PROBLEM - - -	3
1.3	GENERAL OBJECTIVES OF THE STUDY -	4
1.4	THE SCOPE OF THE STUDY - - -	5
1.5	SPECIFIC PROPOSALS OF THE WORKING HYPOTHESIS	5
1.6	EVOLUTION OF WOUND CARE MANAGEMENT	7
1.7	MAGGOT DEBRIDEMENT THERAPY (MDT) -	10
1.8	HISTORICAL BACKGRUOND OF MDT - -	11
1.9	MDT CLINICAL TRIALS - - - -	12
1.10	PHYSIOLOGY OF WOUND HEALING - -	22
1.11	WOUND HEALING BY SECONDARY INTENTION (WOUND WITH SEPARATED EDGE) - -	22

1.12 THE MEDICAL ENTOMOLOGY OF MDT - 23

1.13 PROTEOLYTIC AND MECHANICAL DEGRADATION OF NECROTIC TISSUE BY MAGGOTS - - 25

1.14 PROTEOLYTIC ACTIVITY IN MDT - - 25

1.15 ADMINISTRATION OF MAGGOTS ONTO WOUNDS 26

1.16 ANATOMY OF BEETLES - - - - 27

1.17 REPRODUCTION IN BEETLES - - - 29

1.18 MEDICAL ENTOMOLGY OF BEETLES - - 30

CHAPTER TWO
LITERATURE REVIEW

2.1 LITERATURE REVIEW - - - - - 32

2.2 PREPARATION OF MAGGOTS - - - - 35

2.3 IMPLANTATION - - - - - - 35

2.4 ADMINISTRATION OF MAGGOTS UNTO WOUND - 36

2.5 PROTEOLYTIC ENZYMES - - - - 36

2.6 FIBRINOLYTIC ACTIVITY- - - - - 39

2.7 CONTROL OF INFECTIONS - - - - 40

CHAPTER THREE
MATERIAL AND METHODS

3.1 MATERIAL AND METHODS - - - - 45

3.2 EXTRACTION OF LARVAL SECRETION- - - 45

3.3 METHOD OF HARVESTING LARVAL SECRETION AND MEASUREMENTS OF THE PH AND POH - - 46

3.4 QUALITATIVE TEST FOR AMMONIA - - - 47

3.5 QUANTITATIVE DETERMINATION OF PROTEIN - 47

3.6 QUALITATIVE DETERMINATION OF THE PRESENCE OF BLOOD ELEMENT (HAEMOGLOBIN) BY MEDI-TEST STRIP METHOD - - - - - - - 49

3.7 STUDY OF THE ANTIMICROBIAL EFFECTS OF THE EXTRACT OF THE ARTHROPOD LARVAE - - - - 49

CHAPTER FOUR
RESULTS

4.1 RESULTS - - - - - - - 54

4.2 INCREASE pH IN MAGGOT'S MEDIUM (LARVAL SECRETION HARVESTED) WITH TIME - - - 60

4.3 DECREASES IN CALCULATED POH CHANGE IN EFFLUENT FROM MAGGOTS (LARVAL SECRETION) WITH TIME - - - - - - - 62

4.4 INCREASE IN BASE CONCENTRATION IN EFFLUENTS FROM MAGGOTS (LARVAL SECRETION) - - 64

4.5 CHANGES IN PH AND POH IN MAGGOTS MEDIUM (LARVAL SECRETION) WITH TIME - - - 65

4.6 TIME DEPENDENT INCREASE IN HAEMOGLOBIN CONCENTRATION IN EFFLUENTS FROM MAGGOTS' MEDIUM (LARVAL SECRETION - - - - 67

4.7 MAGGOT FACTOR INDUCED PROTEOLYSIS MEASURED AGAINST NATURAL 'DECAY OF PROTEIN ALL AT ROOM TEMPERATURE - - - - - 69

4.8 MAGGOT FACTOR INDUCED PROTEOLYSIS MEASURED AGAINST NATURAL DECAY AT CONTROL TEMPERATURE OF 37°C - - - - - - - 71

4.9 MAGGOT ENHANCED RATE OF PLASMA PROTEIN DEGRADATION (PROTEOLYSIS) - - - - 73

4.10 PRESENCE OF AMMONIA BY QUALITATIVE ANALYSIS IN MAGGOT'S SECRETION - - - 77

4.11 QUALITATIVE TEST SHOWING PRESENCE OF BLOOD ELEMENTS (HAEMOGLOBIN) - - - - 79

4.12 TEST IN HARVEST FROM MAGGOTS GROWN IN THE LABORATORY IN MIXTURE OF WASHED SAND AND GROUNDNUT POWDER - - - - - 79

4.13 pH MEASUREMENT ON CONTROL NORMAL SALINE SAMPLES IN AN ATMOSPHERE OF PUTRID MEAT - 79

4.14 QUALITATIVE ANTIBIOGRAM / ANTIMICROBIAL EFFECT OF MAGGOT SECRETION - - - 79

4.15 UREA- ELECTROLYTE AND ENZYME PROFILE IN MAGGOT'S SECRETION - - - - - 80

CHAPTER FIVE
DISCUSSION, CONCLUSION, SUMMARY AND CONTRIBUTION TO KNOWLEDGE

5.1 DISCUSSION - - - - - - - 97
5.1.1 Growing Maggots in the Laboratory - - - - 97
5.1.2 Rise in pH and Alkalinity in Maggot's Secretion - - 98
5.1.3 Antibiogram of Maggot's Secretion - - - - 99
5.1.4 Haemoglobin in Faecal Grown Maggots - - - 99
5.1.5 Increased Proteolysis by Maggot's Secretion - - - 100
5.1.6 Antinecrosis Effect of Maggots on Pigskin - - - 101

5.2 CONCLUSION - - - - - - 102

5.3 SUMMARY OF FINDINGS - - - - - 103

5.4 CONTRIBUTION TO KNOWLEDGE - - - 104

REFERENCES - - - - - - - 106

APPENDIX - - - - - - - 111

LIST OF TABLES

TABLE		PAGE
1	Changes in mean weight of non-infested pigskin samples with time	54
2	Changes in mean weight of infested pigskin samples with time	57
3	pH change in larval secretion with time - - -	61
4	Change pOH with time in the larval secretion - -	63
5	Changes in Base concentrations with time from larva -	64
6	Changes with time, the pH and pOH in larval secretion -	65
7	Time dependent increase in haemoglobin concentration- -	66
8	Total protein concentration by Biuret method, at room temperature	68
9	Total protein by Biuret method on control and replicate test plasma at 37^0c - - - - - - -	70
10	Total protein Biuret method on replicate test plasma samples at room temperature - - - - - -	72
11	Protein changes in control and plasma test samples at control temperature of 37^0c - - - - - -	73
12	Protein changes in control and test sample of plasma at room temperature - - - - - -	74
13	Plasma protein change in control and plasma test sample. -	75
14	Maggot induced mean plasma protein change at room and control temperature - - - - - - -	76
15	Qualitative Tests on Effluent from Maggots (Larval Secretion)	78
16	Showing Urea- Electrolyte and Enzyme Profile in Maggot's Secretion	81

LIST OF FIGURES

FIGURE		PAGE
1	Change in mean weight of non infested pig skin samples with time	55
2	Changes in mean weight of maggot pig skin samples with time	58
3	Changes in mean weight of non-infested and maggot infested samples	59

LIST OF PLATES

PLATE		PAGE
I	Diagram showing life cycle and metamorphosis in *Phaenicia sericata*	14
II	Picture showing venous ulcer in the leg. Courtesy Dr Ronald Sherman	15
III	Picture showing maggots applied unto chronically infected and necrotic ulcer - - - - - - -	16
IV	Within less than 2 weeks, the dead tissue has been cleaned by the maggots - - - - - - - -	17
V	Photo of leg 3 months later - - - - -	18
VI	Prior to maggot therapy - - - - - -	19
VII	Indeed, his underlying wound was more extensive than could be appreciated by looking at the surface - - - -	20
VIII	His wound is seen here, just about completely covered with healthy skin - - - - - - -	21
IX	Plastic buckets with net covers used in culturing maggots - -	52
X	Histology of Maggot Infested Pig Skin Sample At O Hour -	82
XI	Histology of Non- Infested Pig Skin Sample At 0 Hour - -	83
XII	Histology of Maggot Infested Pig Skin Sample After 48 Hour -	84
XIII	Histology of Non- Infested Pig Skin After 48 Hours - -	85
XIV	Histology of Maggot Infested Pig Skin Sample After 96 Hour -	86
XV	Histology of Non- Infested Pig Skin Sample After 96 Hour -	87
XVI	Histology of Maggot Infested Pig Skin Sample After 144 Hour -	88
XVII	Histology of Non- Infested Pig Skin Sample After 144 Hour -	89
XVIII	Histology of Maggot Infested Pig Skin Sample After 288 Hour -	90
XIX	Histology of Non-Infested Pig Skin Sample After 288 Hours -	91
XX	Histology of Non- Infested Pig Skin Sample Showing Severe Necrosis After 336 Hours - - - - - -	92

XXI Maggot Infested-Pig Skin Samples Labelled With Different Coloured Rubber Bands - - - - - - 93

XXII Maggot-Infested Pig Skin Samples Lebelled With Different Coloured Rubber Bands - - - - - - 94

XXIII Picture of larvae of *Scarabaeus sacer* (Dung Beetle larvae) - 95

ABSTRACT

Wound healing is a normal Physiological process and its failure is what is considered a disease. Bacterial infection and the presence of necrotic tissue within a wound grossly impair its normal healing. Infestation of non-healing wounds by maggots of the species of fly Phaenicia sericata has been known for ages to be beneficial to wound healing. The larvae of dung beetle, *Scarabaeus sacer* (Grub/scarab) are consumed by a lot of communities all over the world. Their nutritional value has suggested possible therapeutic uses. The aim of the study was to develop a modified method of growing the maggots in a controlled laboratory conditions and study their secretion, chemical components and effect on experimental pigskin. The Naturally obtained grubs were analyzed for their nutritional contents and possible therapeutic uses. A piece of putrid meat with wide surface area was exposed to the atmosphere to attract wild flies that then deposited their eggs. The eggs were allowed to hatch in a dark moist condition on washed sharp sand mixed with groundnut powder. Three day old larvae were chemotactically stimulated by exposure to smell of another putrid meat. The five different groups of maggots each sixty in number were washed with normal saline after every thirty minutes and the pH of the effluents measured, corresponding to larval secretion with no contaminants. Qualitative antibiogram showed significant zones of inhibition in *Klebsiella* spp, *Pseudomonas aeroginosa* and *Candida albican* and not *E.coli* cultures. Two groups of test and control sample pigskin each containing ten specimen were allowed to decompose with no maggot infestation and with maggot infestation respectively. The weight of the specimen and a replicate experiment for histology were conducted. Results showed an increasing alkalinity in maggots' secretion that tested positive for ammonia and also less acanthloysis in pigskin

specimens that had been infested with maggots. The rich calcium 51.2 %, protein 28.5 %, carbohydrate 0.3%, fat 7.1% and Iron 12.8% contents were shown in the proximate analysis of dried sample of the Scarab which suggest use to avert or treat medical condition associated with deficiency of calcium, iron and protein.

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Abstract	Achieving the universal objective of a rights-based approach to educatio towards addressing the problem of out of school children require nations to ado appropriate legislative measures coupled with an effective machinery for i enforcement. The need to eliminate all obstacles that stand in the way towar realising the existence of the right of a child to education and the enjoyme thereof must also be adequately addressed. Disparities within national boundari necessitate diverse approaches. The research work has therefore examined th need for an agenda for the promotion and protection of the right of the child education in Borno State of Nigeria. It examined the culture of the people, th historical development of education in the state with vivid statistical evidence, th general nature, form and content of child's right as well as the historical evolutio of the social, economic and cultural rights to education. The work identified an evaluated the domestic, international and regional legal and institution framework for the promotion and enforcement of child's right to education Nigeria and Borno State and the various impediments to the realization of th child's right. The essence of the analysis is to bring out the relevance and th adequacy of existing legal instruments and institutions designed to promote an protect the right of the child to education and the work has made a strong case f reform. The thesis adopted the doctrinal method of research by examining number of domestic, regional and international instruments, reviewing textbooks, journals, government publications and documents, statistical dat reports obtained from specialised agencies. In addition, analysis of judici authorities relevant to the research was undertaken. Findings have shown th effective promotion and protection of the child's right to education is the mo critical factor in a child's overall wellbeing and development. Though there is place is host of legal instruments and institutions at the international, regional an domestic level to provide the required backing for the protection of the child, th non-adoption and incorporation of these legal instruments into domestic la

	non-adoption of the Child's Rights Law in Borno State, non-justiciability of th constitutional provision in relation to child's rights to education in Nigeria an absence of effective enforcement mechanism of the available State Educatio Laws pertaining to the right of the child have rendered ineffective the nob objective of promoting and protecting the right of the child to education in Born state of Nigeria. The implication of this is that there is a strong need to take som revolutionary steps towards addressing the situation. It is imperative, therefore, make justiciable in the constitution, child's right to education, pass into Law Borno State the Child's Right Bill to set the basic minimum standard for th promotion and enforcement of the child's right to education in the state; put place appropriate institutions for the enforcement of the right of the child Borno State.
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CHAPTER ONE
INTRODUCTION

1.1 BACKGROUND TO THE STUDY

The role of antibiotics in modern day practice cannot be over emphasized (Marples and Reith 1992). Bacterial infections complicate both surgical and non-surgical wounds. It is thus not a surprise that one important preoccupation of wound care specialist is control of infection along side the removal of necrotic debris that may impair healing. Surgical debridement of wound is thus a common item on the operation list of many Hospitals's Department of Surgery. In removing necrotic tissue, the Surgeon entirely depends on the sense of seeing in a rather subjective manner to decide which tissue is necrotic and which is not. The efficiency of such is highly debatable. Is there a natural phenomenon that has evolved and could be of higher precision than the Human sense which can be exploited in debridement? The answer is yes. Maggots have historically had role in management of wounds.

The use of maggots in cleansing wound is one of most popular aspect of Biotherapy. The larvae (Maggots) of some specie of fly are long known to exert some beneficial effects on chronic, infected and often necrotic wounds (Stoddard, Sherman, Mason and Pelsang, 1995). The benefits are due to a number of factors that include antimicrobial effect of the maggot secretion on the wound as well as the break down of the structural protein in the necrotic tissue leading to debridement. The antibacterial effect of maggots' factor is a result of an evolutionary adaptation to wipe off competition by bacteria in a maggots infested environment including wounds of Human Being. This adaptation is no different from the process of antibiosis, a phenomenon in Fungus that lead to production of a Biomolecules called antibiotics

that kill bacteria to wipe off competition in the fungal colony, according to Iqbal, Craig and Brady (2014). This is exploited in Modern therapeutics by administration to patients of these relatively harmless antibiotics to kill invading bacteria. Hence the discovery and use of antibiotics may as well be grouped under Biotherapy. Biotherapy by definition is the use of Biological material for the diagnosis and treatment of diseases. Manipulation of Biological phenomena has now advanced to molecular level where transfections of certain host organisms using plasmids or retroviruses to insert genes for certain desirable biomolecule to biosynthetically obtain the useful material for use by Humans, Brown and Terry (2006). This has given birth to modern recombinant DNA technology in the field of Biotechnology.

To appreciate better the concept of maggot debridement therapy (MDT), a Biosurgery option, it is important to review the problems of non-healing wounds due to the presence of necrotic debris and infection and in the light of the history of the efforts to tackle these problems. The need for proper management of wound to effect speedy healing has been in the mind of physicians since the time of Galen in ancient Egypt. Before the advent of antibiotics, voraciously infected wound in a significant percentage of patients is synonymous with death warrant, as the overwhelming sepsis results in systems failure and the demise of the patient. This explains why the 1928 discovery of penicillin by Sir Alexander Fleming in England and the subsequent effort by Dr Howard Florey, a Scottish pathologist, and his team to extract purify and gets penicillin on clinical trial, is seen as a historical breakthrough in medicine, Hugh TB (2002). The discovery of penicillin, the so-called “magic bullet” revolutionized the practice of medicine, as the outcome becomes better of an otherwise fatally infected wounds of injured allied force soldiers from the battlefield of the second world war

from 1939 to 1945. The process of wound healing is an orderly physiological process, which includes inflammatory changes, chemical mediators of inflammation, polymorphonuclear cell infiltration and reconstruction of damaged tissue among others.

Cushing and Philips (2013) affirmed that surgical debridement of wound and the use of antibiotics were chiefly used to offset necrotic tissues impeding wound healing and for radical treatment and/ or prophylaxis against invading bacteria respectively.

Dung beetle larvae (*Scarabaeus sacer*) are used by a number of communities in Africa and especially in Nigeria, as medicinal product for jaundice and fever and as food by many communities all over the world. A study in the nutritional content and what possible use they may be in disease prevention as intervention for nutritional deficiency are of importance.

1.2 STATEMENT OF THE PROBLEM

The use of the larvae of *Lucilia sericata* for debridement of wound is an age long and widely acceptable option for the debridement of non-healing chronically infected wound. But the growth of these maggots in a control laboratory condition present some challenges. Sherman and Wyle (1996) described an elaborate method of growing maggots in the laboratory using saw-dust and honey. The high cost of honey in Nigeria means difficulties in cultivation of large quantity of maggots for use in research and for treatments in hospitals. This raises the critical question on the exploration of an alternative method or an appropriate modification of Sherman and Wyle’s method that can be cost effective.

The secretion from the maggots has to be harvested with no contamination for any informative biochemical analysis to be conducted. This has to involve the careful partitioning of the group of maggots from the offensive smelling putrid meat serving as stimulants for the chemotaxis dependent release of maggots' saprophagous secretion. Not very much is known about the composition of maggots' secretion and thus the study on the biochemical nature of the secretion is of paramount importance.

Inflammatory process after injury that results in a wound formation has the outpouring of protein rich exudates as a sign, Chang and Gabison (2001). The soluble proteins of plasma constitute the source of the protein in the exudates. In the process of healing of the wound the plasma proteins need to be cleared along with the structural protein of any necrotic tissue present. Proteolysis of plasma protein is thus part of the mechanism of wound healing. How much of it can be enhanced by maggots' secretion remains to be seen and this study has some relevance to that.

Ordinarily, it may be assumed that non-healing chronically infected wounds undergoing treatment using maggots may have faster liquefaction than non-infested wounds. But the possible massive breakdown of tissue due to the presence of bacteria has made such hypothesis to be questioned subject to real experiments that compare the two.

The larvae of dung beetle, *Scarabaeus sacer* (Grub/scarab) are consumed by a lot of communities all over the world. Their nutritional value has suggested possible therapeutic uses.

1.3 GENERAL OBJECTIVES OF THE STUDY

The general objectives of this study are:

1 To develop a laboratory method of growing the larvae of *Luclia sericata*. This would pave the way for the harvesting of the secretion from these saprophagous maggots.

2 To device a simple way of harvesting the secretion from the maggots.

3 To study the biochemical properties of the maggots' secretion both qualitatively and quantitatively.

4 To study the effects of maggots on experimental pigskin model. The weight and histological changes in biopsy samples would be monitored.

5 To study the possibility of growing the larvae of *Scarabaeus sacer* (Scarab/Grub) in the laboratory.

6 To study the nutrient content of the Scarab

1.4 THE SCOPE OF THE STUDY

The scope of the research spanned the growth of the maggots in the laboratory, harvesting of the secretion from the larvae and undertaking in vitro analyses of its components both qualitatively and quantitatively. The qualitative analyses were largely dependent on the facilities available and accessible. The best possible outcome was to be obtained within the allocated resources. High performance liquid chromatography machine and facilities for the assays of purine and pyrimidine bases would have been useful but resources certainly constituted a stumbling block.

1.5 SPECIFIC PROPOSALS OF THE WORKING HYPOTHESIS

1) To use putrid meat to attract wild flies that would deposit their eggs. The eggs are to be hatched in a newly developed culture medium consisting of washed sharp sand and groundnut powder. With the moist putrid meat on top of the

half bucketful of sharp sand and a net cover the plastic bucket, the eggs are expected to hatch and migrate to the moist sharp sand.

2) At the end of metamorphosis and from the stock of the adult flies, the appropriate species of *Lucilia sericata* is selected and another piece of putrid meat introduced to the group in captivity (trapped by net over the bucket). The eggs laid are allowed to hatch too and the larvae are definitely of the selected 2nd generation parent stock.

3) To use the principle of chemotaxis and stimulate the larvae feeding frenzy by saturating their local atmosphere with the smell of putrid meat in an improvised plastic jar. Half hourly washed off effluent with normal saline would provide a rich maggots' secretion.

4) pH measurement of the larval secretion are to be conducted. A Null hypothesis proposing that there is no significant variation and correlation of pH with time is made and subjected to statistically analysis. Correlation analysis and ANOVA test are to be used. The Null hypothesis is to be rejected when $p<0.05$ and to be accepted when $p<0.05$.

5) The weights of individual specimen of pigskin from two different groups of maggots' infested and non-infested groups undergoing necrosis are measured forty eight hourly (two days). A Null hypothesis supposes there is no significant change in weight of sample pigskin with time. The Null hypothesis is to be rejected when $p<0.05$ and to be accepted when $p<0.05$.

6) To test the effect of the larval secretion on plasma protein samples by mixing the two and measuring the change in specific gravity. The measured specific gravity of plasma is to be used for estimating the plasma protein concentration

by More and Van Slyke's method. Null hypothesis is proposed stating that there is no significant change in plasma protein concentration with time in the test group incubated with larval secretion and in a control group without the larval secretion. Five replicates are used and the whole series of tests are to be repeated under room temperature and then at control temperature of $37^{o}C$. The Null hypothesis is to be rejected when $p<0.05$ and to be accepted when $p<0.05$.

7) To conduct qualitative and quantitative tests for Na^+, K^+, $HCO3^-$, $Cl^-$and the enzyme amylase, and metabolite like creatinine, protein and bilirubin. A Null hypothesis proposes that there is no difference in concentration of these with time. The Null hypothesis is to be rejected when $p<0.05$ and to be accepted when $p<0.05$.

8) To test for the presence of blood products in larvae grown in decomposing tissue mixed faeces using Medi-test Combi 9 kit.

1.6 EVOLUTION OF WOUND CARE MANAGEMENT

The role of antibiotics in modern day practice cannot be overemphasized. Bacterial infections complicate both surgical and non-surgical wounds. It is thus not a surprise that one important preoccupation of wound care specialist is control of infection along side the removal of necrotic debris that may impair healing. Surgical debridement of wound is thus a common item on the operation list of many Hospitals' Department of Surgery (Cushing and Philips, 2013). In removing necrotic tissue, the Surgeon entirely depends on the sense of seeing in a rather subjective manner to decide which tissue is necrotic and which is not. The efficiency of such is highly debatable. Is there a natural phenomenon that has evolved and could be of higher

precision than the Human sense which can be exploited in debridement? The answer is yes. Maggots have historically had role in management of wounds.

Jaundice is the yellowish discolouration of especially the Sclera of the eye due to staining of elastic/connective tissue of the body by elevated blood level of bilirubin is a major concern in neonatology. Jaundice, is a sign of a number of disease entities rather than a disease itself. Among the aetiologic factors in jaundice are neonatal jaundice (jaundice of immaturity) due to immaturity of the liver. It is important to clear bilirubin and to prevent damage to the brain of the newborn. The poorly developed blood-brain barrier may not prevent bilirubin staining the basal ganglia and causing brain damage (Kernecterus). Supporting the liver by stimulating macrosomal enzymes is of importance.

Viral hepatitis especially Hepatitis B virus infection (serum hepatitis) is considered a very infectious and quite a deadly disease. One of the signs of hepatitis B is jaundice. Often the cytotoxic drugs used to reduce the viral load are themselves toxic and side effects may be overwhelming. The search for a safer Biomolecule, capable of helping the liver by minimizing damage and promoting functions as well as reducing the viral load, would seem a good idea.

Damage to the parenchymal cells of the liver (hepatocyte) may be a product of chronic ingestion of high level of alcoholic drink (ethanol) which has to be metabolized by alcohol dehydrogenase enzyme to the excretable metabolites acetaldehyde and then acetate all in the liver in an overwhelming manner. The search for Biomolecules, capable of inducing macrosomal enzymes of the liver and promoting its efficiency in clearing Bilirubin and other ingested materials like alcohol that may harm the liver, is an ongoing task in biomedical research.

Dung beetle larvae (*Scarabaeus sacer*) are used by a number of communities in Africa and especially in Nigeria, as medicinal product for jaundice and fever and as additive in alcoholic beverage by some communities in Niger Delta for "for health promotion".

The above paragraphs thus introduce us to the subject of Biotherapy. The larvae (Maggot) of some species of flies are long known to exert some beneficial effects on chronic, infected and often necrotic wounds. The benefits are due to a number of factors that include antimicrobial effect of the maggot secretion on the wound as well as the break down of the structural protein in the necrotic tissue leading to debridement. The antibacterial effect of maggot factor is a result of an evolutionary adaptation to wipe off competition by bacteria in maggot infested environments including wounds of Human Beings. This adaptation is not different from the process of antibiosis; a phenomenon in Fungus that leads to production of a Biomolecules called antibiotics that kill bacteria to wipe off competition by the bacteria in the fungal colony. This is exploited in Modern therapeutics by administration to patients of these relatively harmless antibiotics to kill invading bacteria. Hence the discovery and use of antibiotics may as well be grouped under Biotherapy. Biotherapy by definition is the use of Biological material for the diagnosis and treatment of diseases. Manipulation of Biological phenomena has now advanced to molecular level where transfections of certain host organisms using plasmids or retroviruses to insert genes for certain desirable biomolecule to biosynthetically obtain the useful material for use by Humans. This has given birth to modern recombinant DNA technology in the field of Biotechnology.

To appreciate better the concept of maggot debridement therapy (MDT), one of the Biosurgeries under Biotherapy, it is important to review the problems of non-healing wounds due to the presence of necrotic debris and infection and in the light of the history of the efforts to tackle these problems. The need for proper management of wound to effect speedy healing has been in the mind of physicians since the time of Galen in ancient Egypt. Before the advent of antibiotics, voraciously infected wounds in a significant percentage of patients was synonymous with death warrant, as the overwhelming sepsis resulted in systems failure and the demise of the patient. This explains why the 1928 discovery of penicillin by Sir Alexander Fleming in England (Zakeri B. and Luk TK, 2003) and the subsequent effort by Dr Howard Florey, a Scottish pathologist, and his team to extract purify and get penicillin on clinical trial, is seen as a historical breakthrough in medicine Hugh TB (2002). The discovery of penicillin, the so-called “magic bullet” revolutionized the practice of medicine, as the outcome became better for otherwise fatally infected wounds of injured allied force soldiers from the battlefield of the second world war from 1939 to 1945. The process of wound healing is an orderly physiological process, which includes inflammatory changes, chemical mediators of inflammation, polymorphonuclear cell infiltration and reconstruction of damaged tissue among others.

Surgical debridement of wound and the use of antibiotics were chiefly used to offset necrotic tissues impeding wound healing and for radical treatment and/ or prophylaxis against invading bacteria respectively.

1.7 MAGGOT DEBRIDEMENT THERAPY (MDT)

To most people, maggot debridement therapy is the medical use of live maggots (fly larvae) for cleaning wound (Sherman and Pectar, 1988). Stedman’s

medical dictionary states that maggot therapy is ‘’an obsolete ‘’ therapy of wound debridement and removal of abscessed tissue by use of sterilized maggots. With current and abundant literatures on the re-emergence and success of MDT, it leaves no one in doubt that the word “obsolete” in reference to MDT does not fit.

1.8 HISTORICAL BACKGRUOND OF MDT

The medical benefits of maggots have been historically documented where military surgeons noted that soldiers whose wounds became infested with maggots did better and had a much lower mortality rate than did soldiers with similar but non-infested wound (Le Clerg 1990, Sherman and Hall et al 2000). This observation was also made earlier during Napoleonic era when injuries sustained on the battlefield seemed to obey similar order.

William Baer at Johns Hopkins University Baltimore Maryland was the first physician in the United States to actively promote maggot therapy and his results published posthumously by his colleagues in 1932.

MDT was successfully and routinely performed in over 300 hospitals until the mid-1940’S when its use was supplanted by the techniques that were believed to be superior to MDT. Maggot therapy was occasionally used during the 1970’s and 1980’s when antibiotics, surgery and other modalities of modern medicine failed. In 1989, Physicians at the veteran affairs medical centre in long Beech argued that if maggot therapy was effective enough to treat patients who otherwise would have lost limbs despite modern surgical and antibiotics treatments, then we should be using maggots therapy before the wounds progress that far and not only as a last resort. This signalled a comeback for maggot therapy especially in the treatments of chronic wounds infected with antibiotic resistant bacteria (Wolf and Hanson1999).

1.9 MDT CLINICAL TRIALS

Clinical studies, which began in 1989 at Veteran's Affair Medical Centre and the University of California, are underway. Results thus far demonstrate that maggot therapy is more efficient at debridement (cleaning) infected and gangrenous wounds than any other non-surgical treatment prescribed by the hospital wound care team. Additionally, wounds treated with MDT healed more quickly by (several folds) than they had been healing prior to initiating MDT.

In June 2000 Ronald Sherman of the Department of Pathology University of California Irvine, presented the preliminary results of his prospective trials of conventional wound care followed by maggot therapy at a wound healing society symposium in Toronto, Canada. 43 maggot treated wounds were debrided faster and more completely than they had been during conventional treatment. The impressive results suggested that maggots can provide a cost-effective alternative to conventional treatments for necrotic venous ulcer, which involve considerable nursing time and expenses. In a randomized trial on 12 patients with sloughing venous ulcer, Michael Walker (West Cumberland Hospital White Haven in U.K) found that maggot therapy debride ulcers more quickly and effectively than standard hydrogen dressing. Patients treated with maggot therapy had their ulcers successfully debrided with a single application whereas two of the six hydrogen treated patients still needed dressing a month later. Walker calculated that, taking nursing time into account, maggot therapy cost a little more than half as much as hydrogel (UK ₤78 VS ₤136).

Although trial was small and non-masked, Walker was hugely impressed by the result. The patients happily accepted maggot therapy "although a few reported nipping sensation". People with diabetes are among those that will benefit most from

Maggot Debridement Therapy. Certain pathology of diabetes such as restricted circulation that can lead to gangrene in extremities as well as neuropathy (nerve damage) can result in the dead flesh that maggot therapy is used to remove.

The most common problems seen in diabetic foot are the direct results of anaesthesia and trauma of the affected area and these two are the reflection of vasculopathy and neuropathy. A second problem people with diabetes have is impaired wound healing and often is going to be a site where infection can occur and reoccur. In almost all the cases, Maggots have been able to clean the wounds without damaging underlying healthy tissues; the most positive aspect of maggot therapy. In some cases of surgical debridements there are fears of making wounds bigger by extensive removal of even healthy tissues in the process which result in bigger and even more complicated Wounds (Mumacuoglu et al, 1998).

(maggots) are the larvae of
Phaenicia sericata

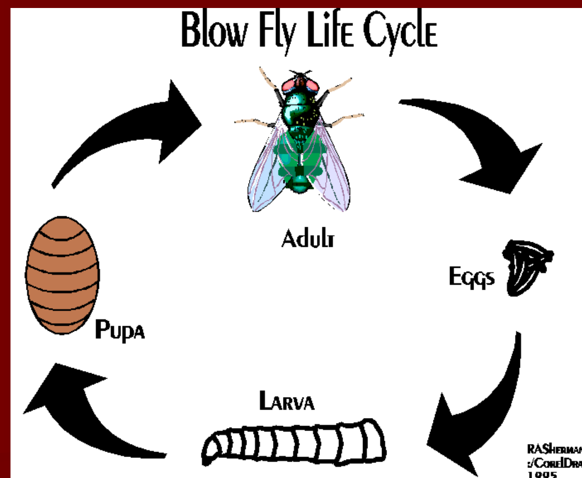


Plate I: Diagram showing life cycle and metamorphosis in *Lucilia sericata*

A 60 year-old man with diabetes and recurrent venous stasis ulcers was admitted to the hospital for worsening leg ulcers, despite outpatient care and antibiotics.

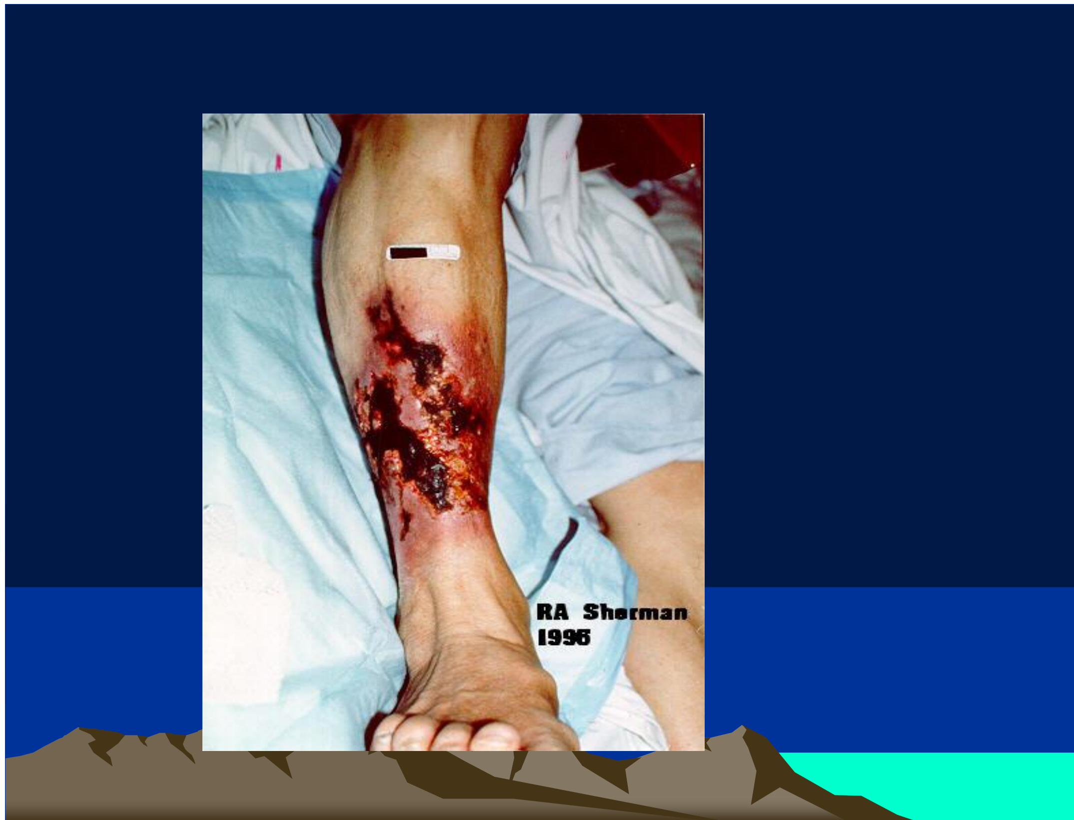
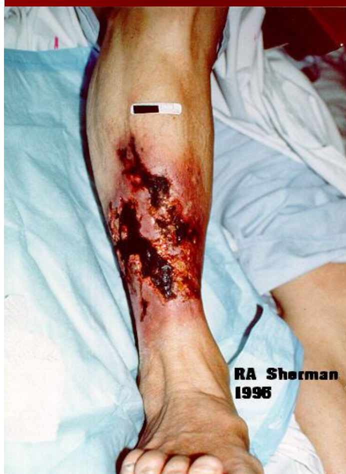


Plate II: Picture showing venous ulcer in the leg. Courtesy Dr Ronald Sherman.

His gangrenous venous stasis ulcers can be seen here, before maggot therapy. At this time, he had already been hospitalized for 5 weeks, receiving intravenous antibiotics, as well as regular medical and surgical care. He had no significant improvement.

Plate III: Picture showing maggots applied unto chronically infected and necrotic ulcer.



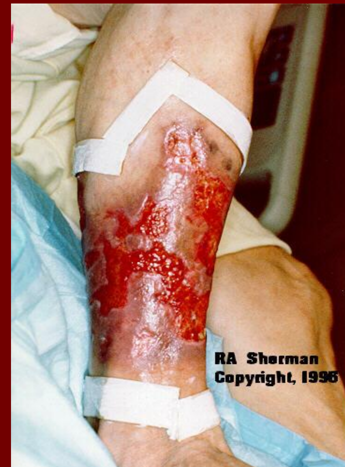
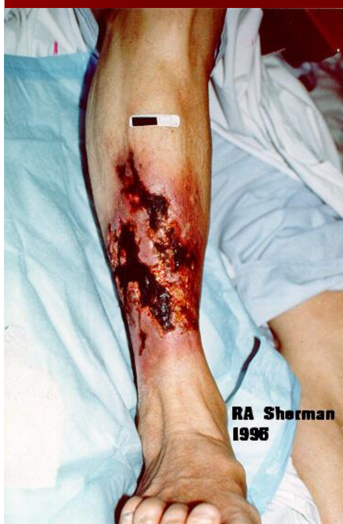


Plate IV: Within less than 2 weeks, the dead tissue has been cleaned by the maggots, revealing a healthy bed of red tissue beneath. This tissue will eventually give rise to skin, which will cover over the wound. The picture courtesy of Ronald Sherman.

Chronic non healing venous ulcer and MDT



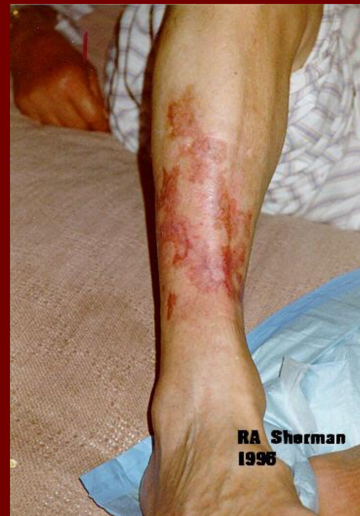


Plate V: Photo of leg 3 months later; healing after maggot therapy has already completed. Skin has completely covered the wound, without the need for grafting. The pink scars will continue to resolve, and his leg will achieve normal colour over the coming months

Case 2 ~

A 70 year-old man with foot ulcers for over 3 years had been treated unsuccessfully by his internists, podiatrists, and orthopedic surgeons. He requested an evaluation for maggot therapy.

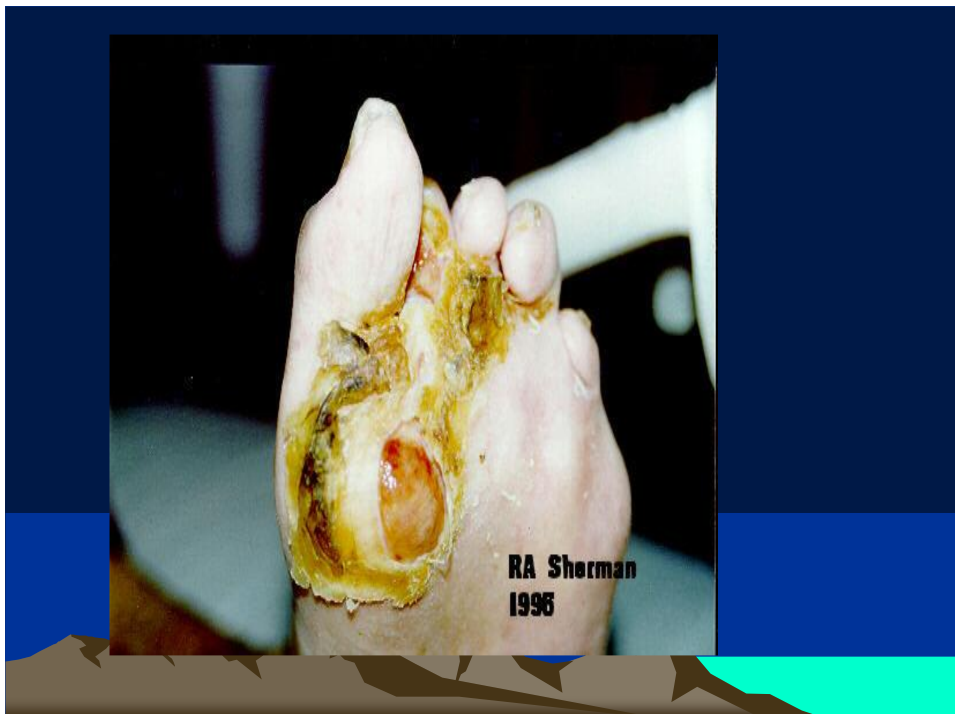


Plate VI: Prior to maggot therapy, his ulcer was dirty, and was surrounded by thick callus. It was impossible to tell what was healthy skin, and what was not. Podiatrists and orthopedic surgeons had trimmed the wound, but the callous quickly recurred, and the doctors were afraid to debride (clean) the wound too vigorously, lest he end up with an even bigger wound, which still might not heal. The picture courtesy of Ronald Sherman.

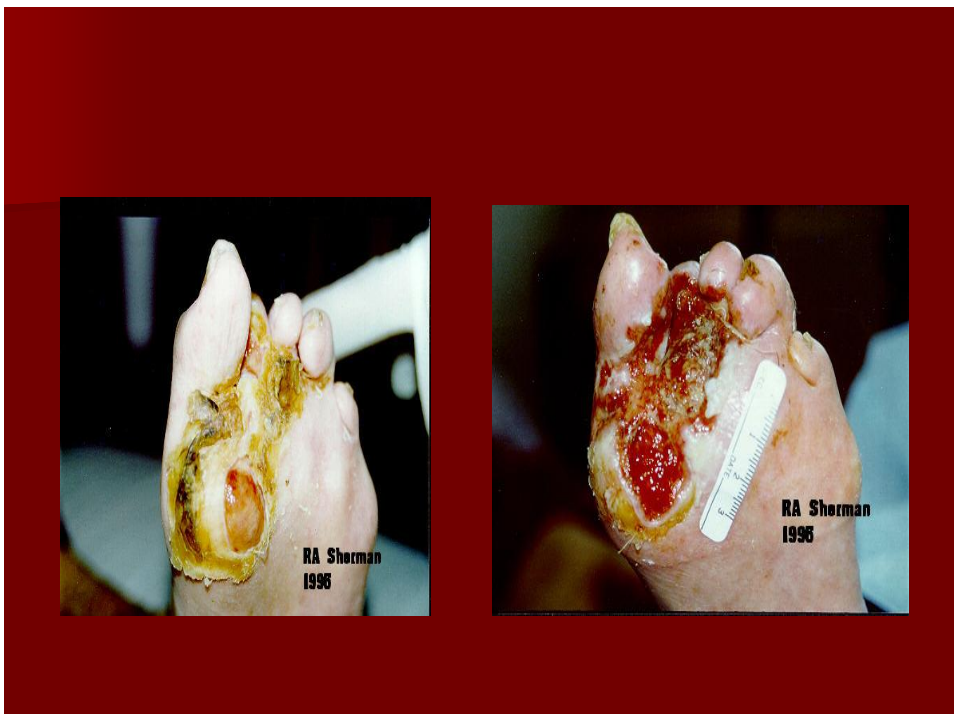
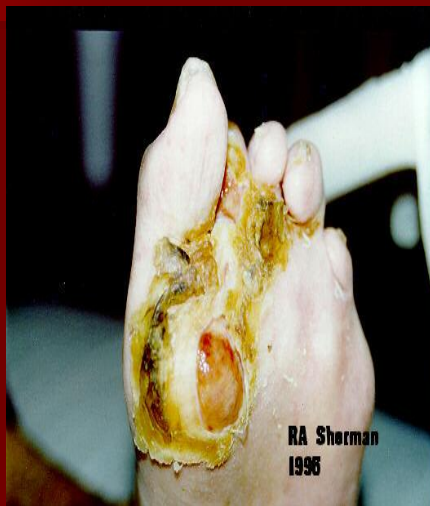


Plate VII: Indeed, his underlying wound was more extensive than could be appreciated by looking at the surface. This fact was apparent one week after maggot therapy was initiated, because the maggot debridement removed the entire callus and dead, infected tissue. What was left was a big hole; but it was clean, healthy, well-vascularized, and able to heal itself. The picture courtesy of Ronald Sherman.

MDT and chronic non healing decubitus ulcer



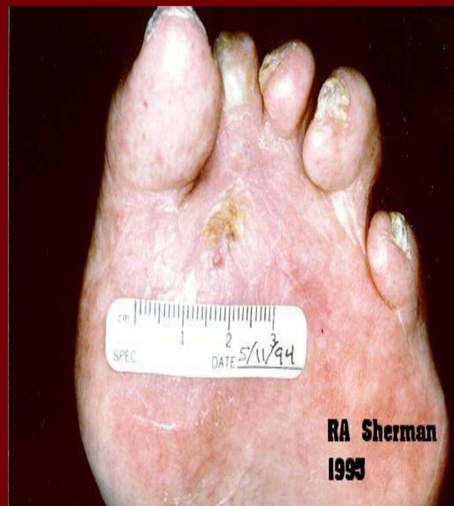


Plate VIII: His wound is seen here, just about completely covered with healthy skin.

To this day, two years after healing, his wound has not recurred.

1.10 PHYSIOLOGY OF WOUND HEALING

The physiology of wound healing is a complex but orderly phenomenon involving a number of processes (Clark, 1996).

- Induction of an acute inflammatory process by the initial injury.
- Regeneration of parenchyma cell.
- Migration and proliferation of both parenchymal and connective tissue cell.
- Synthesis of extracellular matrix (ECM)
- Remodeling of connective tissue and parenchymal components.
- Collagenization and acquisition of wound strength.

The mechanisms underlying most of these events are well studied and understood and involve the mediators of acute inflammation, growth factor, cell ECM interaction in cell migration, proliferation, and differentiation; and the mechanisms of angiogenesis and fibrosis.

1.11 WOUND HEALING BY SECONDARY INTENTION (WOUND WITH SEPARATED EDGE)

When there is more extensive loss of cell and tissue, as occurs in infarction, inflammatory ulceration, abscess formation, and surface wound that create large defects, the reparative process is more complicated. The common denominator in these entire situations is a large tissue defect that must be filled. Regeneration of parenchymal cell cannot completely reconstitute the original architecture. Abundant granulation tissue grows in from the margin to complete the repair. This form of healing is referred to as secondary union or healing by secondary intention. Secondary healing differs from primary healing in several respects.

1. Inevitably, large tissue defect initially has more fibrin and more and more necrotic debris and exudates that must be removed. Consequently the inflammatory reaction is more intense.
2. Much larger amounts of granulation tissue are formed. When a large defect occurs in deeper tissue, such as in viscus, granulation tissue with its numerous scavenger white cells bears the full responsibility for its closure because drainage to the surface cannot occur.
3. Perhaps the feature that most clearly differentiates primary from secondary healing is the phenomenon of wound contraction, which occurs in large surface wounds. Large defect in the skin of a rabbit are reduced in approximately 6 weeks to 5 to 10% of their original size, largely by contraction. Contraction has been ascribed, at least in part, to the presence of myofibroblasts-altered fibroblast that have the ultrastructural characteristics of smooth muscle cell.

Repair of tissue involves two distinct processes:

1. Regeneration, denoting the replacement of injury cells by cells of the same type, sometimes leaving no trace of the previous injury.
2. Replacement by connective tissue called fibroplasia or fibrosis-which leaves a permanent scar involving the following processes: (a) cell migration (b) proliferation (c) differentiation.

An orderly regeneration requires – basement membrane.

1.12 THE MEDICAL ENTOMOLOGY OF MDT

Maggot debridement therapy is just another method of treatment akin to the phenomenon of antibiosis and is an exploit of the natural relation between the metamorphosing larvae of flies and decomposing organic matter, including dead tissue

of animals. The larvae are the decomposer in this simple illustration of food chain. With the understanding of this, it could be summarized that when maggots infest the body it is called myiasis. Naturally occurring myiasis can be beneficial but sometimes it can be harmful, depending upon the type of maggot and the circumstances surrounding the infestation. Maggots frequently furnish important legal information and are used to help solve crimes, because their age of development can be an indicator of the time of death or more specifically, the post mortem interval. The presence of maggots or other insects on a body (live or dead) can also provide information about the location and /or circumstances of a crime. The study of maggots and other insects in this role is called forensic entomology.

Radford and Paul (1994) describe myiasis as infestation of living animals by flies (Dipteria) and that it may be classified according to anatomical sites, so there are dermal subdermal, nasopharyngeal, orbital, ophthalmic, aural, urogenital and intestinal myiasis. Many species of flies can cause myiasis, but the majority are opportunists whose saprophagous larvae normally dwell in and feed on decaying organic matter such as carrion. There are those fly larvae that are equipped to flourish in necrotic wounds. Such maggots usually confine themselves to dead tissue and hence are able to even benefit the healing process.

The choice of the maggot species for debridement therapy is very important because some maggots attack living tissue as against necrotic tissue and that makes them useless and dangerous for debridement. Examples of such include the old world screw–worm Chrysomya bezziana, also known as the Old World screwworm fly or screwworm of African and southern Asia. Wohlfaht’s wound myiasis caused by *Wohlfartia magnifica* of North Africa and the new World screw-worm called

Cochliomyia hominivorax of the Americas are also principal causes of malign myiasis in man . Larvae are obligated parasites of living tissue. Batches of eggs are laid on wounds in ears and on mucus membrane. The larvae burrow in groups into healthy tissue, causing widespread destruction that may be mutilating or fatal (Lice Busvine, 1978).

1.13 PROTEOLYTIC AND MECHANICAL DEGRADATION OF NECROTIC TISSUE BY MAGGOTS

Steve Thomas (Princes of Wales Hospital Bridge U.K) describes maggot Larvae as living chemical factories that once applied to the wound, and covered with a fine nylon net (to provide escape) during maggot debridement therapy, move over the surface ‘’secreting “ proteolytic enzymes that breakdown dead tissue and turn it into a soup which they then ingest (Visitants et al1981). Proteolytic enzymes are enzymes that prom*ote* proteolysis and proteolysis is the hydrolysis of the peptide bonds of protein with formation of smaller polypeptide. Those enzymes appear to be responsible for debriding or liquefying action of the maggot upon the dead (necrotic) tissue. By isolating, identifying and characterizing these maggot-derived factors, it may be possible some day to provide the benefit of maggot induced wound healing without requiring the maggots. But for now whole live maggots are necessary to deliver these exceptional wounds care. The isolation and characterization of maggot derived factors is preliminarily following an already established fact on the role of fibrinolytic system, proteolysis and antibacterial activity on wound healing

1.14 PROTEOLYTIC ACTIVITY IN MDT

There are many ways of assessing the level of proteolytic activity and this includes measurement of plasma protein carbonyl concentration and total body protein.

This type of test is important in in-vivo monitoring of proteolytic activity (Abu Ziden et al 1995). In milk, plasmin activity and the proteose peptone content are used as proteolysis indices (Le Roux and Colin et al 2002). Incubation of maggot derived factors with plasma may be a tool in measurement of extent of proteolysis by monitoring the changes in total plasma protein and/or plasma protein carbonyl concentration.

1.15 ADMINISTRATION OF MAGGOTS ONTO WOUNDS

The maggots should be left in the wound for three to seven days depending on the temperature and amount of necrotic tissue. They will then crawl out on their own and into the gauze (see figure 1). The wound can be flushed with saline and wiped dry. The dressing should be changed every 4-8 hours. A course of several treatments may be needed. A 24-48 hours rest maybe needed if local irritation becomes a problem. When the wound has been completely treated, it should be bandaged and treated as clean open wound. Sugar paste or honey can than be employed.

One Biotherapeutic agent in the field of Medical entomology is Beetle. Beetles are the most diverse group of insects. Their order, Coleoptera (meaning "sheathed wing"), has more described species in it than in any other order in the animal kingdom. Forty percent of all described insect species are beetles (about 350,000 species), and new species are regularly discovered. Estimates put the total number of species, described and undescribed, at between 5 and 8 million. This is why when J. B. S Haldane, a Scottish geneticist, was asked what his studies of nature revealed about God, he replied, "An inordinate fondness for beetles".

1.16 ANATOMY OF BEETLES

The general anatomy of beetles is quite uniform, though specific organs and appendages may vary greatly in appearance and function between the many families in the order. Beetle bodies are divided into three sections: the head, the thorax, and the abdomen. Like all insects, beetles are segmented organisms, and all three of the major sections of the body may themselves be composed of several further segments, although these are not always readily discernable.

Beetles are generally characterised by a particularly hard exoskeleton and hard forewings (elytra). The beetle's exoskeleton is made up of numerous plates called sclerites, separated by thin sutures. This design creates the armoured defences of the beetle while maintaining flexibility. The elytra are not used for flight, but tend to cover the hind part of the body and protect the second pair of wings (alae). Elytra must generally be raised in order to move the hind flight wings. A beetle's flight wings are crossed with veins and, after landing, are folded, often along these veins, and stored below the elytra. In some cases the ability to fly has been lost, most notably in the ground beetles (family Carabidae) and the true weevils (family Curculionidae), but also in some desert and cave-dwelling species of other families. Many of these species have the two elytra fused together, forming a solid shield over the abdomen. In a few families both the ability to fly and the elytra have been lost, with the best known example being the glowworms of the family Phengodidae, in which the females are larviform throughout their lives.

Beetles have mouth parts similar to those of grasshoppers. Of these parts, the most commonly known are likely the mandibles, which appear as large pincers on the front of some beetles. The mandibles are a pair of hard, often tooth-like structures that

move horizontally to grasp, crush, or cut food or enemies (Predation). Two pairs of finger-like appendages are found around the mouth in most beetles, serving to move food into the mouth. These are the maxillary and labial palpi.

The eyes are compound, and may display remarkable adaptability, as in the case of whirligig beetles (family Gyrinidae), in which the eyes are split to allow a view both above and below the waterline. Other species also have divided eyes (some Cerambycidae and Curculionidae), while many beetles have eyes that are notched to some degree. A few beetle genera also possess ocelli, which are small, simple eyes usually situated farther back on the head (on the vertex).

Beetle antennae are primarily organs of smell, but may also be used to physically feel out a beetle's environment. Further, they may be used in some families during mating, or among a few beetles for defence. Antennae vary greatly in form within the Coleoptera, but are often similar within any given family. In some cases males and females of the same species will have different antennal forms. Antennae may be clavate (flabellate and lamellate are sub-forms of clavate, or clubbed antennae), filiform, geniculate, moniliform, pectinate, or serrate. The larvae of beetles are usually the principal feeding stage of the lifecycle. Larvae tend to feed voraciously once they emerge from their eggs. Some feed externally on plants, such as those of certain leaf beetles, while others feed within their food sources (most metallic wood-boring beetles and longhorn beetles). The larvae of many beetle families are predatory like the adults (ground beetles, lady beetles, rove beetles). The larval period varies between species but can be as long as several years.

Beetle larvae can be differentiated from other insect larvae by their hardened, often darkened head, the presence of chewing mouthparts, and spiracles along the

sides of the body. Like adult beetles, the larvae are varied in appearance, particularly between beetle families. Beetles whose larvae are somewhat flattened and are highly mobile are the ground beetles, some rove beetles, and others whose larvae are described as campodeiform. Some beetle larvae resemble hardened worms with dark head capsules and minute legs. These are elateriform larvae, and are found in the click beetle and darkling beetle families. Some elateriform larvae of click beetles are known as wireworms. Beetles in the families of the Scarabaeoidea have short, thick larvae described as scarabaeiform, but more commonly known as grubs.

All beetle larvae go through several instars, which are the developmental stages between each moult. In many species the larvae simply increase in size with each successive instar. In some cases, however, more dramatic changes occur. Among certain beetle families or genera, particularly those that exhibit parasitic lifestyles, the first instar (the planidium) is highly mobile in order to search out a host, while the following instars are more sedentary and remain on or within their host. This is known as hypermetamorphosis. Examples include the blister beetles (family Meloidae) and some rove beetles, particularly those of the genus *Aleochara*.

As with all endopterygote insects, beetle larvae pupate for a period of time, and from the pupa emerges a fully formed, sexually mature adult beetle, or imago. Adults have an extremely variable lifespan, from weeks to years, depending on the species.

1.17 REPRODUCTION IN BEETLES

Beetles may display extremely intricate behaviour when mating. Smell is thought to be important in the location of a mate.

Conflict can play a part in the mating rituals of species such as burying beetles (genus *Nicrophorus*) where conflicts between males and females rage until only one of

each is left, thus ensuring reproduction by the strongest and fittest. Many beetles are territorial and will fiercely defend their small patch of territory from intruding males.

Pairing is generally short but in some cases will last for several hours. During pairing sperm cells are transferred to the female to fertilize the egg.

Among striped love beetles *Eudicella gralli* from the forests of Central Africa, the iridescent wing cases are used in marriage ceremonies.

Parental Care

Parental care varies between species and this may include the simple laying of eggs under a leaf. Certain scarab beetles, which construct impressive underground structures complete with a supply of dung to house and feed their young.

Predation

Beetles and their larvae have a variety of strategies to avoid being eaten. The larvae of scarab and other large ground beetles go on the attack, using their strong mandibles to dissuade predators.

1.18 MEDICAL ENTOMOLGY OF BEETLES

Cantharidin, the blister-causing oil found in several families of beetles, was accepted by the FDA in 2004 as treatment for warts and other skin problems. It also has historical use by the Greeks and Romans and is used as an aphrodisiac in some societies. Another extract from Blister beetles has also been used to treat problems of the Urogenital system.

Cantharidin is secreted by many species of blister beetle, and most notably by the Spanish fly, *Lytta vesicatoria*.

Cantharidin was first isolated by Pierre Robiquet in 1810. It is an odourless and colourless solid at room temperature. It is secreted by the male blister beetle and given

to the female during the mating. Afterwards the female beetle covers its eggs with it as a defence against predators. The complete mechanism of the biosynthesis is currently unknown. If cantharidin is ingested by human males, as it is excreted by the kidneys, it irritates the urinary tract causing spectacular swelling called priapism, but this is quite painful and does not result in arousal.

Diluted, it can be used to remove warts and tattoos. In addition, it can be used to treat the small papules of *Molluscum contagiosum*. However, when ingested, 10 mg is usually a fatal dose.

Chemical name	2,6-Dimethyl-4,10-dioxatricyclo-[5.2.1.0^{2,6}]decane-3,5-dione
Other names	Cantharidin
Chemical formula	$C_{10}H_{12}O_4$
Molecular mass	196.20 g/mol
CAS number	[56-25-7]
Density	1.41 g/cm^3
Melting point	212 °C

There are much more to be discovered of the medicinal use of Beetle and in this study the larvae of *Scarabaeus sacer.* On the basis of folk medicine, there are possibilities of scarab use in the treatment of Protein Energy Malnutrition (PEM) and in the management of hopocalcaemia by dietary modification.

CHAPTER TWO
LITERATURE REVIEW

2.1 LITERATURE REVIEW

Kiortosis et al (1989) have extensively reviewed and also proffered explanations on the evolutionary nature of wound healing efforts by the body of living organism. The mechanisms have developed from simple membrane repair of unicellular organisms to a more complex repair and regenerative processes in higher multicellular organisms. Human efforts to understand and influence the rate and order of wound healing is as old as man himself. The time of Galen witnessed some unusual concept and approach to wound care, where it was thought that formation of pus was a prerequisite to normal healing of wound (Weissman, 1992). Subsequently this idea changed and the effort to control infection to achieve optimal wound healing emerged. Easmond and Goodfellow et al (1990) made a review which brings to light the negative effect of infection and necrotic tissue on wound healing. Majno et al (1960) describe the pathology of necrosis, probably made worse by infection and the need for wound debridement that may enhance the speed and orderly nature of wound healing. But a much earlier indirect observation on the beneficial effect of debridement of wound by maggots was made from biblical time of Job, whose massive surface lesion of the skin was infested by maggots and the miraculous cure that followed (the story of Job in the old testament). The maggots most likely playing the role of debridement agent. Sherman and Hall et al (2000) have traced the history of medicinal maggots, an ancient remedy for contemporary affliction, to Napoleonic era when it was observed that maggot infested wounds of soldiers from the battlefield did better than similar non-infested wounds. Baer (1931) showed in his work the value of maggot

debridement therapy in the treatment of chronic osteomyelitis. Osteomyelitis has been extensively studied and the associated chronic features identified. These include sequestrum formation, which is a dead piece of bone formed as a result of suppurative and ischaemic injury. The chronic inflammatory responses in chronic osteomyelitis result in osteoclastic bone resorption, ingrowths of fibrous tissue, and deposition of reactive bone in the periphery.

In the presence of sequestrum the reactive woven or lamellar bone may be deposited as a sleeve of living tissue known as the involucrum (Ray and Bassed 1985). It also goes without saying that other morphologic variants of chronic osteomyelitis like Brodie's abscess and sclerosing osteomyelitis of Garre are but similar in aetiopathogenesis. While the former is a small intraosseous abscess that frequently involves the cortex and is being walled off by reactive bone, the latter develops in the jaw and is associated with extensive new bone formation that obscures much of the underlying osseous structure.

In an attempt to study and understand the pathogenesis of chronic osteomyelitis using chick osteoblast in culture, Hudson and Ramp et al (1995) examineed two strains of *Staphylococcus aureus* that were ingested by the osteoblast to different extents suggesting strain difference in uptake. Initial association of *S. aureus* with osteoblast was independent of the presence of matrix collagen produced by the osteoblast.

It was found that internalization of bacteria required live osteoblast, but not live *S. aureus*, indicating that osteoblast are active in ingesting the organisms. Viable bacteria were cultured several hours after ingestion, also indicating that the bacteria were not killed by the osteoblast. Jain and Sherman et al (1995), suggest the strong

need to remove sequestra when successive radiographs of diaphyseal sequestra show no reduction in size of the sequestrum after treatment with antibiotics for at least 6 to 8 weeks and an increase in the amount of the involucrum. Sequestrum and involucrum constitute impediments to healing in osteomyelitis.

This fact is further buttressed by the study of Tornetta and Demarco (1995) where the presence of halosequestrum was listed as strict contra-indication to secondary intramedullary nailing because of the high risk of having non-union. These and many other findings have shown the need to debride (remove) the dead tissue in chronic osteomyelitis in order to achieve healing. This is where MDT comes into relevance in osteomyelitis.

The pioneering effort of William Baer in the United States using MDT to treat osteomyelitis was followed by the work of Hewitt (1932) and Mckeevar (1933) as the benefit of MDT in osteomyelitis becomes clinically clearer.

Sherman and Pechter described the interesting re-emergence of MDT in a review in 1988 on the therapeutic applications of fly larvae in human medicine especially for treating osteomyelitis. But osteomyelitis is not the only pathological condition with debris and necrotic tissue in which MDT has application.

Teich and Myers (1986) have studied the therapeutic effect of MDT for severe skin infection to effect healing with less scaring and thus of more cosmetic value to the patients. MDT has also found place in plastic surgery as illustrated by the work of Namias et al (2000), where limb salvage after fourth degree burns was achieved. Mumcuoglu et al (1997) also studied the biodebriding effect of maggot in gangrenous wounds with an impressive result. Stoddard and Sherman et al (1995) successfully demonstrated in their work, the applicability of MDT in non-healing leg ulcers of

diabetics. It is in the opinion of the team that with the use of MDT in some diabetic ulcers and gangrene, surgery could be avoided.

Neuropathy and vasculopathy in diabetic patients result in ulcers due to anesthesia and small repeated trauma and then gangrene due to restricted circulation. Maggots remove the dead tissue. Mumcuoglu and Inger et al (1998) further supported the earlier work of Stoddard. Thus, MDT has found a place in podiatry (see figure 5 to 7) and diabetic care.

It is not all species of fly larvae that are used in MDT. Some species do attack normal tissue thereby causing destruction and usually are avoided for use in MDT. Examples are the Old World screw worm, Wohfaht's wound myiasis etc (Lice - Busvine, 1978). Blowflies rather than common houseflies are commonly used now.

The species *Lucilia sericata* is one of three species that was regularly used in maggot therapy early in the century. Thomas and Jones et al (1996) and Sherman and Pechter (1988) describe how the fly larvae are mixed into a wound dressing and the area is covered with gauze.

2.2 PREPARATION OF MAGGOT

After the maggots were duly prepared, the wound is also prepared for MDT. Wounds have to be opened and cleaned as much possible but antiseptic must be used with caution and flushing with sterile water is one of the best pre-treatment. It is recommended that maggots be implanted when bleeding has stopped and that in acute infection maggots should not be implanted until at least eight days later.

2.3 IMPLANTATION

Maggots are transferred from the container by adding sugar-saline, which will make them float to the top. They can be gathered on a sterile gauze pad, which is then

placed on the wound. The population should be 6-10 per square centimetre. They can also be gently scraped into the wound. This should be covered loosely with gauze, an air permeable cover of fine mesh material such as nylon stocking or Gove-Tex protects the surface. The maggots must be able to breathe while cleaning the wound and the liquefied necrotic tissue must be able to drain. The dressing must be changed every four hours to prevent it from becoming impermeable and suffocating the maggots. Itching and local irritation can occur during treatment. The patient must be prevented from fretting or scratching the area. Redness around the site and rise in temperature can also be expected. Excessive temperature must be treated as a separate condition that may require systematic antibiotics.

2.4 ADMINISTRATION OF MAGGOTS ONTO WOUND

The maggots should be left in the wound for three to seven days depending on the temperature and amount of necrotic tissue. They will then crawl out on their own and into the gauze (see figure 1). The wound can be flushed with saline and wiped dry. The dressing should be changed every 4-8 hours. A course of several treatments may be needed. A 24-48 hours rest maybe needed if local irritation becomes a problem. When the wound has been completely treated, it should be bandaged and treated as clean open wound. Sugar paste or honey can than be employed.

2.5 PROTEOLYTIC ENZYMES

Current focus by scientists is on the biochemistry and molecular biology underlying the wound healing benefits of medicinal maggots. Wald and Olejar et al (2001) have investigated the functions of trypsin and chymotrypsin beyond the gastrointestinal digestion of protein diet. Using the mixture of these two proteolytic enzymes and papain, they were able to demonstrate their antimetastatic effect on B_{16}

melanoma cells and melanoma extirpation in C_{57} B_{16} mice. A complex function of proteolytic enzymes in contrast to antimetastatic activity is shown by the work of Chabowski and Sulkowska et al (2001), where Cathepsin D a proteolytic enzyme is evaluated immunohistochemically in colorectal adenocarcinoma. A statistical correlation was found between high Cathepsin D expression in low differentiated cancer cell of the main mass of the cancer and low Cathepsin D expression in low differentiated cancer cell which formed nest at border of cancer invasion. Just as proteolytic enzymes do help wound healing by degrading necrotic tissue, so also they may be a promoting factor in certain metastasizing tumors (Goldmann and Moorkamp et al 2001). In an analysis of 53 specimens from primary squamous cell carcinomas of the skin for the expression of collagenase iv and Cathepsin D, statistical analysis revealed significant differences for the overall expression of Cathepsin D ($P < 0.05$) expression of cathepsin at the invading front ($P < 0.05$) and the tumour thickness ($P < 0.01$).

Interestingly, other types of proteolytic enzymes seem to have favourable effect on the patient as shown by the work of Nikkola and Vihinen et al (2001) where high collagenas-1 expression correlates with a favourable chemoinmunotherapy response in human metastatic melanoma. Matrix metalloproteineses (MMPs) are proteolytic enzymes that can degrade extracellular Matrix and thus enhance metastasis. The expressions of two collagenolytic (MMPs) in 37 samples obtained from 26 patients treated for metastatic melanoma were obtained. The samples showed a different expression pattern of collagenase –1 (MMP-1) and collagenase-3 (MMP-13). The samples with high levels of MMP-1 (n=18) were more frequently MMP-13

negative (14 out of 18), whereas those with low expression level of MMP-1 (n=15) were predominantly positive for MMP-3 (9 out of 15), (P=0.027).

High expression levels of MMP-1 were associated with a favourable response to chemoimmunotherapy. Patients responding to treatment (η=13) frequently had intensively MMP-1 expressing metastases (9 out of 13), especially those who achieved a complete response (5 out of 6). Response failures (η= 7) (P=0.019). There was tendency toward longer survival among the patients with intensively MMP-1 expressing tumours (median 14.3 versus 6.7 months, P=0.068).

It could be arguably stated that the use of MDT in chronic, non-healing ulcers and the tendency of chronic ulcers to undergo malignant transformation are important factors to be studied in the light of the activity of proteolytic enzymes of the maggots and all the possible outcomes.

The work of Furaya and Ishikura et al (2000) involving analyses of Matrix metalloproteineses and their inhibitors (TIMP-2/MMP-2 ratio) in cyst fluid of serous ovarian tumours, show that matriolytic enzymes such as MMP-2, MMP-7 and MMP-9 are secreted into cyst fluid from serous adenocarcinoma tissue. In part, the aggressive invasion of serous adenocarcinoma cells may be explained by the expression of metriolytic enzymes.

The negative effect of excessive proteolytic activity has also been shown by the need to use inhibitors of proteolytic enzymes in chemical and thermal injury of the eyes (Reim and Redbrake et al 2001).

This according to the authors is based on pathophysiological findings on the role of proteolytic enzymes in aggravating corneal, scleral and conjuntival ulcerations, tissue proliferation and scarification. Chang and Gabison et al (2001) have also

expressed the opinion that the pathogenesis of neovascularization may be influenced by matrixmetelloproteinases and other proteolytic enzymes.

In a review by Neurath (2001), he stated that 50 years of research on proteolytic enzymes (trypsin, carboxypeptidases, and mast cell protease) have seen a lot of advances in the molecular biology and physiological chemistry researches.

Another factor worth considering is the ability of proteolytic enzymes to enhance the formation of thrombus.

These enzymes are stimulated by inflammatory cells particularly T-lymphocytes and this process has been identified in the pathogenesis of atherematous plaque formation within coronary arteries (Reiner and Tedeschi, 2001).

The proteolytic enzymes cause degradation of fibrous tissue cap resulting in thrombus and occlusion of artery. Stable plaques have thick fibrous cap and few inflammatory cells while vulnerable plaque has numerous inflammatory cells that stimulate proteolytic enzymes.

Proteolytic enzymes released by maggots act topically. The extent of possible absorption into the systemic circulation, if any is yet to be ascertained and fully established and MDT is still considered relatively safe as the risk of thrombus formation has not been identified as a contra-indication, yet.

2.6 FIBRINOLYTIC ACTIVITY

Fibrin is formed in response to damaged blood vessels after injury is sustained. It prevents bleeding by partaking in the clotting of blood. The lysis of fibrin is also part of normal repair and healing process. Maurer (2001) has recently shown that substances such as Bromelain which is a crude extract from pineapple has fibrinolytic effect and has among other benefits, a place in healing surgical traumas.

Organization and remodeling requires cellular infiltration (fibroblast and osteoblast) and breakdown of haematomas (fibrinolysis). According to Thomson and Proctor (1989), fibrinogen consists of polymer whose links have to be digested by plasmin to convert the insoluble fibrin to fibrin (fibrinogen) degradation product and fibrin split product (FDP and FSP respectively). The concentration of these products is directly proportional to the degree of fibrinolysis.

Plasminogen is the precursor of plasmin. Plasmin activators are present together with plasminogen in the euglobin fraction of the plasma whereas the natural inhibitors of plasminogen conversion are not. Thrombin is used to clot the euglobulin fraction of the plasma and the lysis time is a reflection of the activity of plasminogen activators.

Incubation of the euglobin fraction of the plasma and maggot derived factors may show results that can explain the biology and chemistry of the beneficial effect of MDT.

2.7 CONTROL OF INFECTIONS

The 19th century made modern surgery possible by means of two great discoveries: safe anaesthesia, and control of wound infection (Duffy 1990). A Boston dentist, William Morton, discovered that inhalation of diethyl ether would render a person unconscious and incapable of perceiving pain. As surgeons began using anaesthesia to perform longer and more intricate operations, however, the benefit of Morton’s discovery began to be diminished by wound infection, or sepsis, caused by the entrance of bacteria into the bloodstream. In Vienna, Hungarian physician Ignaz Semmelweis had been insisting that puerperal sepsis, a usually fatal infection experienced by some women after childbirth, was due to infection of the birth canal by

the hands of hospital attendants. This theory was ridiculed, because at that time no scientific reason was known that supported it. When Pasteur showed that micro-organisms in the air and on the hands could produce disease, however, the British surgeon Joseph Lister began his epochal work on infection published in 1857. This work showed that surgery was made safer by using antiseptics such as phenol to sterilize equipment and the surrounding environment. Nowadays, Infection is a well known and established factor that impairs normal wound healing.

Staphylococcus aureus remains the most common cause of wound infection after surgery or trauma not involving the mucous membranes with their rich anaerobic commensal flora (Vandenesch and Etienne et al 1993). The clinical presentation of staphylococcal wound infection varies from minimal erythema and serous discharge, through small abscesses (often in relation to sutures), to marked cellulitis (with deep pus and wound dehiscence) with considerable pain and systemic disturbance. Maggots are said to secrete proteolytic enzymes and ammonia rich secretion that kill and inhibit the growth of these bacteria thereby controlling the infections that impair wound healing.

A review by Despin and Axtel (1995) summarizes the uses and importance of beetles and their larvae in the ancient civilization of Egypt as evident on hieroglyphics as well as the present day uses. The work of Hellberg (1995) added credence to the use the beetle poison Cantharidin ordinarily secreted by the male beetles as a contribution to the females for their use to protect their eggs against predators. The evaluation of the Physical and chemical properties of Cantharidin became a platform on which it was suggested for possible use in the treatment of warts, a new alternative to the more corrosive and dangerous eye irritants podophyllin for similar purpose. A wider scale

study later gave reason for its approval by the US FDA (United State Food and Drug Administration). Cantharidin is now licensed for use in the treatment of warts. This is considered as another important Modern Biotherapeutic achievement.

Despin and Axtel (1995) refer to the use of insects' larvae as nutritious source of protein by many indigenous communities in Asia, Africa and Latin America. These insects' larvae include those of beetle especially the larger sized dung beetle larvae. Their work revealed increased in weight of chicks fed on a mix of beetle larvae and starter feed better than those fed with starter feed or beetle larvae along. On analysis the beetle larvae were found to be 68% composite protein. Dung beetle larvae also called Scarab or Grub are used in alcoholic beverages as elixir for health. Other communities especially in the Northern part of Nigeria use water based crude extract from gut section of the scarab for treatment of jaundice and related fever.

The highly organic nature of these larvae may serve as rich source of proteins and enzymes that may stimulate biological effect(s) on especially the liver. A review article by Karp W. (1979) highlighted earlier work that suggested the targeting of the liver to induce its microsomal enzymes to enhance functions for such reasons as neonatal jaundice. An efficient enzyme system means more efficient bilirubin clearance from the plasma leading to reduction in the depth of jaundice and related problems.

Watchko and Oski (1992) made a summary of the different causes of neonatal hyperbilirubinaemia and jaundice in neonate and the concern about the likely effects on their vulnerable and immature blood brain barrier to cause bilirubin encephalopathy. Staining of the basal ganglia and other parts of the brainstem has been reported to be the fundamental damage that causes kernecterus, a disorder

characterized by mental sub- normality as well as motor dysfunctions. Percentage distributions of the causes of jaundice of the newborn was given by Newman and Maisels (1992) with following: Physiological jaundice accounting for about 45% cause of the jaundice, Rhesus incompatibility 29%, ABO incompatibility 13% and others 2%.

It was in 1980 that Telzrow wrote that young experimental Rats brains are susceptible to staining and damage by hyperbilirubinaemia. He referred to an earlier work published that further put credence to Human autopsy findings in neonate published by Karp (1979) where extensive damages were reported in the basal ganglia and other parts of the pyramidal areas because of the easy permeation by the excess hydrophobic bilirubin crossing the immature blood brain barrier in their brain.

Though photodegradation of bilirubin has been extensively studied and the behaviour of infants undergoing phototherapy written, several workers prominent among which include Ostrow (1972) and Telzrow et al 1980. Lucey (1972) highlighted the concerns on the effect of the Ultra-violet radiation on the eye of the baby undergoing phototherapy and other side effects. Ahlfors (1994) and Maisels (1996) further raised concern on the use of exchange blood transfusion as a method of managing neonatal hybilirubinaemia and the need to have extensive phototherapy and pharmacologic treatment to minimize the use of the blood transfusion. The work of Martinez (1999) showed the useful though limited value of the administration of mesoporphyrin in neonatal hyperbilirubinaemia. Hence the search for biologically active molecules that can safely degrade bilirubin into soluble derivative and or stimulate the liver microsomal enzymes especially UDP-glucoronyl transferase seems

an effort in the right direction in view of the acceptable multidimensional approaches to the management of neonanatal hyperbilirubinaemia and its complications.

CHAPTER THREE

3.1 MATERIAL AND METHODS

Material used for various aspects of this work included:

The experimental animals. A population of 5 day old larvae of green bottle fly (Phanaecia sericata) obtained from 2nd generation of the laboratory grown flies, a population of the larvae from natural environment (faeces and putrid meat), Metlar H80 Balance (Gallenlamp) was used for weighing chemicals and other substances, test tube beakers, conical flasks, 150cl plastic bottle, filter barriers a piece of putrid meat a plastic perfusion tube and heparin. The equipment used in harvesting the larval secretion include 150cl glass bottle a stand, plastic filter barriers within the column of the bottle, a beaker and conical flask, an effluent recycling plastic tube. A piece of putrid meat, an isotonic fluid medium, 0.9% sodium chloride (Normal saline) obtained from May & Baker chemicals, 800 ml of plasma sample was obtained from healthy volunteers; 3 males and 3 females. Combi 9 strip and Biuret Reagent. 10 % stock solution of sodium chloride (Dacie and Lewis 1968) and Permission for the research by the university of Jos teaching hospital ethical committee was obtained and the Helsinki declaration on ethical use of experimental animals duly observed.

3.2 EXTRACTION OF LARVAL SECRETION

The theory behind the extraction of secretion from maggot is Pavlovian-like condition reflex. Perfusion of maggots after 1 hour of starvation inside a well-ventilated plastic Jar with a piece of putrid meat introduced into the atmosphere of the maggots' environment stimulates feeding frenzy. This is expected to chemotactically stimulate Pavlovian-like reflex and the Normal saline solution is used to perfuse the colony of the maggots to harvest product of this stimulation. The effluent is recycled

about 5 times in quick succession, through a plastic perfusion tube improvised to obtain a greater concentration of the secretion from the maggots for any given harvest (See figure 8)

3.2.1 Change in Weight of Infested and Non-Infested Experimental Pigskin Samples

The material used include twenty units of pig skin sample cut to average size of 35 grams, four plastic buckets two of which with tightly covered outlet with clothing, twenty rubber bands, twenty five Litre of Distilled water, Metlar Balance, formal-saline, ten Tissue specimen bottles, Dissecting kit and Pairs of surgical gloves and disposable gloves.

Ten of the twenty pig Skin samples were deliberately infested with laboratory grown larvae of *Lucilia sericata*. The other Ten were control samples and all of them were allowed to decompose naturally under the same room temperature in moist environment within the two plastic Jars. Each sample was weighed serially on alternate days with Metlar balance for two weeks (384 hours). Replicate of the experiment of a sample each were conducted with samples for histology taken from both the maggot infested and the non-infested Pigskin samples for histology. The results were analyzed using SPSS and MS Excel software

3.3 METHOD OF HARVESTING LARVAL SECRETION AND MEASUREMENTS OF THE PH AND POH

Using infusion giving set (DANA), a total of 30 cycles was achieved, 5 cycles a sample was obtained for measurement. PH mater was used for measurement of pH of each extract sample after standardizing the metre by immersing the probe in buffer solutions of pH 4 and 9.The Qualitative test for ammonia was conducted by using

hydrochloric acid solution and the total protein content in determined by Biuret method.

3.4 QUALITATIVE TEST FOR AMMONIA

To 2ml of the larval harvest in test tube, 1ml of 0.1 M solution of Hcl was added and a clear bright daylight was used to examine for whitish fume.

3.5 QUANTITATIVE DETERMINATION OF PROTEIN

For the purpose of serial measurement of total protein for the estimation of the rate of proteolysis, the protein content of the plasma of the blood can be calculated from the observed value of the specific gravity of the plasma with sufficient exactness. This correlation was shown by Moore and Van Slyke, who found that the relationship is expressed by the formula:

Grams total protein per 100 cc. plasma equal 343 (G - 1.0070),

G being the observed specific gravity. They included measurements of the protein content of the plasma of normal individuals which were in agreement with those published by Linder, Lundsgaard and Van Slyke in 1924 and by Salvesen in 1926. These observers also published data showing that the plasma of individuals analyzed over a long period of time exhibits variations in the total protein content. That there are alterations in the direction of a decrease in the protein content of the plasma of the blood in pathological states has been shown by Linder, Lundsgaard and Van Slyke. Decreases both in specific gravity and in protein content of the plasma have been shown to be present in nephritis with oedema.

For the purpose of standardization, a double check method on total protein determined, Biuret method is employed. Quantitative estimation of protein by Biuret method. Protein standard 40mg BSA/ml. Biuret reagent was obtained dissolving 3g of

CuS04.5H20 and 9g of Na.K Tartarate in 560ml of 0.2ml/1 NaOH, addition of 5g of K1 and made up to 1liter with 0.2 ml/1 of sodium hydroxide. Water-bath at 37° C was used.

METHODS

5.0ml of Biuret reagent was added to 0.1 of sample mix and warmed at 37^{0}C for 10minutes and cooled. Similarly react 0.1ml of 40mg/protein standard as last, and 0.1ml of water as blank with Biuret reagent as for sample. Need observance of both sample and standard against blank at 540mm.

1ml of the crude extract was obtained and mixed with equal volume of Biuret reagent the optical density (OD) of this test solution measured using colorimeter as optical density the optical density ready made standard solution in measured.

The Biuret method was used to determine the total protein in plasma incubated with the larval extract. This test is based on reaction of peptide bonds of protein with cupric ions in alkaline solution to form a coloured product. The absorbance obtained in the coloured product is measured at 540nm and it is directly proportional to the total protein concentration of the specimen assayed. The total protein in five replicate controlled plasma sample and equal number of test replicate plasma samples. These were incubated with 0.2 ml of larval extract at room temperature and at controlled temperature of 37°C. Twenty-four hourly plasma protein concentration determinations follow another four days after the initial estimation (day one).

Protein concentration = O.Dtest × Concentration of standard. ÷_O.DStd.

Protein concentration = O.Dtest /O.DStd × Concentration of standard used × 100 ml / Volume of sample used × 1/1000 Sample.

The Protein concentration was obtained in gram/100ml. Concentration of protein standard. 60mg is in 1ml (60mg/ml) 6mg was in 0.1ml. Since 0.1ml of protein standard was used the concentration (amount of protein standard more appropriately was 6mg). Dividing by the Volume of sample used changed the amount of protein in test to mg per ml. Multiplication by 100 was to convert the amount of test protein to mg/100ml. Multiplication by 1/1000 was to convert the amount of test protein to gram /100ml.

3.6 QUALITATIVE DETERMINATION OF THE PRESENCE OF BLOOD ELEMENT (HAEMOGLOBIN) BY MEDI-TEST STRIP METHOD

Medi-Test combi 9 strips by Machery-Nagel, rue Gutenberg-F-67722 Hoerdt Germany, **wa**s used to test the harvest in the maggot medium. The principle for this test is based on pseudoperoxidative activity of haemoglobin and myoglobin, which catalyze the oxidation of an indicator by an organic hydroperoxide, producing a green colour. The Colour intensity is directly proportional to the concentration of the blood element

Two groups of maggots were used for this test i.e. ones that are grown in natural environment (mixture of putrid meat and fish with Human faeces) and the group grown in groundnut paste mixed with washed Sand.

3.7 STUDY OF THE ANTIMICROBIAL EFFECTS OF THE EXTRACT OF THE ARTHROPOD LARVAE

A. **SPECIMENS:** Five specimens selected randomly were used for the study, They were labeled 1, 4, 8, 10 and U

B. **MICROORGANISMS USED**: Five different microorganisms that were isolated from wound swabs were used for the studies. These are *staphylococcus aureus*, *Eschericia coli*, Klebsiella species, *Pseudomonas aeruginosa*, and candida species.

C. **LABORATORY INVESTIGATIONS:** The antimicrobial property of the five crude extracts was carried out using the punch plate method. Each of the microorganisms at concentration of 105 cfu/ml was used in inoculating the dried diagnostic sensitivity agar (DST agar). Five holes each were punched on the inoculated DST plate. The crude extracts were then pipetted into the labelled holes. The plates were then incubated at 37 ^{o}C for 18 - 24 hours after which they were examined for zones of inhibitions around the holes.

PROTEOLYTIC EFFECT OF LARVAE OF LUCILIA SERICATA ON SAMPLES OF PIGSKIN

Pig skin samples were obtained from the abattoir. Special precaution was taken to prevent the skin samples from exposure to hot water as often may be the practice that may damage the tissue. The skin samples were cut into sixteen approximately similar sizes weighing between twenty to thirty grams. Eight of the sixteen Pigskin samples were allowed to stay moist and aerated at room temperature in a plastic jar covered by net to prevent wild flies from depositing eggs as they progressively underwent necrosis. The other eight remaining were incubated moist with the larvae of *Lucilia sericata* at room temperature in a plastic jar covered with a net. Each of these sample was weighed after every forty eight hours. Two of the sample Pigskin were also incubated separately, one without the larvae and the other with the larvae in similar manner to the first two to enable specimen collection for histology. Pigskin

sample was then cut from the stock for histology after every forty eight hours. Pigskin was chosen because it is the closest to human skin (Sullivan and Eaglstein 2001).

OBTAINING SCARABS FOR STUDIES

Scarabs or Grubs are the larval form of the Dung Beetle *Scarabaeus sacer*. They are hatched from the eggs laid by the female Beetle in moist organic rich material mostly cow dung. Cow dung provides hiding and food source for the larvae that usually ingest a lot of it. Scarabs are sensitive to light (negatively phototrophic) and tend to burrow in soil to avoid it.

Forty units of the Scarabs between ten and twenty gram weight were obtained from dung of cows from their grazing area in Bassa L.G.A area. Dark and moist humus soil was used to temporarily store them before sacrifice.

DISSECTION AND PROCESSING OF SCARAB FOR CRUDE EXTRACTION OF THE LARVAL FACTOR

The larvae were frozen instantly by immersion into ice cold water in a freezing compartment of a deep freezer. Their guts were cut open and the head, legs and other appendages were left intact. The body parts were dried in desiccators for four days. Protein and mineral content were determined. All results were recorded and tabulated and statistically analyzed using Microsoft Excel and SPSS 10.0 computer software.

Plate IX: Plastic buckets with net covers used in culturing maggots

CHAPTER FOUR
RESULTS

The following are the results of changes in the weight of samples of pigskin undergoing necrosis with infesting maggots on one side and a control group with no maggots on the other. Ten pigskin samples were allowed to decompose in a normal saline moist open environment at room temperature and weight every other day (48 hourly). The results in table I showed an initial decrease of the mean weight from 35.19 grams to 34.48 grams by the sixth day (144 hours) followed by a transient increase to 36.59 grams by the eighth day (192 hours). The decrease thereafter continued steadily to the sixteenth day (384 hours). The decrease in weight with time has negative correlation and Analysis of Variance (ANOVA) that is statistically significant ($p<0.05$). The decrease in weight is attributable to autolysis and tissue breakdown by microbial activity. Transient increase in weight at the eighth day is probably due to imbibing of water by the tissue samples.

Line graph in figure I demonstrated the same events. The ‘kink’ or elevation at the 192 hours conspicuously shows the transient increase in weight mentioned earlier.

4.1 RESULTS

TABLE 1: SHOWING CHANGES IN MEAN WEIGHT OF NON-INFESTED PIGSKIN SAMPLES WITH TIME

S/NO	Time of Measurement of Pig skin weight (Hours)	Mean Weight of Non-infested Pig Skin samples Measured (Gram)	Standard error of mean +/- (SEM)
1	0	35.19	5.258631
2	48	34.36	4.796547
3	96	33.67	5.287728
4	144	34.48	5.108143
5	192	36.59	4.965988
6	240	33.67	4.345498
7	288	32.28	4.742322
8	336	28.22	4.67758
9	384	26.79	4.953504

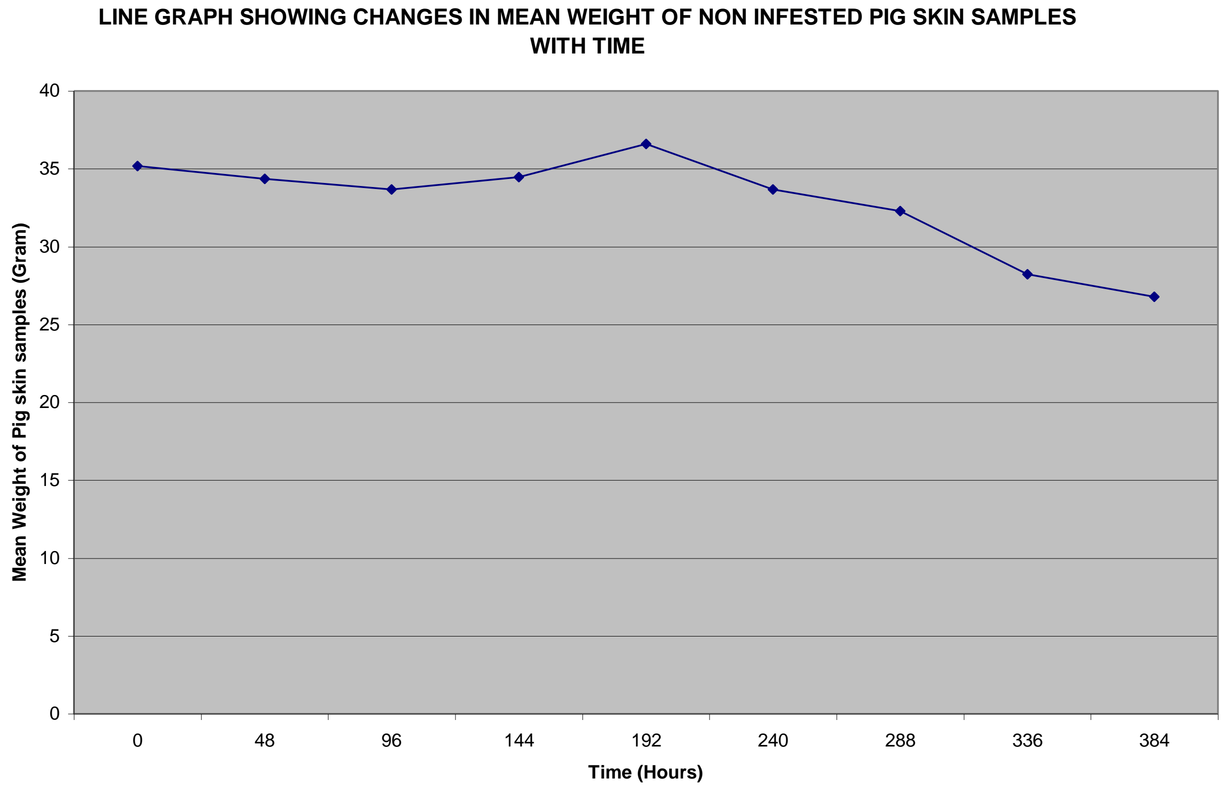


Figure I: Line Graph showing Changes in mean weight of Non-Infested Pig Skin Samples with Time

Ten pigskin samples that were allowed to decomposed with maggots feeding on them in a normal saline moisten and opened environment at room temperature and weight every other day (48 hourly). The results in table II showed an initial decrease of the mean weight from 35.82 grams to 34.93 grams by the sixth day (144 hours) followed by a transient increase in weight to 36.90 grams from 34.20 grams from the fourth (96 hours) to the eighth day (192 hours). The decrease thereafter continued steadily to the sixteenth day (384 hours). The decrease in weight with time has negative correlation and Analysis of Variance (ANOVA) that is statistically significant ($p<0.05$). The decrease is weight is attributable to autolysis, tissue breakdown by microbial activity and tissue degrading activity of the maggots of *Lucilia sericata*. Transient increase in weight with time is probably due to imbibing of water by the tissue samples.

Line graph in figure II demonstrated the same events. The ‘kink’ or elevation at the 144 and 192 hours conspicuously showed the transient increase in weight mentioned earlier. A comparative graph show that the maggot infested pigskin possibly has earlier onset of water imbibitions than the non-infested pigskin samples.

TABLE II: SHOWING CHANGES IN MEAN WEIGHT OF MAGGOT'S INFESTED PIGSKIN WITH TIME

S/NO	Time of Measurement of Infested Pig skin weight (Hours)	Mean Weight of Maggot infested Pig samples Skin Measured (Gram)	Standard error of mean +/- (SEM)
1	0	35.82	5.894338
2	48	34.83	5.024358
3	96	34.20	4.983379
4	144	34.93	4.692875
5	192	36.90	4.455811
6	240	34.04	3.943335
7	288	31.82	4.169813
8	336	28.66	4.139736
9	384	27.32	4.153935

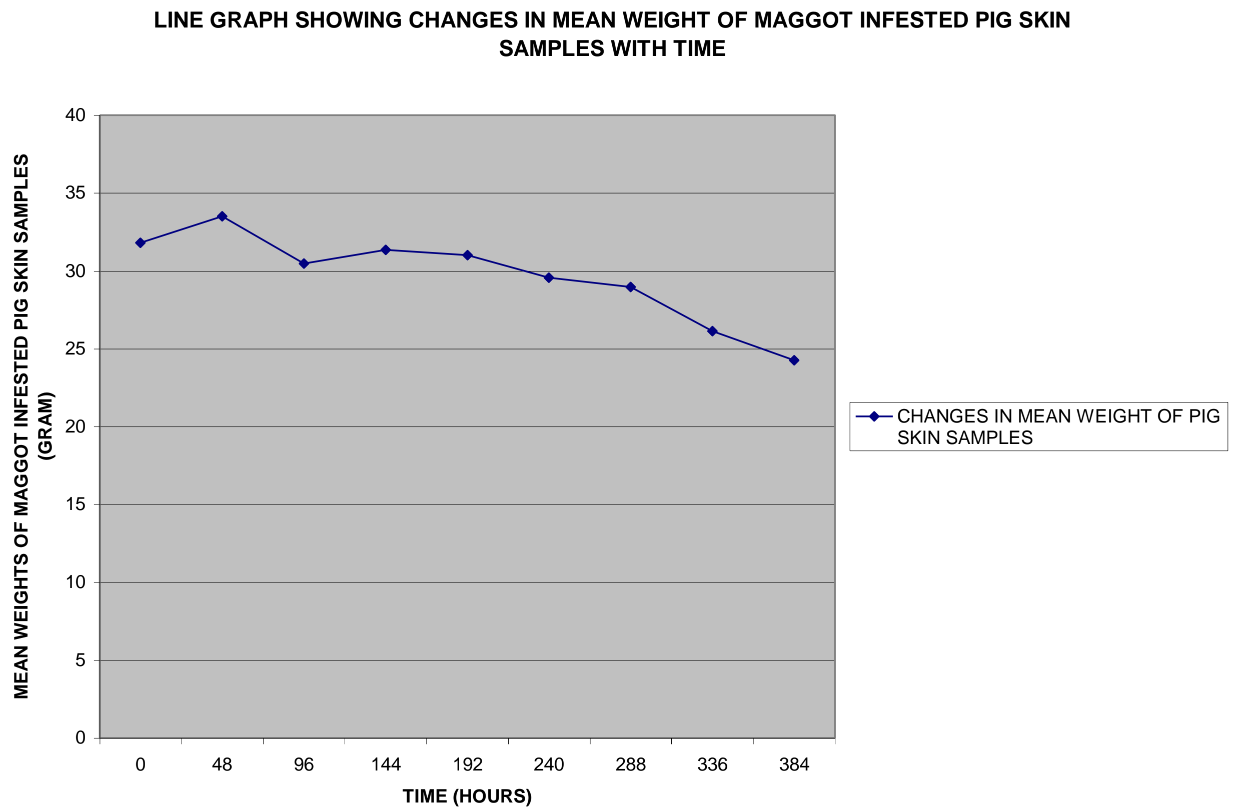


Figure II: Line Graph showing Changes in mean weight of Maggot Infested Pig Skin Samples with Time

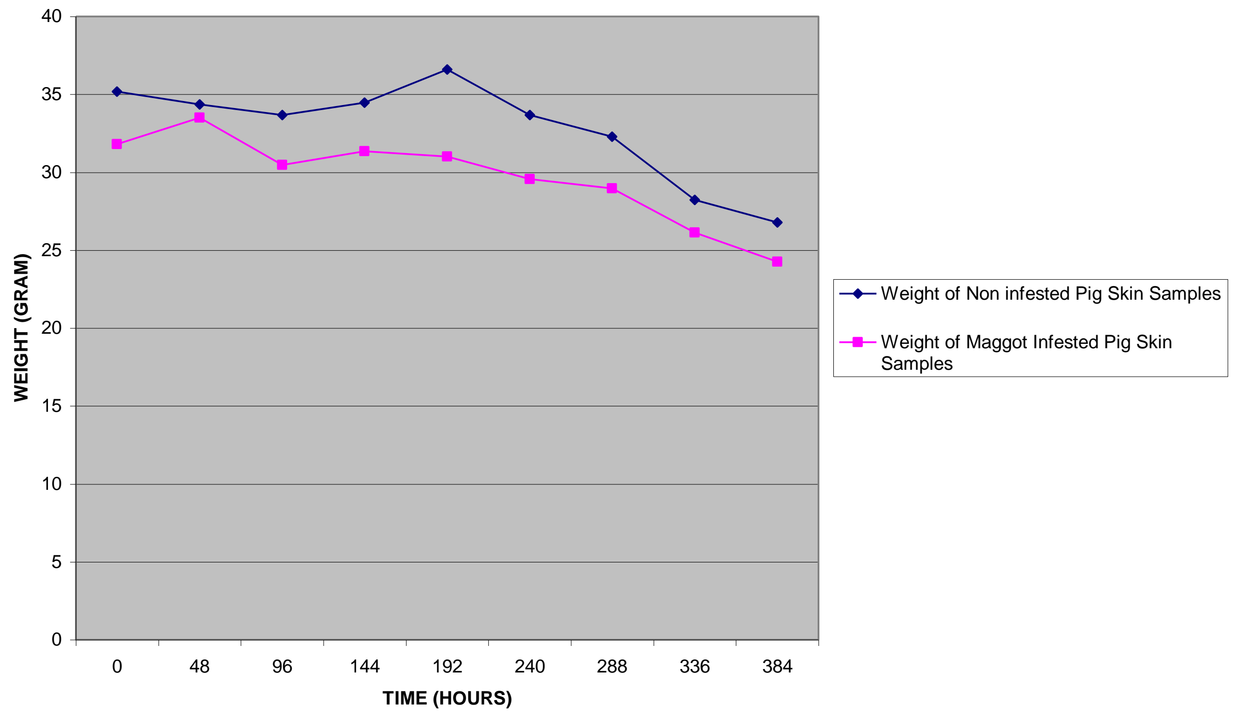


Figure III: Line Graph showing Comparative Changes in mean weights of Non-Infested and Maggot Infested Pig Skin Samples with Time

4.2 INCREASE pH IN MAGGOT'S MEDIUM (LARVAL SECRETION HARVESTED)

WITH TIME

Six big size test tubes were used to flood and wash off the corresponding six groups of equal number of maggots dwelling in an atmosphere saturated with the smell of putrid meat and periodically at the interval of thirty minutes that each group of maggots was washed off. The pH values of the effluents from the six groups were individually measured. Table III showed that there is progressive increase in the pH of the effluents from the maggots' environment that contain their secretion. From an initial pH of 5.93 the mean pH went up to 7.85 indicating clear alkalinizing changes from maggots' secretion. The formula that relates pH and pOH is used to indirectly estimate the pOH that clearly shows decrease with time (Table IV). There is significant and positive correlation between pH of the effluents and time ($P<0.05$). Conversely, there is a negative and significant correlation ($P<0.05$) between pOH and time. Table V showed that the calculated equivalent base concentration $[OH^-]$ has positive correlation with time that is statistically significant ($P < 0.05$).

TABLE III OF MEASURED $_{p}H$ CHANGE OF LARVAL SECRETION WITH TIME

S/NO	TIME MINUTE	MEASURED $_{P}$H	SEM
1.	0	5.93	± 0.00
2	30	7.12	± 0.16
3	60	7.50	± 0.13
4	90	7.60	± 0.14
5	120	7.70	± 0.14
6	150	7.79	± 0.45
7	180	7.82	± 0.13
8	210	7.85	± 0.13

4.3 DECREASES IN CALCULATED pOH CHANGE IN EFFLUENT FROM MAGGOTS (LARVAL SECRETION) WITH TIME

Using the relationship pH + pOH = 14, the calculated pH is used to evaluate the pOH.

pOH = 14 - pH.

A progressive decrease in pOH signifies increase in alkalinity. From a baseline measurement at zero minute (0 minute) to the two hundred and tenth minute (210 minutes) the pOH changed from 8.07 to 6.15 as shown in table IV. Using the calculated pOH, the estimated hydroxyl ion concentrations [OH-], have been calculated using the formula:

pOH = - log $[OH^-]$

Table V shows the pH, pOH and $[OH^-]$.At zero degree centigrade (0 ^oC) and

ordinary pressure, one volume of water dissolves about 1000 volumes of ammonia gas but only a much smaller quantity of the gas react with protons to give a measurable base concentration through the ionic equilibrium in water. Hence the base concentration and most definitely the ammonia concentration estimated from this work is far below the actual huge concentration present.

TABLE IV OF CALCULATED pOH CHANGE WITH TIME IN THE EFFLUENTS FROM MAGGOTS (LARVAL SECRETION)

S/NO	TIME (MINUTE)	pOH	SEM
1	0	8.07	± 0.000
2	30	6.98	± 0.16
3	60	6.51	± 0.13
4	90	6.40	± 0.14
5	120	6.30	± 0.14
6	150	6.22	± 0.45
7	180	6.18	± 0.13
8	210	6.148	± 0.13

4.4 INCREASE IN BASE CONCENTRATION IN EFFLUENTS FROM MAGGOTS (LARVAL SECRETION)

TABLE V OF CALCULATED BASE CONCENTRATION ([0H] IN MOL/DM^3) CHANGES WITH TIME (MINUTE)

S/NO	TIME (MINUTE)	BASE [OH] (MOL/DM^{-3})	SEM
1	0	1.2×10^{-8}	$\pm 2.53 \times 10^{-25}$
2	30	5.8×10^{-6}	$\pm 3.20 \times 10^{-6}$
3	60	3.3×10^{-6}	$\pm 9.78 \times 10^{-7}$
4	90	2.6×10^{-6}	$\pm 8.75 \times 10^{-7}$
5	120	2.1×10^{-6}	$\pm 7.54 \times 10^{-7}$
6	150	1.7×10^{-6}	$\pm 6.56 \times 10^{-7}$
7	180	1.6×10^{-6}	$\pm 1.28 \times 10^{-7}$
8	210	2.4×10^{-6}	$\pm 2.02 \times 10^{-6}$

4.5 CHANGES IN PH AND POH IN MAGGOTS MEDIUM (LARVAL SECRETION) WITH TIME

TABLE VI OF MEASURED pH and pOH CHANGES WITH TIME AND THE CORRESPONDING CALCULATED BASE CONCENTRATION [OH] IN LARVAL SECRETION

S/No	TIME (MINUTE)	PH	POH	BASE [OH] (MOL/DM^{-3})	SEM
1.	0	5.93	8.07	1.2×10^{-8}	$\pm 2.53 \times 10^{-25}$
2.	30	7.12	6.88	5.8×10^{-6}	$\pm 3.20 \times 10^{-6}$
3.	60	7.50	6.50	3.3×10^{-6}	$\pm 9.78 \times 10^{-7}$
4.	90	7.60	6.40	2.6×10^{-6}	$\pm 8.75 \times 10^{-7}$
5.	120	7.70	6.30	2.1×10^{-6}	$\pm 7.54 \times 10^{-7}$
6.	150	7.78	6.22	1.7×10^{-6}	$\pm 6.56 \times 10^{-7}$
7.	180	7.82	6.18	1.6×10^{-6}	$\pm 1.28 \times 10^{-7}$
8.	210	7.85	6.15	2.4×10^{-6}	$\pm 2.02 \times 10^{-6}$

TABLE VII: H and pOH CHANGES WITH TIME AND THE CORRESPONDING CALCULATED ACID BASE CONCENTRATION $[OH^-]$ IN EFFLUENT FROM MAGGOTS (LARVAL SECRETION)

TABLE OF MEASURED pH and CALCULATED pOH CHANGES WITH TIME AND THE CORRESPONDING CALCULATED ACID BASE CONCENTRATION $[OH^-]$ IN EFFLUENTS FROM MAGGOTS (LARVAL SECRETION)

TIME (minute)	**PH**	**SEM**	**POH**	**SEM**	**$[H^+]$Mol/dm^{-3}**	**SEM**	**$[OH^-]$ Mol/dm^{-3}**	**SEM**
0	5.93	± 0.00	8.07	± 0.00	7.7×10^{-5}	$\pm 2.04\times 10^{-5}$	1.2×10^{-8}	$\pm 2.53\times 10^{-25}$
30	7.12	± 0.16	6.88	± 0.17	1.4×10^{-6}	$\pm 3.00\times 10^{-6}$	5.8×10^{-6}	$\pm 3.20\times 10^{-6}$
60	7.50	± 0.13	6.51	± 0.13	3.2×10^{-7}	$\pm 8.79\times 10^{-8}$	3.3×10^{-6}	$\pm 9.78\times 10^{-7}$
90	7.60	± 0.14	6.40	± 0.14	4.1×10^{-7}	$\pm 1.55\times 10^{-7}$	2.6×10^{-6}	$\pm 8.75\times 10^{-7}$
120	7.70	± 0.14	6.30	± 0.14	5.2×10^{-7}	$\pm 2.82\times 10^{-7}$	2.1×10^{-6}	$\pm 7.54\times 10^{-7}$
160	7.79	± 0.45	6.22	± 0.45	6.9×10^{-7}	$\pm 1.88\times 10^{-7}$	1.7×10^{-6}	$\pm 6.56\times 10^{-7}$
180	7.82	± 0.13	6.18	± 0.13	7.3×10^{-7}	$\pm 2.00\times 10^{-7}$	1.6×10^{-6}	$\pm 1.28\times 10^{-7}$
210	7.85	± 0.13	6.15	± 0.13	1.0×10^{-5}	$\pm 2.63\times 10^{-5}$	2.4×10^{-6}	$\pm 2.02\times 10^{-6}$
Degree of Freedom	$F_{7, 40}$		$F_{7, 40}$		$F_{7, 40}$		$F_{7, 40}$	
F_{cal}	144.118				81.409		9.063	
F_{tab}	2.24				2.24		2.24	

There is significant difference in $[OH^-]$ Concentration with time $p < 0.05$

4.6 TIME DEPENDENT INCREASE IN HAEMOGLOBIN CONCENTRATION IN EFFLUENTS FROM MAGGOTS' MEDIUM (LARVAL SECRETION

The maggots (larvae of *Lucilia sericata*).grown in natural environment of faeces and putrid meat were obtained, washed thoroughly with normal saline and disinfected with Sodium hypochlorite. They were changed to a confined new environment where they were stimulated by the 'smell' of putrid meat in the confinement. Effluents by washing them with normal saline were obtained and they showed the qualitative presence of haemoglobin by Combi 9 strip test which were then quantitatively and colorometrically assayed. Table VIII shows progressive increase in haemoglobin from the effluent of maggots' grown in natural environment. From a baseline at zero minute (0 minute), the haemoglobin concentration increased from 2.85 gram/dl to 5.66 gram/dl at the two hundred and tenth minute (210 minutes).

TABLE VIII OF COLOROMETRIC DETERMINED AND TIME DEPENDENT INCREASE IN HAEMOGLOBIN CONCENTRATION, FROM EFFLUENT OF LARVAE GROWN IN NATURAL ENVIRONMENT

S/NO	TIME (MINUTE)	HAEMOGLOBIN CONCENTRATION (GRAM/DL)
1	0	2.846
2	30	3.77
3	60	4.267
4	90	4.689
5	120	5.070
6	150	5.344
7	180	5.51
8	210	5.663

R = +0.81471

4.7 MAGGOT FACTOR INDUCED PROTEOLYSIS MEASURED AGAINST NATURAL 'DECAY OF PROTEIN ALL AT ROOM TEMPERATURE

Lucilia sericata secretion concentrate (effluent) obtained at the two hundred and tenth minute (210 minutes) was incubated with replicate sample of plasma at room temperature. The plasma protein levels were assayed by Van Slyke's method and the obvious fall in protein concentrations with time were tabulated. Table IX show the progressive proteolysis across a time span of one hundred and twenty minutes (120 minutes) as protein concentration decreases in both the control and the replicate test samples. The means and standard errors were calculated and displayed in table XI. Analysis of variance shows there is a significance difference between the test and the control ($p<0.05$) samples. The extent of protein breakdown is higher in the test sample than the control samples at room temperature.

TABLE IX OF TOTAL PROTEIN CONCENTRATION BY MOORE AND VAN SLYKE METHOD, IN REPLICATES OF PLASMA TEST SAMPLE MIXED WITH MAGGOTS' FACTOR AT ROOM TEMPERATURE

S/No		CHANGES IN TOTAL PLASMA PROTEIN CONCENTRATION (G/DL) IN SERIAL SAMPLES AGAINST TIME (HOURS)					
		0 Hour	24 Hours	48 Hours	74 Hours	96 Hours	120 Hours
1.	Control plasma sample 1	5.6	5.58	5.20	5.10	4.10	3.94
2.	Control plasma sample 2	5.6	5.42	5.40	5.00	4.84	4.80
3.	Control plasma sample 3	5.6	5.20	4.80	4.20	3.80	3.80
4.	Control plasma sample 4	5.6	5.32	5.42	5.40	5.40	5.36
5.	Control plasma sample 5	5.6	5.48	5.19	5.08	4.90	4.80
6.	Replicate1 (test larval extract)	4.83	2.14	1.20	0.55	0.50	0.40
7.	Replicate2	4.60	3.40	0.68	0.54	0.40	0.30
8.	Replicate3	4.33	4.20	4.10	3.60	2.10	0.50
9.	Replicate4	4.27	4.00	2.40	0.80	0.64	0.64
10.	Replicate5	4.20	3.84	3.12	1.07	0.85	0.84

4.8 MAGGOT FACTOR INDUCED PROTEOLYSIS MEASURED AGAINST NATURAL DECAY AT CONTROL TEMPERATURE OF 37°C

Lucilia sericata secretion concentrate (effluent) obtained at the two hundred and tenth minute (210 minutes) was incubated with replicate test samples of plasma at a control temperature of 37°C. The plasma protein levels were assayed by Moore and Van Slyke's method and the obvious fall in protein concentrations with time were observed and tabulated. Table X shows the progressive proteolysis across a time span of one hundred and twenty minutes (120 minutes) as protein concentration decreases in both the control and the replicate test samples. The means and standard errors were calculated and displayed in table XII. Analysis of variance shows there is a significance difference between the test and the control ($p<0.05$) samples. The extent of protein breakdown is higher in the test samples than the control samples at a control temperature of 37°C. The comparison between the means of the tests at room temperature in the month of June and those of control temperature showed no significant difference

TABLE X OF CHANGES IN TOTAL PROTEIN CONCENTRATION DETERMINED BY MOORE AND VAN SLYKE'S METHOD FOR CONTROL AND REPLICATE TEST PLASMA SAMPLES MIXED WITH LARVAL SECRETION AT 37 ^{O}C.

		CHANGES IN TOTAL PLASMA PROTEIN CONCENTRATION (G/DL) IN SERIAL PLASMA SAMPLES AGAINST TIME (HOURS).					
S/No		**0 Hour**	**24 Hours**	**48 Hours**	**96 Hours**	**72 Hours**	**120 Hours**
1	Control plasma sample 1	5.6	5.01	4.05	3.95	4.00	3.02
2	Control plasma sample 2	5.6	5.23	5.16	3.92	5.02	3.88
3	Control plasma sample 3	5.6	5.40	5.18	4.49	4.89	3.87
4	Control plasma sample 4	5.6	4.98	4.83	3.51	4.71	3.05
5	Control plasma sample 5	5.6	5.32	5.10	4.09	4.77	3.66
6	Replicate1(with larva extract)	4.51	3.84	0.95	0.37	0.48	0.21
7	Replicate2	4.42	3.57	1.05	0.28	0.35	0.18
8	Replicate3	4.73	4.49	1.23	0.40	0.46	0.37
9	Replicate4	4.83	3.63	0.88	0.49	0.73	0.21
10	Replicate5	4.67	3.66	1.37	0.38	0.42	0.19

4.9 MAGGOT ENHANCED RATE OF PLASMA PROTEIN DEGRADATION (PROTEOLYSIS)

TABLE XI OF TOTAL PROTEIN CONCENTRATION BY MOORE AND VAN SLYKE'S METHOD ON REPLICATE TEST PLASMA SAMPLES MIXED WITH MAGGOT SECRETION AT ROOM TEMPERATURE

		PROTEIN CONCENTRATION IN CONTROL PLASMA SAMPLE SERIES		PROTEIN CONCENTRATION IN PLASMA TEST SAMPLE SERIES INCUBATED WITH MAGGOT'S FACTOR	
S/No	TIME (HOUR)	SAMPLEMEAN (G/DL)	S.E.M	SAMPLE MEAN	S.E.M
1	0	5.6	0	4.446	0.2627
2	24	5.4	0.1308	3.516	0.8237
3	48	5.202	0.2229	2.3	1.3926
4	72	4.956	0.4017	1.312	1.2973
5	96	4.608	0.5792	0.898	0.6928
6	120	4.54	0.5857	0.536	0.2114

TABLE XII OF MEAN TOTAL PROTEIN CHANGE IN CONTROL AND PLASMA TEST SAMPLES MIXED WITH MAGGOTS' SECRETION. AT CONTROL TEMPERATURE OF 37 ^{O}C

		PLASMA PROTEIN LEVEL IN CONTROL SAMPLES (G/DL)			PLASMA PROTEIN LEVEL IN TEST SAMPLES WITH CRUDE LARVAL EXTRACT (G/DL)		
S/No	TIME (HOUR)	SAMPLE MEAN (G/DL)	S.E.M	% CHANGE IN MEAN	SAMPLE MEAN (G/DL)	S.E.M	% CHANGE IN MEAN
1	0	5.6	0	0	4.63	0.17	0
2	24	5.19	0.186	7.32	3.82	0.39	17.49
3	48	4.86	0.476	1.32	1.09	0.20	76.46
4	72	4.68	0.397	16.43	0.49	0.14	89.42
5	96	3.99	0.35	28.75	0.38	0.08	91.79
6	120	3.49	0.43	37.68	0.23	0.08	95.03

**TABLE XIII OF THE CHANGES IN MEANS OF PLASMA PROTEIN IN CONTROL AND TEST SAMPLES OF PLASMA INCUBATED WITH MAGGOT FACTOR
AT ROOM TEMPERATURE**

S/No	TIME (HOUR)	MEAN PLASMA PROTEIN CONC. IN CONTROL SAMPLES G/DL (G/DL)	MEAN OF PLASMA PROTEIN IN TEST SAMPLE WITH LARVAL EXTRACT. (G/DL)	DEVIATION	$(DEVIATION)^2$ D^2
1.	0	5.60	4.63	0.97	0. 9409
2.	24	5.19	3.82	1.37	1. 876
3.	48	4.86	1.09	3.77	14. 213
4.	72	4.68	0.49	4.19	17. 56
5.	96	3.99	0.38	3.61	13. 03
6.	120	3.49	0.23	3.26	10. 63
		ΣX	Σy	ΣD=17.17	$\Sigma(D^2)$ = 58.249
				=2.86166	

TABLE XIV OF MEANS OF PLASMA PROTEIN CHANGES IN CONTROL AND PLASMA TEST SAMPLES MIXED WITH LARVAL SECRETION

S/NO	TIME (HOUR)	MEAN PLASMA PROTEIN CONC. IN CONTROL SAMPLES (G/DL)	MEAN PLASMA PROTEIN CONC. WITH MAGGOT FACTOR IN SERIAL TEST SAMPLES (G/DL)	DEVIATION (D)	(DEVIATION) (D^2)
1.	0	5.6	4.45	1.15	1.32
2.	24	5.4	3.52	1.88	3.53
3.	48	5.20	2.30	2.90	8.41
4.	72	4.96	1.31	3.65	13.32
5.	96	4.61	0.89	3.72	13.84
6.	120	4.54	0.536	4.00	16.00
		ΣX	ΣY	$\Sigma D = 17.30$	$\Sigma D^2 = 56.42$

4.10 PRESENCE OF AMMONIA BY QUALITATIVE ANALYSIS IN MAGGOT'S SECRETION

QUALITATIVE TEST FOR THE PRESENCE OF AMMONIA

TEST

0.1M hydrochloric acid solution was added to to 0.5 ml of effluent from maggot (secretion) from maggots

OBSERVATION

Cloudy vapour clearly seen on the edge of the test tube

INFERENCE

Presence of ammonia confirmed

TABLE XV: QUALITATIVE TESTS ON EFFLUENT FROM MAGGOTS (LARVAL SECRETION)

S/NO	TEST	OBSERVATION	INFERENCE
1	0.1M hydrochloric acid solution was added to to 0.5 ml of effluent from maggot (secretion) from maggots	Cloudy vapour clearly seen on the edge of the test tube	Presence of ammonia confirmed
2	Medi-Test Combi-9 strip was dipped into the samples containing maggots' secretion from larvae grown human faeces and putrid meat and another batch grown in the laboratory	the colour of blood element (haemoglobin) testing segment changed from colourless to green only from the maggots grown in natural environment	Haemoglobin presence and possibly adsorbed initially from human faeces and putrid meat used to grow the maggots in their natural environment
3	pH measurement on control normal saline samples in an atmosphere of putrid meat	initial pH of 7.2 that has not change within the 300 minutes of the experiment	The smell of the putrid meat that stimulates the maggots does not change pH of effluent from maggots. Any change in pH is due maggot secretion.

4.11 QUALITATIVE TEST SHOWING PRESENCE OF BLOOD ELEMENTS (HAEMOGLOBIN)

MAGGOTS GROWN IN NATURAL ENVIRONMENT (MIXTURE OF FAECES AND PUTRID MEAT)

Dipping of Medi-Test Combi-9 strip was dipped into the samples containing maggots' secretion effluent change the colour of blood (haemoglobin) testing segment from colourless to green showing the presence of the blood element, haemoglobin.

4.12 TEST IN HARVEST FROM MAGGOTS GROWN IN THE LABORATORY IN MIXTURE OF WASHED SAND AND GROUNDNUT POWDER

Medi-Test shows no change in colour on dipping into harvest from this group.

4.13 pH MEASUREMENT ON CONTROL NORMAL SALINE SAMPLES IN AN ATMOSPHERE OF PUTRID MEAT

This has shown an initial pH of 7.2 that has not change within the 300 minutes of the experiment.

4.14 QUALITATIVE ANTIBIOGRAM / ANTIMICROBIAL EFFECT OF MAGGOT SECRETION

1. *Staphylococcus aureus* isolate was sensitive to maggots'secretion samples _abeled 4,8,10,and U. It was resistant to number 1
2. *E. coli* isolates was resistant to all the five crude extracts.
3. Klebsiella Spp. Was sensitive to extracts number 1,4.and 10. It was resistant to number 8 and U.
4. *Pseudomonas aeruginosa* was sensitive to crude extracts 1 and 10. It was resistant to 4,8 and U.

5. Candida spp. Was sensitive to crude extracts number 1,4,8 and 10. It was resistant to U.

4.15 UREA- ELECTROLYTE AND ENZYME PROFILE IN MAGGOT'S SECRETION

Using Spectrophotometer in the Chemical pathology laboratory, the effluents from maggots (secretion) grown in the laboratory, were analyzed qualitatively and quantitatively. At the baseline the zero minute, the measurements are reflective of the composition of normal saline used to wash off and obtain secretion from the maggots. Sodium ions and Chloride ions were present at 39 and 25 Mol/L concentrations respectively. Other components from the maggots started showing up after thirty minutes from the maggots. Urea, creatinine, total protein, bilirubin and amylase were detected and assayed to the two hundred and tenth minute as shown in table XVII. All the components increased and analysis of variance showed that the increases in the substances were statistically significant ($P<0.05$).

TABLE XVI SHOWING UREA- ELECTROLYTE AND ENZYME PROFILE IN MAGGOT'S SECRETION

	Mol/L								
TIME (Minutes)	Na^+	K^+	Cl^-	HCO_3^-	UREA	CREAT	TP	Bil	Amylase
0	39	0	25	<10	0	0	0	0	0
30	42	0.7	29	<10	2.4	0	1.1	0	40
60	45	0.9	34	<10	3.1	56	1.8	0	69
90	48	1.3	37	<10	3.5	7.9	1.8	0.7	74
120	60	1.4	43	<10	3.5	9.8	2.1	0.9	120
150	89	1.9	49	<10	3.9	12.9	2.2	1.2	168
180	108	2.1	87	<10	4.1	16.8	2.2	1.4	194
210	117	2.2	106	<10	4.5	18.8	2.3	1.74	216

Significant $p< 0.05$ and time dependent increase in secretion of electrolytes , metabolites and enzyme.

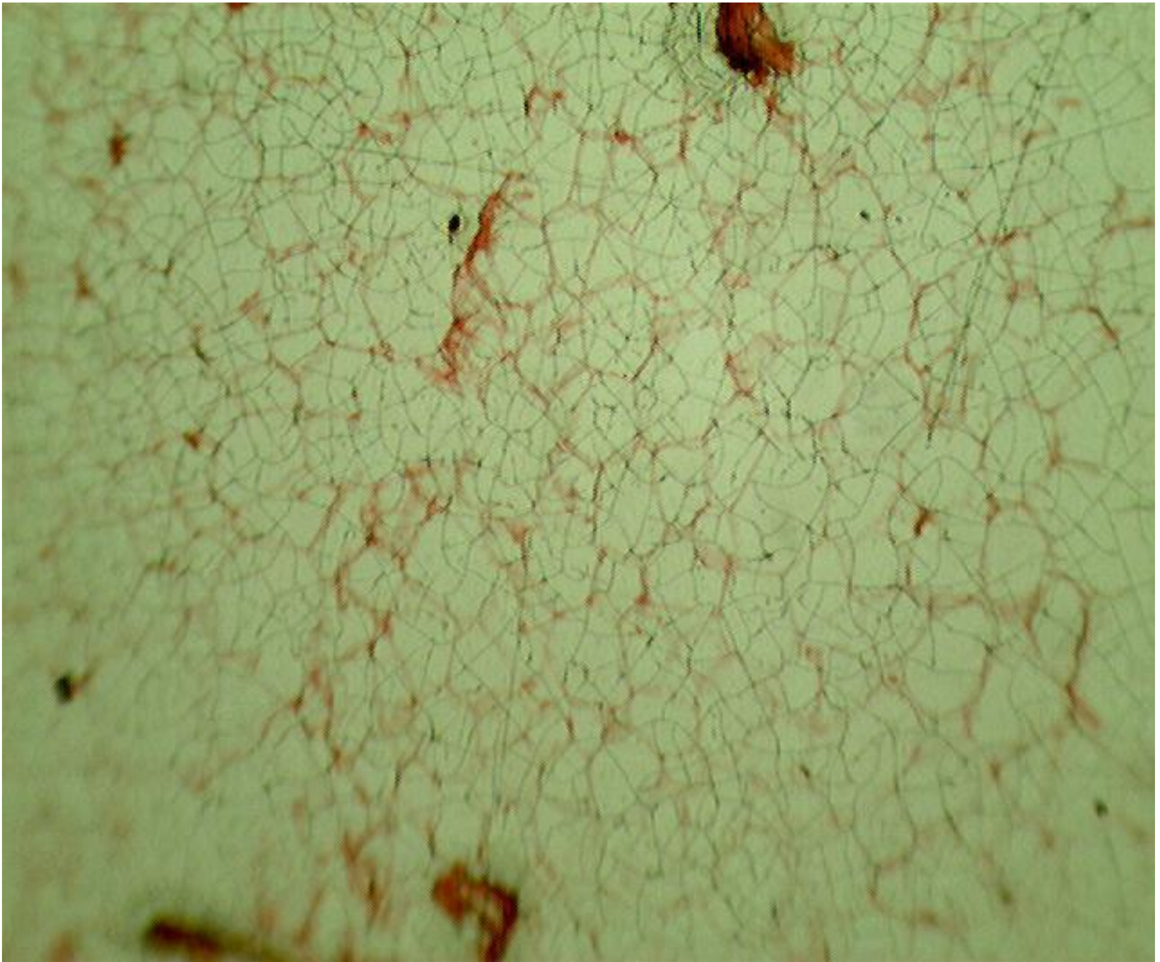


Plate X: Histology of Maggot Infested Pig Skin Sample At O Hour

Faintly staining mature adipocytes. Scattered fragments of eosinophilic necrotic debris are present.

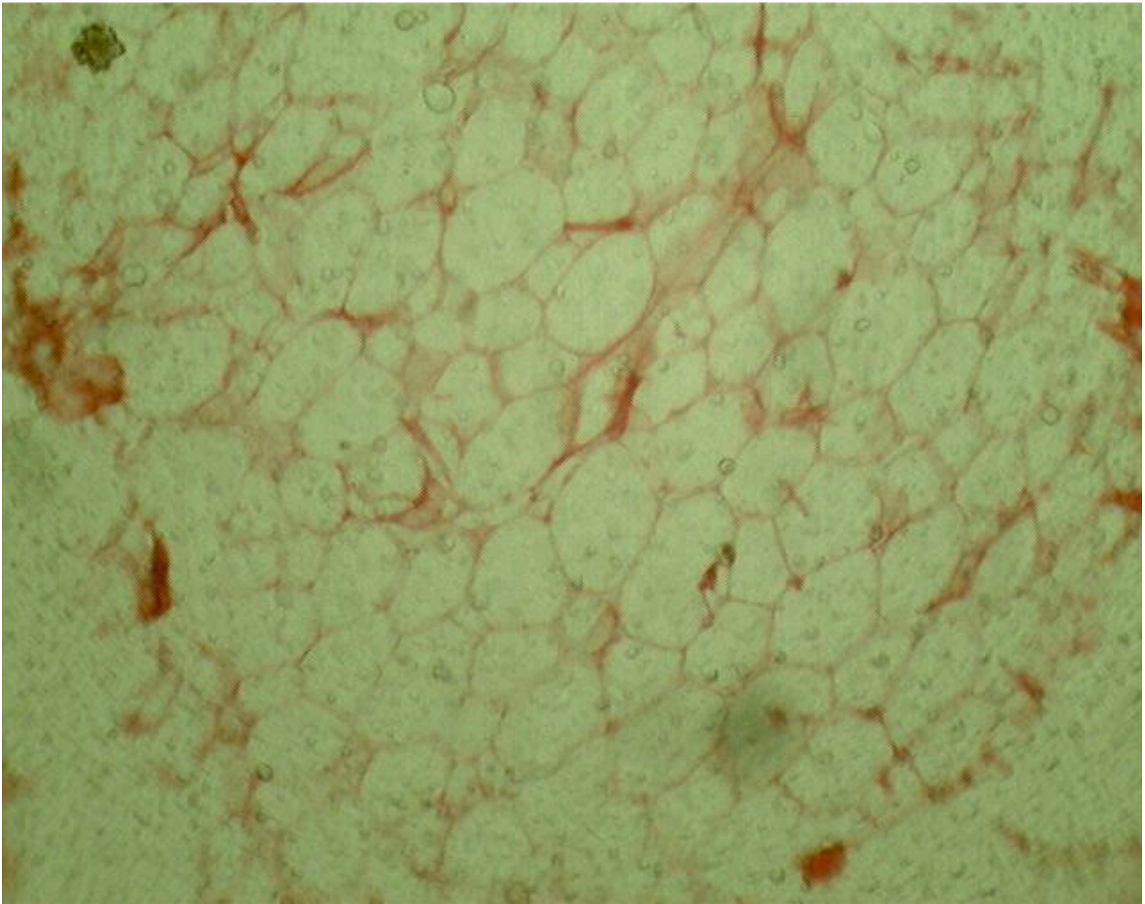


Plate XI: Histology of Non- Infested Pig Skin Sample At 0 Hour

No necrosis. Microscopy shows sheets of large mature adipocytes with clear cytoplasm and peripherally displaced nuclei

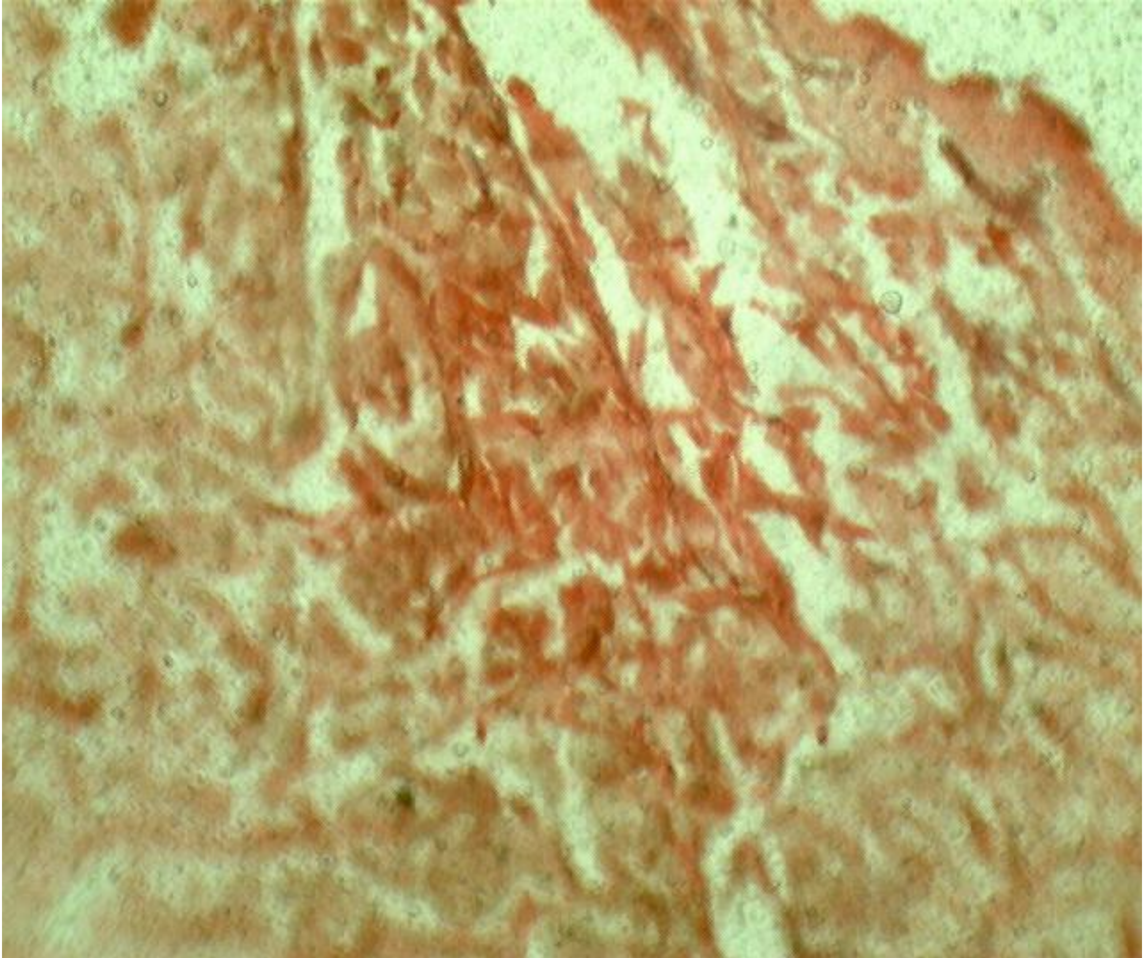


Plate XII: Histology of Maggot Infested Pig Skin Sample After 48 Hour

Moderate necrosis. Detailed cellular structure is not seen. A blood vessel is outlined against a background of a sill cohesive necrotic tissue. The higher magnification show eosinophilic bands of partially digested collagen separated by clear spaces.

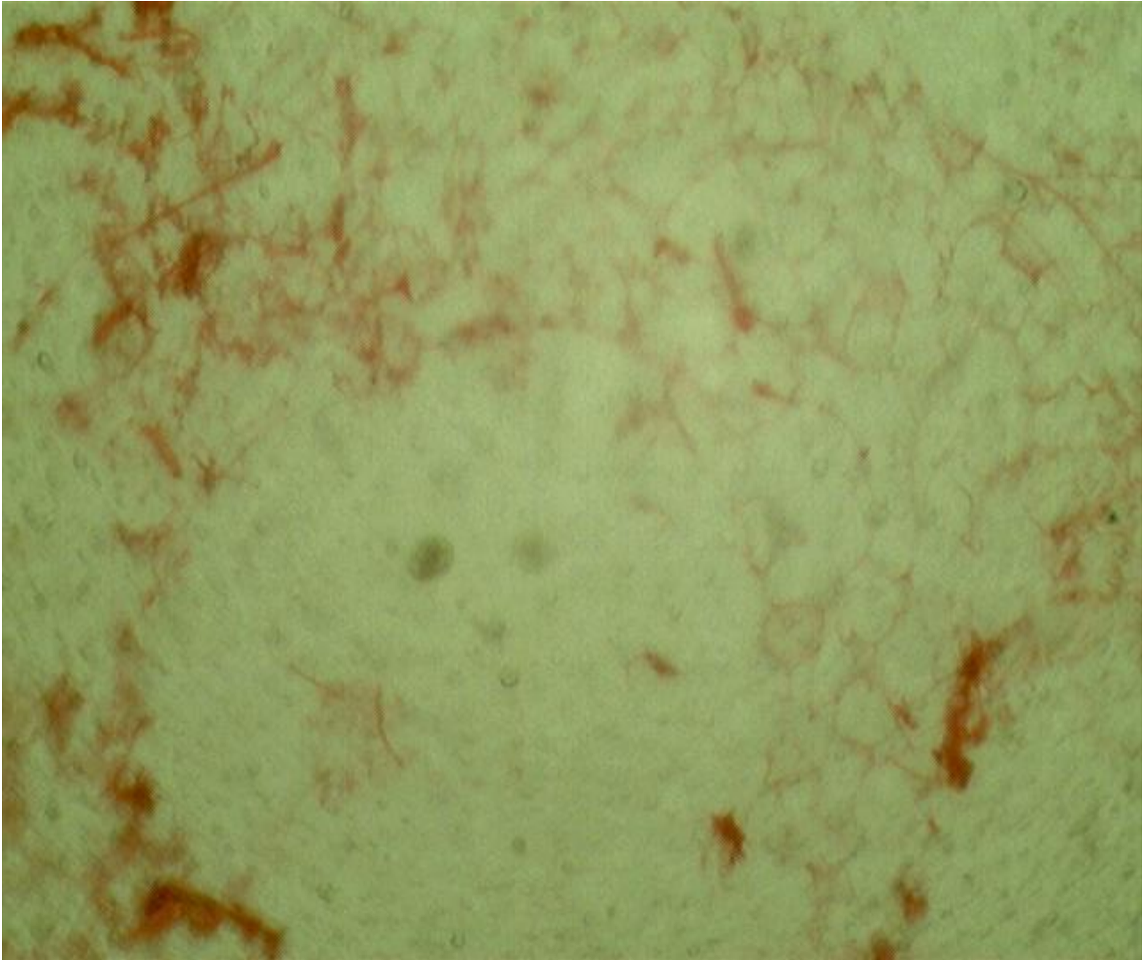


Plate XIII: Histology of Non- Infested Pig Skin After 48 Hours

Mild necrosis. A few mature adipocytes are seen the cytoplasm of some of which have ruptured and coalesced to form microscopic pseudocysts. Few eosinophilic cellular debris are seen.

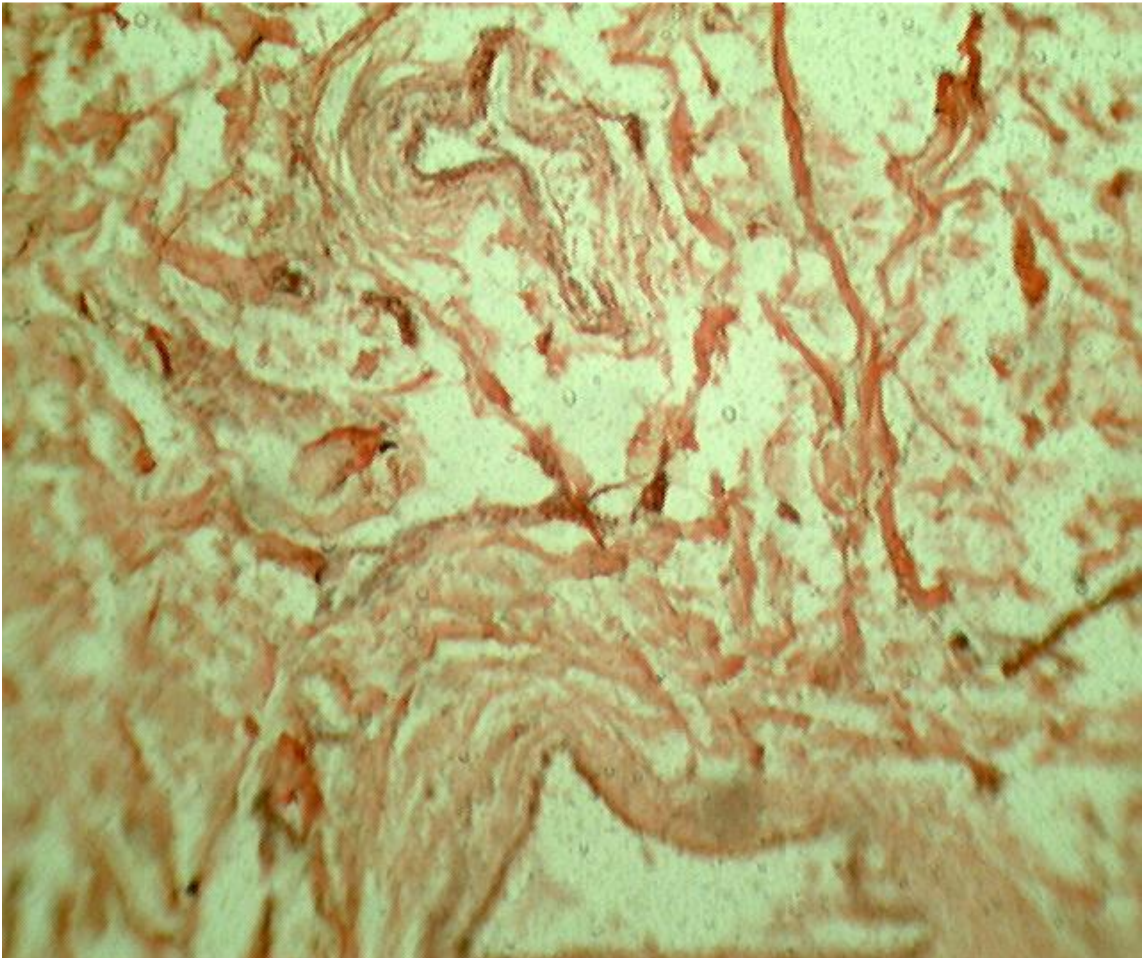


Plate XIV: Histology of Maggot Infested Pig Skin Sample After 96 Hour

Mild necrosis. Partially necrotic fibrocollagenous stroma. Thick walled blood vessels (arteries) are seen. There is marked loss of substance (vacuolation) of the stroma.

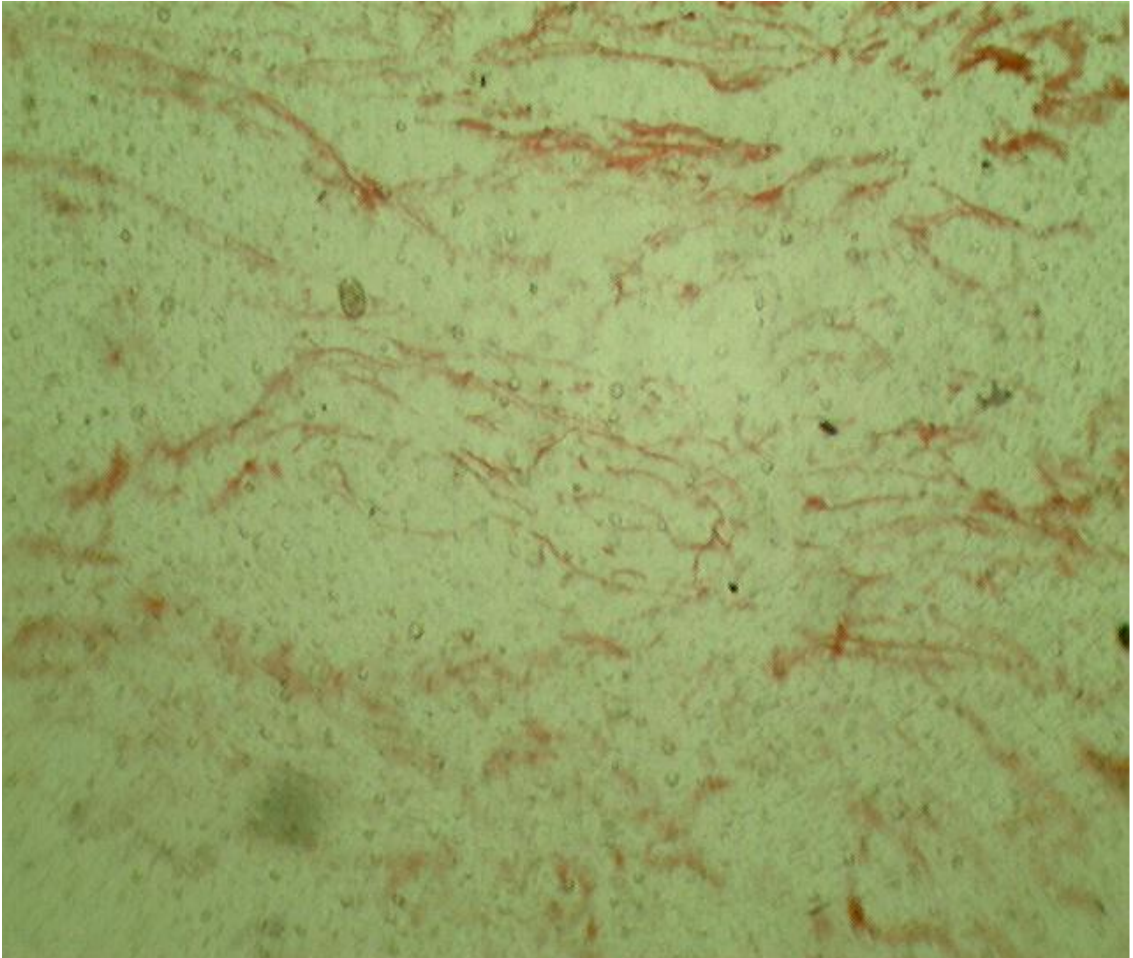


Plate XV: Histology of Non- Infested Pig Skin Sample After 96 Hour

Severe necrosis. Only a few strands of eosinophilic debris is present

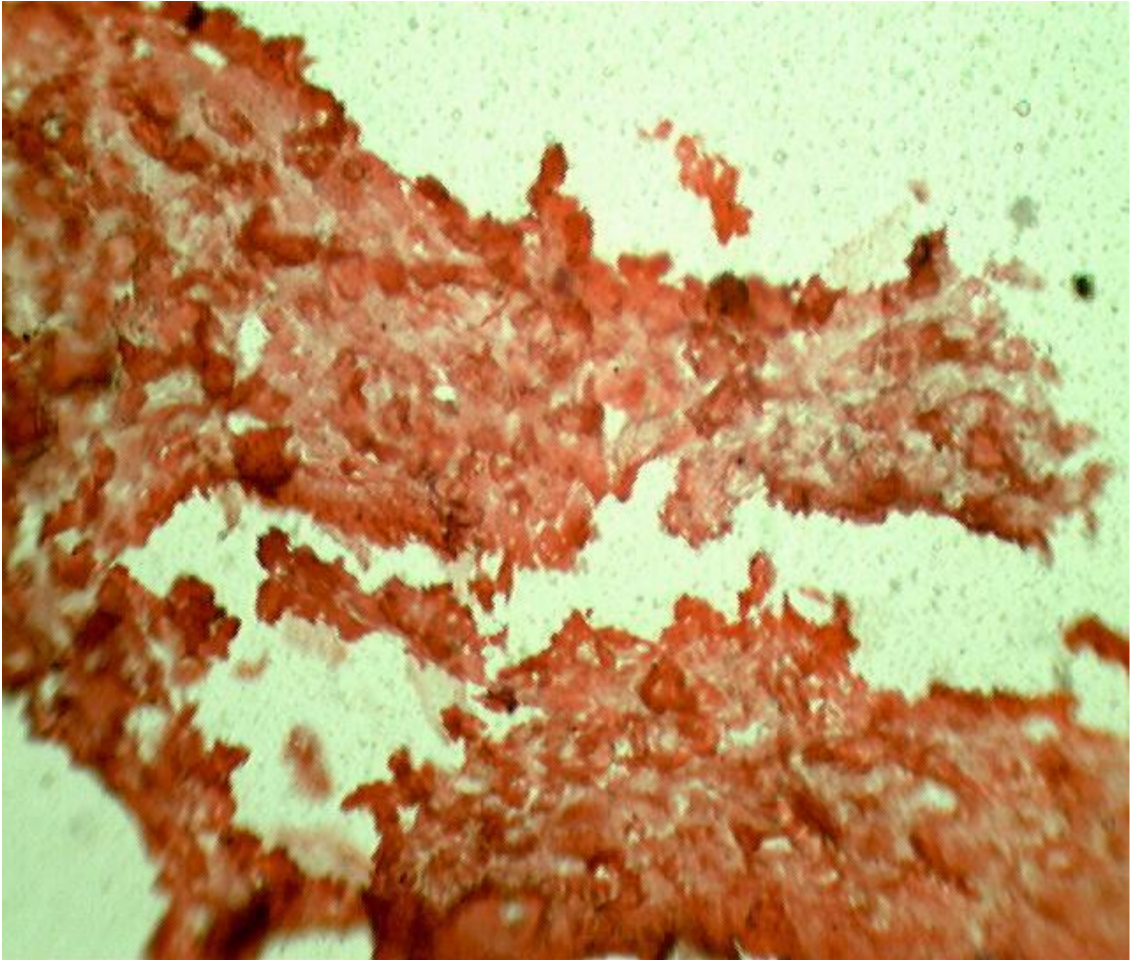


Plate XVI: Histology of Maggot Infested Pig Skin Sample After 144 Hour

Severe necrosis. Microscopy shows a structureless cohesive necrotic debris.

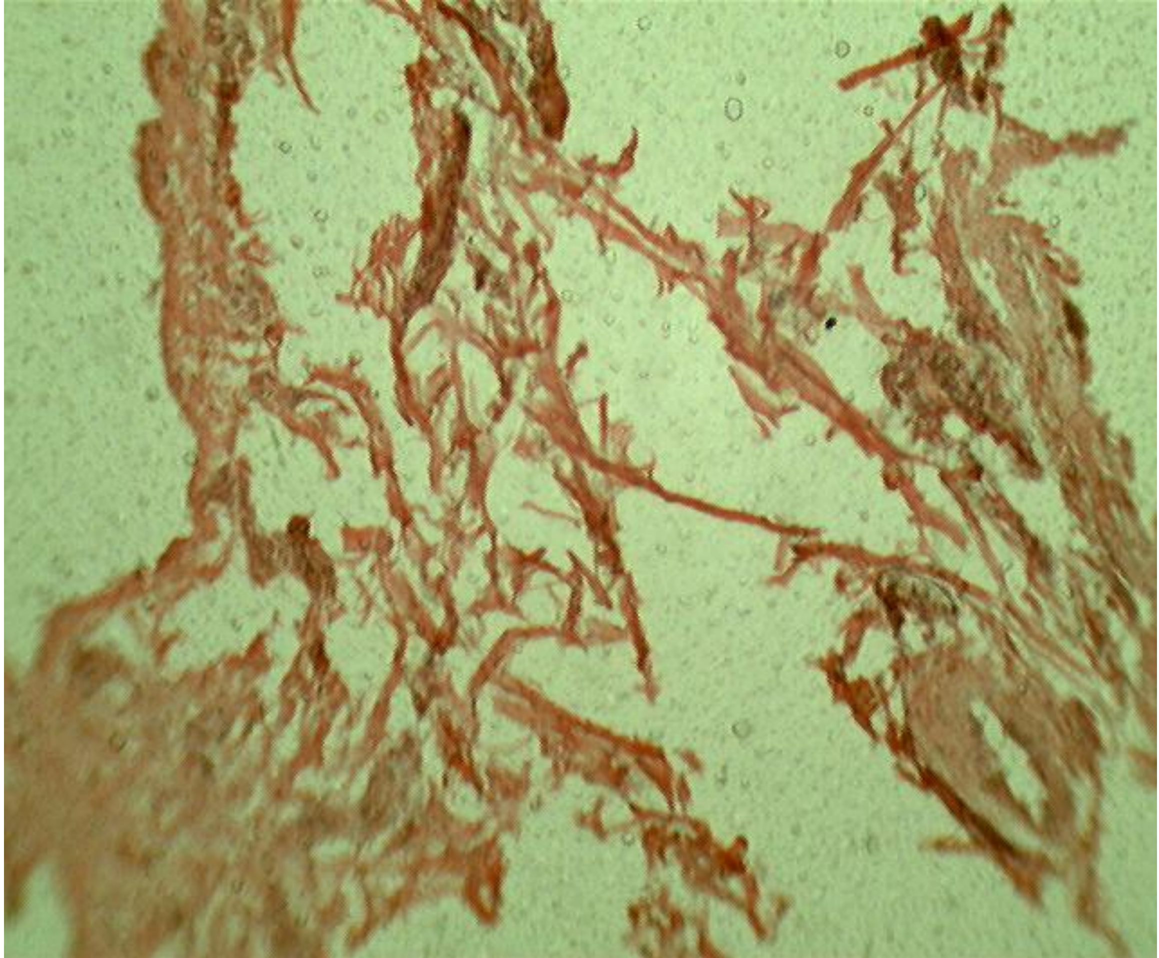


Plate XVII: Histology of Non- Infested Pig Skin Sample After 144 Hour

Moderate necrosis. Moderate amount of necrotic debris present. Remnants of a partially degenerated blood vessel are seen at a higher magnification.

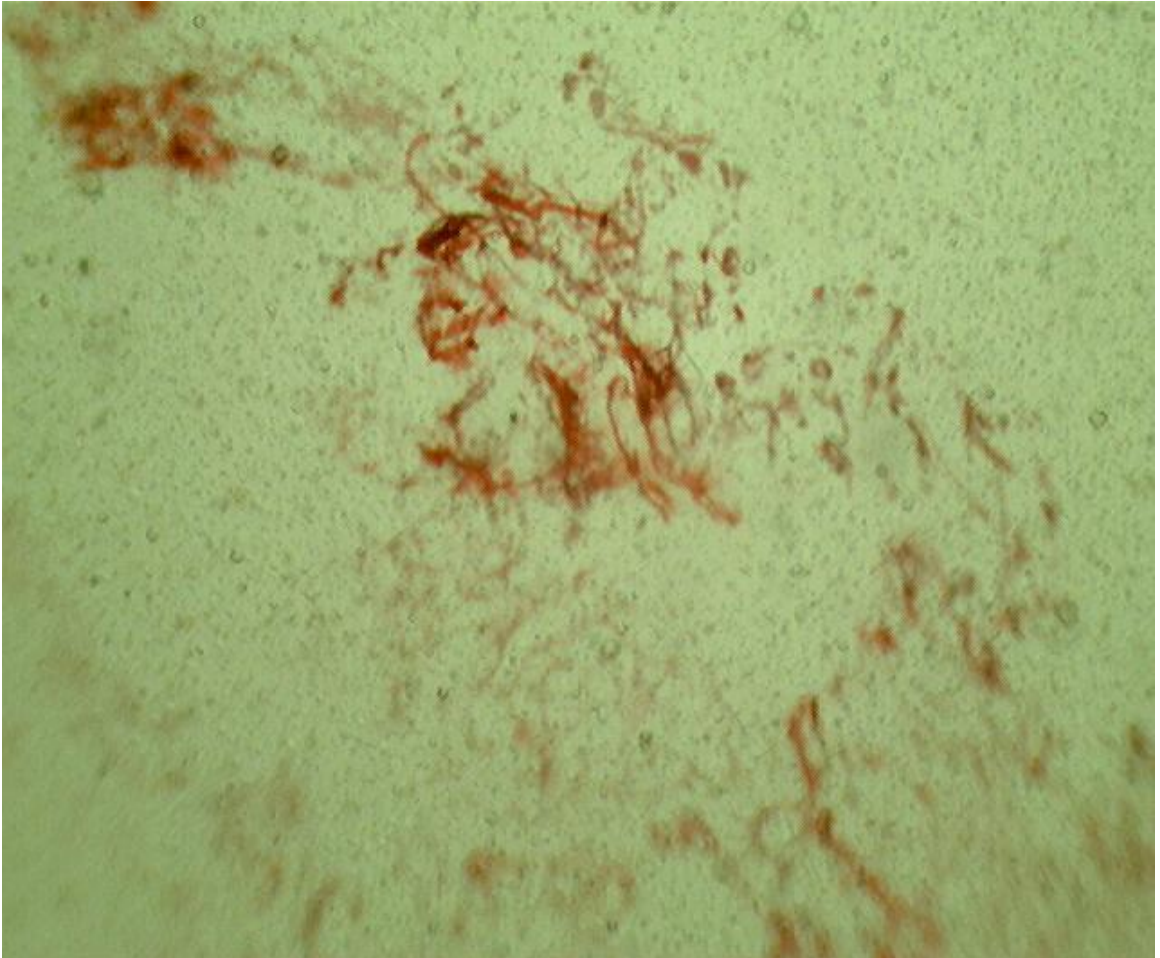


Plate XVIII: Histology of Maggot Infested Pig Skin Sample After 288 Hour

Severe necrosis. Microscopy shows only a few fragments of necrotic cells.

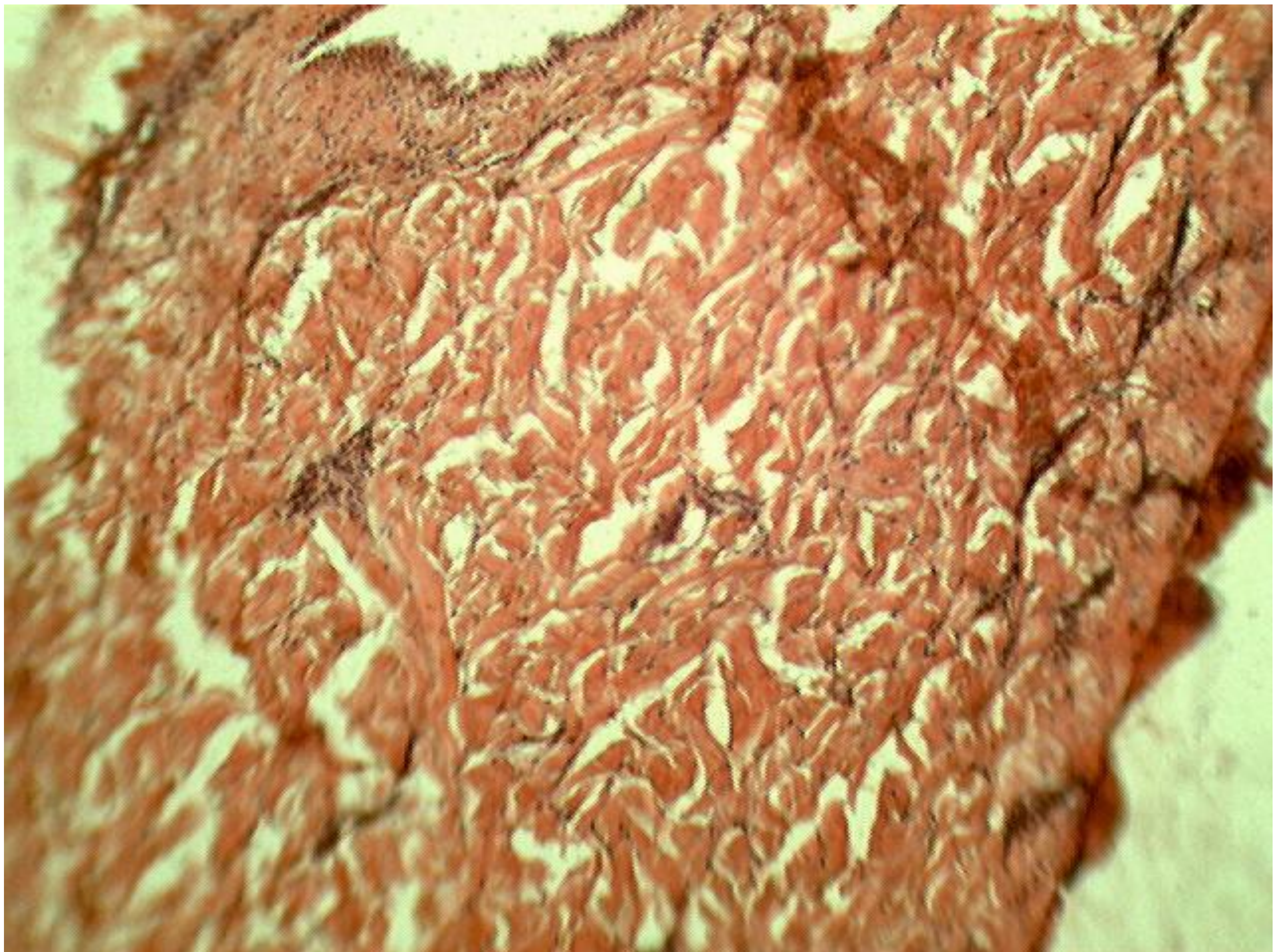


Plate XIX: Histology of Non-Infested Pig Skin Sample After 288 Hours

Microscopy shows structureless fragments of eosinophilic debris with marked variability in intensity of staining.

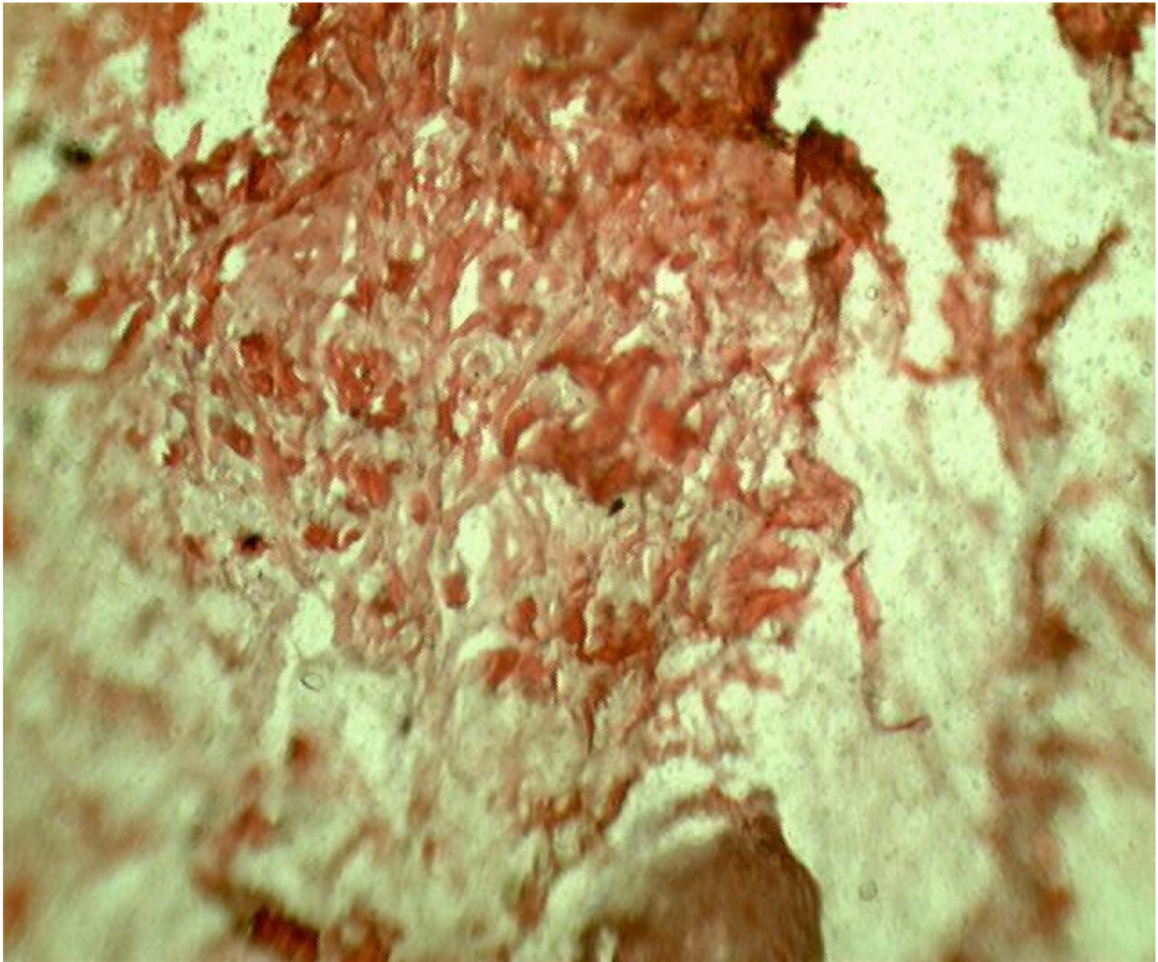


Plate XX: Histology of Non- Infested Pig Skin Sample Showing Severe Necrosis After 336 Hours

Mild necrosis. The nuclei of the cells are recognizable and broad band of collagen band are visible. Chronic inflammatory cells are seen.

Plate XXI: Maggot Infested-Pig Skin Samples Labelled with Different Coloured Rubber Bands

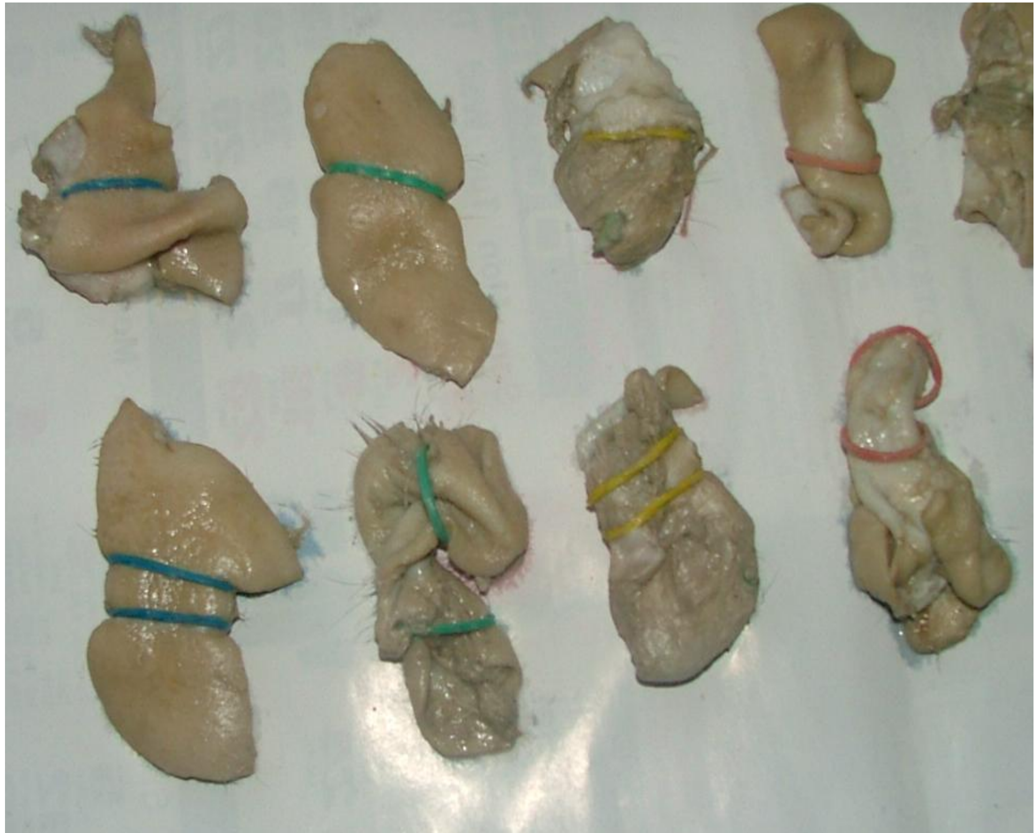


Plate XXII: Maggot-Infested Pig Skin Samples Lebelled with Different Coloured Rubber Bands

Plate XXIII. Picture of larvae of *Scarabaeus sacer* (Dung Beetle larvae).

Result of Proximate Analysis on the Larvae of *Scarabaeus sacer*

Proximate analysis on the dried larvae of *Scabaeus sacer* showed Calcium at 51.2% composition, protein 28.5 %, carbohydrate 0.3%, fat 7.1% and Iron 12.8%.

CHAPTER FIVE
DISCUSSION, CONCLUSION, SUMMARY AND CONTRIBUTION TO KNOWLEDGE

5.1 DISCUSSION

The study on the larvae of *Lucilia sericata* was designed such that a modified method of a laboratory growing of the maggots is introduced and tested and the larval secretion harvested for biochemical and histological studies. The viable methods developed have led to the cultivation of the maggots in the laboratory, the harvesting of their secretion and its biochemical analysis as well as the histology of pigskin model infested by the maggots. Proximate analysis of the larvae of *Scarabaeus sacer* has shown important nutritional measurements that support possible use in correction of some nutritional disorders.

5.1.1 Growing Maggots in the Laboratory

This work successfully carried out the culture of larvae of *Lucilia sericata* in the laboratory. Washed sharp sand provided a protective environment for the larvae to thrive. The silica base sand is a poor conductor and insulated the larvae as they hatched and grew. The porous nature of the sand ensured that water that was sprinkled sieved down to nourish the larvae that burrowed down into the soil. The groundnut powder used provided to the larvae the basic organic compounds and the macronutrients required such as carbohydrate, protein and lipid. This is in contrast to the use of sawdust as base and honey for the growth of the larvae. The method by Sherman and Wylie 1996, was used to attract wild flies to get the initial deposit of eggs of flies. About a ten gram (10 gram) piece of putrid beef was obtained and exposed outside to attract wild flies.

5.1.2 Increase in pH and Base Concentration in Maggot's Secretion

Biological cells/tissues require certain range of pH for optimal function. PH derangements in extreme directions outside the physiological range cause dysfunctions and ultimately result in the death of cell(s). In Humans, the average physiological pH of arterial blood is 7.4 ± 0.05 for optimal function of the body tissues. Maggots in this work have been shown to release alkaline rich factor, which increases the pH of their environment.

A qualitative analysis showed the presence of ammonia in the solution, which may be responsible for the increase in alkalinity of the solution as shown by the increase in pH, decrease in pOH and increase in the calculated amount of base concentration. There was increase in base concentration from 2.5x10-^{9} to 2.63x10^{-9} Mol/dm^{-3} (positive correlation of +0.97724 with the time graded harvest from the maggots medium, $p < 0.001$). The method employed in estimating the base concentration was an indirect one by measurement of pH and calculating pOH. At 0°C and ordinary pressure, one volume of water dissolves about 1000 volumes of ammonia gas but only a much smaller quantity of the gas reacted with protons to give a measurable base concentration through the ionic equilibrium in water. Hence the base concentration and most definitely the ammonia concentration estimated from this work was far below the actual huge concentration present.

The presence of ammonia partly explains the antimicrobial actions of maggots when feeding on necrotic tissue during MDT. It is possible that these antimicrobial actions were by direct toxic effect of NH_3 on bacteria, fungi and other susceptible micro-organisms. The addition by maggots of alkaline rich secretion may also be serving as a modifier of the environmental pH, possibly an alkaline medium more

suitable for enzymic components of maggots' secretion to exert their optimal antimicrobial activity.

An advanced study using more sophisticated technique may reveal the definite mode of action and the exact purpose of this alkalinising property of maggot derived factors.

5.1.3 The Qualitative Antimicrobial Property of Maggots' Secretion

Staphylococcus aureus, klebsiella, pseudomonas aerugenosa and *candida* species were sensitive to crude extract systematically picked from maggot medium at 0, 90, 210, 270 and 300 minutes from the start of the experiment. Significant zones of inhibition (> 2 mm) zones in this double blind study has shown no relationship with the concentration of the maggot factors in virtually all the cases mentioned. *Eschericia-coli* were resistant to all concentrations of the maggot's secretion. This is probably not surprising since both maggots and *Eschericia-coli* share environment and both dwell in faeces and decomposing, the *Eschericia-coli* evolved to become resistant to maggots' secretion. This has implication in the use of maggots for debridement of wound infected with *Eschericia-coli*. Appropriate antibiotics that *E. coli* is sensitive to must be used to prevent further impairment to wound healing by the infection.

5.1.4 Presence of Haemoglobin in Harvest from Maggots Grown in Natural Environment

Qualitative test on maggot grown from natural environment using Medi-test combi-9 strip, has shown the presence of blood elements. Despite prior and thorough washing and disinfections using sodium hypochlorite, blood element(s) (haemoglobin and /or myoglobin) have been shown to be released only by maggots grown in natural environment (mixture of putrid meat, fish and faeces) and not from those grown in the laboratory. This is an important finding as it has provided a warning and restriction as

only laboratory grown maggots may be used in MDT. The inappropriate use of maggots from the natural environment may, in some ways serve as means of transmitting disease through the blood element that could have been absorbed and/or ingested from the natural environment or any other contaminated source only to be released in the subsequent environment, that is the wound. Maggots used for MDT should not be used for subsequent MDT on another patient for the aforementioned reason as this in vitro study has shown. This is contribution to our current stage of knowledge of MDT.

5.1.5 Maggot Derived Factor Significantly Enhances the Rate of Proteolysis of Plasma Protein

Proteolytic activity in MDT is an important mechanism which helps in breaking down structural protein of the necrotic tissue and this results in liquefaction of the necrotic debris. Proteolytic activities also extend to plasma proteins that are abundantly found in as part of the exudates formed on wounds. Exudates are protein rich in nature and are released in reaction to tissue injury in the process of sustaining wounds. The maggot-derived factors here are shown to enhance proteolytic activity by significantly increasing ($p < 0.05$) the rate of plasma protein degradation in test samples with maggot’s extract added. The degree of proteolytic activity at various temperature conditions showed that incubation at 37°C did not enhance the rate of proteolysis. The concept of optimal enzymic activity at optimal temperature for a biological system is not clearly demonstrable here. However, MDT may work even better in controlled and optimal temperature and may possibly give better results in tropical than in temperate climate/regions. It is left to be studied whether warmer tropical climates have better prospect for MDT than cooler temperate climate.

Further, test and analysis may provide further characterization of the maggot derived factor and may ultimately lead to extraction, purification and the creation of maggot's factor impregnated dressing material. But it is already known that maggot's secretion contains allantoin, an important keratolytic agent used extensively in cosmetic product to promote exfoliation of the skin. This material may be a product of enzymic action on degradative products of necrotic tissue derived nucleotide that may include purine and pyrimidine bases. The picture is that of efficiency in biological system where even seemingly waste product is utilized in a useful way(s).

5.1.6 Antinecrobiosis Effect of Maggot Infestation on Experimental Pig Skin

Pig skin samples infested with Maggot would ordinarily be thought to have a faster and more extensive degradation that may be reflected as faster loss of weight and poor texture as well as loss of consistency than in non infested pig skin. This work reveals the contrary. Maggot infested Pig skin showed slower rate of decomposition as well as better consistency than non infested pig skin samples (faster necrobiosis by weight loss) that is statistically significant ($p<0.05$). Histological sections showed mark acantholysis much more in non-infested pig skin sample than infested pig skin sample. These results probably suggest that maggots wipe off the more aggressive necrosis causing microbes on the tissue specimens. These are likely bacteria and other microbes that inhabit the same tissue specimen. This work has already shown the antimicrobial property of maggots' secretion.

Calcium was shown to constitute 51.2 %, protein 28.5 %, carbohydrate 0.3%, fat 7.1% and Iron 12.8% contents were shown in the proximate analysis of dried sample of the Scarab which suggest use to avert or treat medical conditions associated with deficiency of calcium, iron and protein.

5.2 CONCLUSION

This work has successfully developed a cost effective method of growing the larvae of *Lucilia sericata* using washed sharp sand mixed with groundnut powder and tinge of iodized salt. A successful harvest of maggots' secretion was made using the smell of putrid meat to induce chemotaxis and the release of saprophagous secretion. The pH change was an obvious change indicating addition of bodily secretion. Ammonia was demonstrated in the maggots' secretion and this is an antibacterial material that is useful in the control of infection when the larvae are used for maggot debridement therapy (MDT). Maggot infested necrotic tissue certainly have better consistency and breakdown more orderly than non-infested tissue whose breakdown is accelerated by aggressive bacterial activities. This explains further the mechanism of the beneficial effects of maggots on wounds and the information can also be of use to forensic specialists working on decomposing tissue. Maggots' secretion has electrolytes, enzymes and metabolite similar to other body secretions like saliva and bile. The secretion has semblance of digestive juice and serves digestive function.The presence of haemoglobin in secretion of maggots grown in Human faeces and decomposing flesh of animals is an affirmation that such should not be used for treatment on humans. The laboratory grown and sterilized variants are most appropriate for the hospital use.

The rich calcium protein and iron level in larvae of *Scarabaeus sacer* has thrown more light on their possible use for treatment of calcium deficiency disorders where dietary calcium supplementation are required as much as iron deficiency anaemia requiring dietary iron supplementation. In environments where the larvae are

in abundance, they can serve as cheap source of protein for growing children and those suffering from Protein Energy Malnutrition (PEM).

5.3 SUMMARY OF FINDINGS

The larvae of *Lucilia sericata* were successfully grown in successive generations using an innovative and modified adaptation of the method described by Sherman and Wylie (1996). The method involved the use of washed sharp sand mixed with groundnut powder. Water was sprinkled twice daily to keep the sand just moist and not flooded.

The secretion from the maggots was obtained by stimulating them with the smell of putrid meat.

The secretion from maggots has been shown to have alkaline property and it is probably due to the rich ammonia concentration that has been tested qualitatively using Hydrochloric acid. The Alkalinizing property has been shown to be time dependent in a statistically significant level ($P<0.05$).

Maggots' secretion has been shown to have in vitro antibacterial property against *Staphylococcus aureus*, *Candida albican*, *Klebsiella* species. *Pseudomonas aereginosa* but most *Escherichia coli* were resistant to the maggots' secretion.

Maggots' secretion has been found to contain electrolytes like Na^+, K^+, $HCO3^-$, Cl^- and the enzyme amylase, and metabolite like creatinine, protein and bilirubin.

The presence of Maggots on experimental pigskin model demonstrated antinecrobiosis effect histologically. Voracious necrosis was prominent in Non-infested pigskin model. The necrotic slough offs did not significantly alter the variation in the weight of the pigskin specimen in both the non-infested and the maggots infested group of tissue.

Maggots' secretion has been shown to enhance the proteolysis of plasma protein both at room temperature and at 37 ^{o}C all at a statistically significant level ($P<0.05$). There was no significant difference (>0.05) in proteolysis at both the room temperature and the simulated body temperature of 37 ^{o}C.

5.4 CONTRIBUTIONS TO KNOWLEDGE

1 A modified and a cost effective method of growing maggots in the laboratory has been developed in the course of this work. This version uses clean sharp sand (Three Kilogram of the sand to five hundred gram of ground powder and a tinge of iodized salt). These ingredient are substitute to the use of Sawdust and expensive honey by Sherman and Wylie (1996). The larvae thrive excellently.

2 A simple method of harvesting saprophagous secretion of the maggots has been developed and used based on the principle of chemotatic stimulation of the larvae. A plastic bottle is cut to partition the maggot section from the putrid meat section with fenestration to enable air to saturate the maggots.

3 The histology of the nature of tissue necrosis under maggots' infestation has been shown to be more organized with less of the widespread acantholysis and cellular degeneration as seen in non-infested pigskin model by comparison. This is possibly achieved by the maggots' secretion inhibiting the aggressive bacterial effect on tissue breakdown. This finding can be used to improve the explanation on the mechanism of action of Maggots Debridement Therapy (MDT).

4 Maggot secretion has been shown to have constituents similar to other body fluids like saliva, pancreatic juice etc. Electrolyte like K and Na are present and enzymes like amylase were also found.

5 The larvae of *Scarabaeus sacer* when used as food can be a rich source of protein, calcium and iron.

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APPENDIX

IONIC PRODUCT CONSTANT OF WATER.

H_2O --- H^+ + OH^-

$$KC = \frac{[H^+][OH^-]}{[H_2O]}$$

BUT $[H_2O]$ HAS CONSTANT K

$$K_C = \frac{[H^+][OH^-]}{\mathbf{K}}$$

K_C X K = $[H^+][OH^-]$ = K_W = 10^{-14} MOL^2 /DM^{-6} .

K_W is called ionic product constant of water.

PH of water at 330 k is 6.9.

PH = - log [H +]

PH + POH = 14

EXAMPLE: to calculate hydroxyl ion concentration of water at 6.9,

POH = 14 – 6.9 = 7.1

POH = - Log [OH^-]

7.1 = - Log [OH^-]

[OH -] = AntiLog (- 7.1)

1.25×10^{-7} Mol / dm^{-3}

This formula is used to calculate indirectly base [OH^-] concentration in a given solution.

ABBREVIATIONS

< = Less than.

> = Greater than.

N, n = Sample size.

P = Probability.

s.d = Standard deviation.

S.E.M = standard error of mean.

X = mean.

df = Degree of freedom.

Đ = Deviatiation.

Σ = SUM

T = Calculated t – test value.

R = Coefficient of correlation.

√ = Square root

Summarize

Case Processing Summary[a]

	Cases	
	Included	
	N	Percent
Sample Non-infested pig Skin * Time of measurement of Pig skin weight (Hours)	89	89.0%
Weight of Non-infested sample Pig skin * Time of measurement of Pig skin weight (Hours)	89	89.0%

Case Processing Summary[a]

	Cases			
	Excluded		Total	
	N	Percent	N	Percent
Sample Non-infested pig Skin * Time of measurement of Pig skin weight (Hours)	11	11.0%	100	100.0%
Weight of Non-infested sample Pig skin * Time of measurement of Pig skin weight (Hours)	11	11.0%	100	100.0%

a. Limited to first 100 cases.

Case Summaries[a]

			Sample Non-infested pig Skin	Weight of Non-infested sample Pig skin
Time of measurement of Pig skin weight (Hours)	0	1	Pig Skin sample 1	28.80
		2	Pig Skin sample 2	28.60
		3	Pig Skin sample 3	38.11
		4	Pig Skin sample 4	39.01
		5	Pig Skin sample 5	32.12
		6	Pig Skin sample 6	36.70
		7	Pig Skin sample 7	42.64
		8	Pig Skin sample 8	39.53

Case Summaries[a]

				Sample Non-infested pig Skin	Weight of Non-infested sample Pig skin
Time of measurement of Pig skin weight (Hours)	0	9		Pig Skin sample 9	38.04
		Total	N	9	9
	48	1		Pig Skin sample 1	29.60
		2		Pig Skin sample 2	28.80
		3		Pig Skin sample 3	37.00
		4		Pig Skin sample 4	36.80
		5		Pig Skin sample 5	30.55
		6		Pig Skin sample 6	36.15
		7		Pig Skin sample 7	41.28
		8		Pig Skin sample 8	38.10
		9		Pig Skin sample 9	37.90
		10		Pig Skin sample 10	27.40
		Total	N	10	10
	96	1		Pig Skin sample 1	28.35
		2		Pig Skin sample 2	27.10
		3		Pig Skin sample 3	35.69
		4		Pig Skin sample 4	37.05
		5		Pig Skin sample 5	30.06
		6		Pig Skin sample 6	35.29
		7		Pig Skin sample 7	41.15
		8		Pig Skin sample 8	37.92
		9		Pig Skin sample 9	37.93
		10		Pig Skin sample 10	26.13
		Total	N	10	10

Case Summaries[a]

			Sample Non-infested pig Skin	Weight of Non-infested sample Pig skin
Time of measurement of Pig skin weight (Hours)	144	1	Pig Skin sample 1	30.01
		2	Pig Skin sample 2	27.71
		3	Pig Skin sample 3	37.09
		4	Pig Skin sample 4	37.27
		5	Pig Skin sample 5	31.70
		6	Pig Skin sample 6	35.92
		7	Pig Skin sample 7	41.99
		8	Pig Skin sample 8	38.68
		9	Pig Skin sample 9	37.70
		10	Pig Skin sample 10	26.72
		Total N	10	10
	192	1	Pig Skin sample 1	33.50
		2	Pig Skin sample 2	29.59
		3	Pig Skin sample 3	42.18
		4	Pig Skin sample 4	38.10
		5	Pig Skin sample 5	35.82
		6	Pig Skin sample 6	36.22
		7	Pig Skin sample 7	43.54
		8	Pig Skin sample 8	40.01
		9	Pig Skin sample 9	38.47
		10	Pig Skin sample 10	28.49
		Total N	10	10

Case Summaries[a]

				Sample Non-infested pig Skin	Weight of Non-infested sample Pig skin
Time of measurement of Pig skin weight (Hours)	240	1		Pig Skin sample 1	30.01
		2		Pig Skin sample 2	27.09
		3		Pig Skin sample 3	37.27
		4		Pig Skin sample 4	34.52
		5		Pig Skin sample 5	32.48
		6		Pig Skin sample 6	34.27
		7		Pig Skin sample 7	41.61
		8		Pig Skin sample 8	36.81
		9		Pig Skin sample 9	33.98
		10		Pig Skin sample 10	28.69
		Total	N	10	10
	288	1		Pig Skin sample 1	36.92
		2		Pig Skin sample 2	25.09
		3		Pig Skin sample 3	37.37
		4		Pig Skin sample 4	31.97
		5		Pig Skin sample 5	31.02
		6		Pig Skin sample 6	31.11
		7		Pig Skin sample 7	40.01
		8		Pig Skin sample 8	32.42
		9		Pig Skin sample 9	30.82
		10		Pig Skin sample 10	26.09
		Total	N	10	10

Case Summaries[a]

			Sample Non-infested pig Skin	Weight of Non-infested sample Pig skin
Time of measurement of Pig skin weight (Hours)	336	1	Pig Skin sample 1	23.82
		2	Pig Skin sample 2	22.19
		3	Pig Skin sample 3	34.74
		4	Pig Skin sample 4	28.84
		5	Pig Skin sample 5	27.69
		6	Pig Skin sample 6	31.50
		7	Pig Skin sample 7	35.19
		8	Pig Skin sample 8	29.51
		9	Pig Skin sample 9	26.29
		10	Pig Skin sample 10	22.45
		Total N	10	10
	384	1	Pig Skin sample 1	21.49
		2	Pig Skin sample 2	20.92
		3	Pig Skin sample 3	31.93
		4	Pig Skin sample 4	27.51
		5	Pig Skin sample 5	25.50
		6	Pig Skin sample 6	30.91
		7	Pig Skin sample 7	35.20
		8	Pig Skin sample 8	28.23
		9	Pig Skin sample 9	25.63
		10	Pig Skin sample 10	20.58
		Total N	10	10
	Total	N	89	89

a. Limited to first 100 cases.

Descriptives

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation
Sample Non-infested pig Skin	90	1	10	5.50	2.89
Weight of Non-infested sample Pig skin	90	20.58	43.54	32.8054	5.6052
Time of measurement of Pig skin weight (Hours)	89	0	384	194.16	123.63
Valid N (listwise)	89				

Means

Case Processing Summary

	Cases	
	Included	
	N	Percent
Sample Non-infested pig Skin * Time of measurement of Pig skin weight (Hours)	89	89.0%
Weight of Non-infested sample Pig skin * Time of measurement of Pig skin weight (Hours)	89	89.0%

Case Processing Summary

	Cases			
	Excluded		Total	
	N	Percent	N	Percent
Sample Non-infested pig Skin * Time of measurement of Pig skin weight (Hours)	11	11.0%	100	100.0%
Weight of Non-infested sample Pig skin * Time of measurement of Pig skin weight (Hours)	11	11.0%	100	100.0%

Report

Time of measurement of Pig skin weight (Hours)		Sample Non-infested pig Skin	Weight of Non-infested sample Pig skin
0	Mean	5.00	35.9500
	N	9	9
	Std. Deviation	2.74	4.9541
48	Mean	5.50	34.3580
	N	10	10
	Std. Deviation	3.03	4.7965
96	Mean	5.50	33.6670
	N	10	10
	Std. Deviation	3.03	5.2877
144	Mean	5.50	34.4790
	N	10	10
	Std. Deviation	3.03	5.1081
192	Mean	5.50	36.5920
	N	10	10
	Std. Deviation	3.03	4.9660
240	Mean	5.50	33.6730
	N	10	10
	Std. Deviation	3.03	4.3455
288	Mean	5.50	32.2820
	N	10	10
	Std. Deviation	3.03	4.7423
336	Mean	5.50	28.2220
	N	10	10
	Std. Deviation	3.03	4.6776
384	Mean	5.50	26.7900
	N	10	10
	Std. Deviation	3.03	4.9535
Total	Mean	5.45	32.8560
	N	89	89
	Std. Deviation	2.86	5.6164

Oneway

ANOVA

		Sum of Squares	df	Mean Square
Sample Non-infested pig Skin	Between Groups	2.022	8	.253
	Within Groups	720.000	80	9.000
	Total	722.022	88	
Weight of Non-infested sample Pig skin	Between Groups	873.884	8	109.235
	Within Groups	1901.945	80	23.774
	Total	2775.829	88	

ANOVA

		F	Sig.
Sample Non-infested pig Skin	Between Groups	.028	1.000
	Within Groups		
	Total		
Weight of Non-infested sample Pig skin	Between Groups	4.595	.000
	Within Groups		
	Total		

T-Test

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Weight of Non-infested sample Pig skin	32.8560	89	5.6164	.5953
	Time of measurement of Pig skin weight (Hours)	194.16	89	123.63	13.11

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Weight of Non-infested sample Pig skin & Time of measurement of Pig skin weight (Hours)	89	-.451	.000

Paired Samples Test

		Paired Differences		
		Mean	Std. Deviation	Std. Error Mean
Pair 1	Weight of Non-infested sample Pig skin - Time of measurement of Pig skin weight (Hours)	-161.3013	126.2658	13.3841

Paired Samples Test

		Paired Differences	
		95% Confidence Interval of the Difference	
		Lower	Upper
Pair 1	Weight of Non-infested sample Pig skin - Time of measurement of Pig skin weight (Hours)	-187.8995	-134.7032

Paired Samples Test

		t	df	Sig. (2-tailed)
Pair 1	Weight of Non-infested sample Pig skin - Time of measurement of Pig skin weight (Hours)	-12.052	88	.000

Correlations

Correlations

		Weight of Non-infested sample Pig skin	Time of measurement of Pig skin weight (Hours)
Weight of Non-infested sample Pig skin	Pearson Correlation	1.000	-.451**
	Sig. (2-tailed)	.	.000
	N	90	89
Time of measurement of Pig skin weight (Hours)	Pearson Correlation	-.451**	1.000
	Sig. (2-tailed)	.000	.
	N	89	89

**. Correlation is significant at the 0.01 level (2-tailed).

Regression

Variables Entered/Removed[b]

Model	Variables Entered	Variables Removed	Method
1	Time of measurement of Pig skin weight (Hours)[a]	.	Enter

a. All requested variables entered.

b. Dependent Variable: Weight of Non-infested sample Pig skin

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.451[a]	.203	.194	5.0421

a. Predictors: (Constant), Time of measurement of Pig skin weight (Hours)

ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	564.077	1	564.077	22.186	.000[a]
	Residual	2211.752	87	25.422		
	Total	2775.829	88			

a. Predictors: (Constant), Time of measurement of Pig skin weight (Hours)

b. Dependent Variable: Weight of Non-infested sample Pig skin

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients
		B	Std. Error	Beta
1	(Constant)	36.832	.999	
	Time of measurement of Pig skin weight (Hours)	-2.048E-02	.004	-.451

Coefficients[a]

Model		t	Sig.
1	(Constant)	36.867	.000
	Time of measurement of Pig skin weight (Hours)	-4.710	.000

a. Dependent Variable: Weight of Non-infested sample Pig skin

Correlations

Correlations

		Weight of Non-infested sample Pig skin	Time of measurement of Pig skin weight (Hours)
Weight of Non-infested sample Pig skin	Pearson Correlation	1.000	-.451**
	Sig. (2-tailed)	.	.000
	N	90	89
Time of measurement of Pig skin weight (Hours)	Pearson Correlation	-.451**	1.000
	Sig. (2-tailed)	.000	.
	N	89	89

**. Correlation is significant at the 0.01 level (2-tailed).

Summarize

Case Processing Summary[a]

	Cases	
	Included	
	N	Percent
Weight of Non-infested sample Pig skin (Gram) * Replicate sample Non-infested pig Skin	90	90.0%
Time of measurement of Pig skin weight (Hours) * Replicate sample Non-infested pig Skin	90	90.0%
Weight of Maggot infested Pig Skin (Gram) * Replicate sample Non-infested pig Skin	90	90.0%

Case Processing Summary[a]

	Cases			
	Excluded		Total	
	N	Percent	N	Percent
Weight of Non-infested sample Pig skin (Gram) * Replicate sample Non-infested pig Skin	10	10.0%	100	100.0%
Time of measurement of Pig skin weight (Hours) * Replicate sample Non-infested pig Skin	10	10.0%	100	100.0%
Weight of Maggot infested Pig Skin (Gram) * Replicate sample Non-infested pig Skin	10	10.0%	100	100.0%

a. Limited to first 100 cases.

Case Summaries[a]

				Weight of Non-infested sample Pig skin (Gram)	Time of measurement of Pig skin weight (Hours)
Replicate sample Non-infested pig Skin	Pig Skin sample 1	1		28.80	0
		2		29.60	48
		3		28.35	96
		4		30.01	144
		5		33.50	192
		6		30.01	240
		7		36.92	288
		8		23.82	336
		9		21.49	384
		Total	N	9	9
	Pig Skin sample 2	1		28.60	0
		2		28.80	48
		3		27.10	96
		4		27.71	144
		5		29.59	192
		6		27.09	240
		7		25.09	288
		8		22.19	336
		9		20.92	384
		Total	N	9	9
	Pig Skin sample 3	1		38.11	0
		2		37.00	48
		3		35.69	96
		4		37.09	144
		5		42.18	192
		6		37.27	240
		7		37.37	288
		8		34.74	336
		9		31.93	384
		Total	N	9	9
	Pig Skin sample 4	1		39.01	0
		2		36.80	48
		3		37.05	96
		4		37.27	144
		5		38.10	192
		6		34.52	240
		7		31.97	288
		8		28.84	336
		9		27.51	384
		Total	N	9	9
	Pig Skin sample 5	1		32.12	0
		2		30.55	48
		3		30.06	96
		4		31.70	144
		5		35.82	192
		6		32.48	240
		7		31.02	288
		8		27.69	336
		9		25.50	384
		Total	N	9	9

Case Summaries[a]

				Weight of Non-infested sample Pig skin (Gram)	Time of measurement of Pig skin weight (Hours)
Replicate sample Non-infested pig Skin	Pig Skin sample 6	1		36.70	0
		2		36.15	48
		3		35.29	96
		4		35.92	144
		5		36.22	192
		6		34.27	240
		7		31.11	288
		8		31.50	336
		9		30.91	384
		Total	N	9	9
	Pig Skin sample 7	1		42.64	0
		2		41.28	48
		3		41.15	96
		4		41.99	144
		5		43.54	192
		6		41.61	240
		7		40.01	288
		8		35.19	336
		9		35.20	384
		Total	N	9	9
	Pig Skin sample 8	1		39.53	0
		2		38.10	48
		3		37.92	96
		4		38.68	144
		5		40.01	192
		6		36.81	240
		7		32.42	288
		8		29.51	336
		9		28.23	384
		Total	N	9	9
	Pig Skin sample 9	1		38.04	0
		2		37.90	48
		3		37.93	96
		4		37.70	144
		5		38.47	192
		6		33.98	240
		7		30.82	288
		8		26.29	336
		9		25.63	384
		Total	N	9	9
	Pig Skin sample 10	1		28.31	0
		2		27.40	48
		3		26.13	96
		4		26.72	144
		5		28.49	192
		6		28.69	240
		7		26.09	288
		8		22.45	336
		9		20.58	384
		Total	N	9	9
	Total	N		90	90

Case Summaries[a]

				Weight of Maggot infested Pig Skin (Gram)
Replicate sample Non-infested pig Skin	Pig Skin sample 1	1		39.40
		2		39.60
		3		36.50
		4		37.59
		5		35.78
		6		33.82
		7		32.71
		8		30.04
		9		29.19
		Total	N	9
	Pig Skin sample 2	1		33.90
		2		35.00
		3		31.65
		4		32.00
		5		32.00
		6		31.31
		7		30.06
		8		29.29
		9		27.32
		Total	N	9
	Pig Skin sample 3	1		32.20
		2		34.00
		3		31.61
		4		32.39
		5		32.22
		6		29.81
		7		29.23
		8		24.72
		9		22.63
		Total	N	9
	Pig Skin sample 4	1		30.40
		2		32.48
		3		29.59
		4		30.03
		5		30.05
		6		28.11
		7		24.99
		8		20.11
		9		19.18
		Total	N	9
	Pig Skin sample 5	1		23.40
		2		26.30
		3		23.64
		4		25.25
		5		24.88
		6		24.29
		7		23.39
		8		21.79
		9		20.91
		Total	N	9

Case Summaries[a]

				Weight of Maggot infested Pig Skin (Gram)
Replicate sample Non-infested pig Skin	Pig Skin sample 6	1		34.70
		2		36.30
		3		32.68
		4		33.32
		5		33.08
		6		32.08
		7		31.93
		8		31.00
		9		29.89
		Total	N	9
	Pig Skin sample 7	1		27.70
		2		29.45
		3		25.70
		4		27.19
		5		27.17
		6		26.53
		7		27.18
		8		24.62
		9		22.47
		Total	N	9
	Pig Skin sample 8	1		39.60
		2		38.44
		3		35.00
		4		35.78
		5		34.87
		6		32.22
		7		31.80
		8		29.60
		9		28.23
		Total	N	9
	Pig Skin sample 9	1		34.10
		2		38.10
		3		35.66
		4		36.03
		5		36.28
		6		34.44
		7		35.30
		8		29.09
		9		24.08
		Total	N	9
	Pig Skin sample 10	1		22.50
		2		25.45
		3		22.60
		4		23.86
		5		23.71
		6		23.05
		7		23.12
		8		21.12
		9		18.67
		Total	N	9
	Total	N		90

a. Limited to first 100 cases.

Frequencies

Statistics

		Weight of Non-infested sample Pig skin (Gram)	Weight of Maggot infested Pig Skin (Gram)	Time of measurement of Pig skin weight (Hours)
N	Valid	90	90	90
	Missing	10	10	10

Frequency Table

Weight of Non-infested sample Pig skin (Gram)

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	20.58	1	1.0	1.1	1.1
	20.92	1	1.0	1.1	2.2
	21.49	1	1.0	1.1	3.3
	22.19	1	1.0	1.1	4.4
	22.45	1	1.0	1.1	5.6
	23.82	1	1.0	1.1	6.7
	25.09	1	1.0	1.1	7.8
	25.50	1	1.0	1.1	8.9
	25.63	1	1.0	1.1	10.0
	26.09	1	1.0	1.1	11.1
	26.13	1	1.0	1.1	12.2
	26.29	1	1.0	1.1	13.3
	26.72	1	1.0	1.1	14.4
	27.09	1	1.0	1.1	15.6
	27.10	1	1.0	1.1	16.7
	27.40	1	1.0	1.1	17.8
	27.51	1	1.0	1.1	18.9
	27.69	1	1.0	1.1	20.0
	27.71	1	1.0	1.1	21.1
	28.23	1	1.0	1.1	22.2
	28.31	1	1.0	1.1	23.3
	28.35	1	1.0	1.1	24.4
	28.49	1	1.0	1.1	25.6
	28.60	1	1.0	1.1	26.7
	28.69	1	1.0	1.1	27.8
	28.80	2	2.0	2.2	30.0
	28.84	1	1.0	1.1	31.1
	29.51	1	1.0	1.1	32.2
	29.59	1	1.0	1.1	33.3
	29.60	1	1.0	1.1	34.4
	30.01	2	2.0	2.2	36.7
	30.06	1	1.0	1.1	37.8
	30.55	1	1.0	1.1	38.9

Weight of Non-infested sample Pig skin (Gram)

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	30.82	1	1.0	1.1	40.0
	30.91	1	1.0	1.1	41.1
	31.02	1	1.0	1.1	42.2
	31.11	1	1.0	1.1	43.3
	31.50	1	1.0	1.1	44.4
	31.70	1	1.0	1.1	45.6
	31.93	1	1.0	1.1	46.7
	31.97	1	1.0	1.1	47.8
	32.12	1	1.0	1.1	48.9
	32.42	1	1.0	1.1	50.0
	32.48	1	1.0	1.1	51.1
	33.50	1	1.0	1.1	52.2
	33.98	1	1.0	1.1	53.3
	34.27	1	1.0	1.1	54.4
	34.52	1	1.0	1.1	55.6
	34.74	1	1.0	1.1	56.7
	35.19	1	1.0	1.1	57.8
	35.20	1	1.0	1.1	58.9
	35.29	1	1.0	1.1	60.0
	35.69	1	1.0	1.1	61.1
	35.82	1	1.0	1.1	62.2
	35.92	1	1.0	1.1	63.3
	36.15	1	1.0	1.1	64.4
	36.22	1	1.0	1.1	65.6
	36.70	1	1.0	1.1	66.7
	36.80	1	1.0	1.1	67.8
	36.81	1	1.0	1.1	68.9
	36.92	1	1.0	1.1	70.0
	37.00	1	1.0	1.1	71.1
	37.05	1	1.0	1.1	72.2
	37.09	1	1.0	1.1	73.3
	37.27	2	2.0	2.2	75.6
	37.37	1	1.0	1.1	76.7
	37.70	1	1.0	1.1	77.8
	37.90	1	1.0	1.1	78.9
	37.92	1	1.0	1.1	80.0
	37.93	1	1.0	1.1	81.1
	38.04	1	1.0	1.1	82.2
	38.10	2	2.0	2.2	84.4
	38.11	1	1.0	1.1	85.6
	38.47	1	1.0	1.1	86.7
	38.68	1	1.0	1.1	87.8
	39.01	1	1.0	1.1	88.9
	39.53	1	1.0	1.1	90.0
	40.01	2	2.0	2.2	92.2
	41.15	1	1.0	1.1	93.3
	41.28	1	1.0	1.1	94.4
	41.61	1	1.0	1.1	95.6
	41.99	1	1.0	1.1	96.7
	42.18	1	1.0	1.1	97.8
	42.64	1	1.0	1.1	98.9
	43.54	1	1.0	1.1	100.0
	Total	90	90.0	100.0	
Missing	System	10	10.0		
Total		100	100.0		

Weight of Maggot infested Pig Skin (Gram)

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	18.67	1	1.0	1.1	1.1
	19.18	1	1.0	1.1	2.2
	20.11	1	1.0	1.1	3.3
	20.91	1	1.0	1.1	4.4
	21.12	1	1.0	1.1	5.6
	21.79	1	1.0	1.1	6.7
	22.47	1	1.0	1.1	7.8
	22.50	1	1.0	1.1	8.9
	22.60	1	1.0	1.1	10.0
	22.63	1	1.0	1.1	11.1
	23.05	1	1.0	1.1	12.2
	23.12	1	1.0	1.1	13.3
	23.39	1	1.0	1.1	14.4
	23.40	1	1.0	1.1	15.6
	23.64	1	1.0	1.1	16.7
	23.71	1	1.0	1.1	17.8
	23.86	1	1.0	1.1	18.9
	24.08	1	1.0	1.1	20.0
	24.29	1	1.0	1.1	21.1
	24.62	1	1.0	1.1	22.2
	24.72	1	1.0	1.1	23.3
	24.88	1	1.0	1.1	24.4
	24.99	1	1.0	1.1	25.6
	25.25	1	1.0	1.1	26.7
	25.45	1	1.0	1.1	27.8
	25.70	1	1.0	1.1	28.9
	26.30	1	1.0	1.1	30.0
	26.53	1	1.0	1.1	31.1
	27.17	1	1.0	1.1	32.2
	27.18	1	1.0	1.1	33.3
	27.19	1	1.0	1.1	34.4
	27.32	1	1.0	1.1	35.6
	27.70	1	1.0	1.1	36.7
	28.11	1	1.0	1.1	37.8
	28.23	1	1.0	1.1	38.9
	29.09	1	1.0	1.1	40.0
	29.19	1	1.0	1.1	41.1
	29.23	1	1.0	1.1	42.2
	29.29	1	1.0	1.1	43.3
	29.45	1	1.0	1.1	44.4
	29.59	1	1.0	1.1	45.6
	29.60	1	1.0	1.1	46.7
	29.81	1	1.0	1.1	47.8
	29.89	1	1.0	1.1	48.9
	30.03	1	1.0	1.1	50.0
	30.04	1	1.0	1.1	51.1
	30.05	1	1.0	1.1	52.2
	30.06	1	1.0	1.1	53.3
	30.40	1	1.0	1.1	54.4
	31.00	1	1.0	1.1	55.6
	31.31	1	1.0	1.1	56.7
	31.61	1	1.0	1.1	57.8
	31.65	1	1.0	1.1	58.9
	31.80	1	1.0	1.1	60.0
	31.93	1	1.0	1.1	61.1

Weight of Maggot infested Pig Skin (Gram)

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	32.00	2	2.0	2.2	63.3
	32.08	1	1.0	1.1	64.4
	32.20	1	1.0	1.1	65.6
	32.22	2	2.0	2.2	67.8
	32.39	1	1.0	1.1	68.9
	32.48	1	1.0	1.1	70.0
	32.68	1	1.0	1.1	71.1
	32.71	1	1.0	1.1	72.2
	33.08	1	1.0	1.1	73.3
	33.32	1	1.0	1.1	74.4
	33.82	1	1.0	1.1	75.6
	33.90	1	1.0	1.1	76.7
	34.00	1	1.0	1.1	77.8
	34.10	1	1.0	1.1	78.9
	34.44	1	1.0	1.1	80.0
	34.70	1	1.0	1.1	81.1
	34.87	1	1.0	1.1	82.2
	35.00	2	2.0	2.2	84.4
	35.30	1	1.0	1.1	85.6
	35.66	1	1.0	1.1	86.7
	35.78	2	2.0	2.2	88.9
	36.03	1	1.0	1.1	90.0
	36.28	1	1.0	1.1	91.1
	36.30	1	1.0	1.1	92.2
	36.50	1	1.0	1.1	93.3
	37.59	1	1.0	1.1	94.4
	38.10	1	1.0	1.1	95.6
	38.44	1	1.0	1.1	96.7
	39.40	1	1.0	1.1	97.8
	39.60	2	2.0	2.2	100.0
	Total	90	90.0	100.0	
Missing	System	10	10.0		
Total		100	100.0		

Time of measurement of Pig skin weight (Hours)

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	0	10	10.0	11.1	11.1
	48	10	10.0	11.1	22.2
	96	10	10.0	11.1	33.3
	144	10	10.0	11.1	44.4
	192	10	10.0	11.1	55.6
	240	10	10.0	11.1	66.7
	288	10	10.0	11.1	77.8
	336	10	10.0	11.1	88.9
	384	10	10.0	11.1	100.0
	Total	90	90.0	100.0	
Missing	System	10	10.0		
Total		100	100.0		

T-Test

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Weight of Non-infested sample Pig skin (Gram)	32.8054	90	5.6052	.5908
	Weight of Maggot infested Pig Skin (Gram)	29.6717	90	5.2077	.5489

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Weight of Non-infested samples of Pig skin (Gram) & Weight of Maggot infested Pig Skin (Gram)	90	.359	.001

Paired Samples Test

		Paired Differences		
		Mean	Std. Deviation	Std. Error Mean
Pair 1	Weight of Non-infested sample Pig skin (Gram) - Weight of Maggot infested Pig Skin samples (Gram)	3.1338	6.1282	.6460

Paired Samples Test

		Paired Differences	
		95% Confidence Interval of the Difference	
		Lower	Upper
Pair 1	Weight of Non-infested sample Pig skin (Gram) - Weight of Maggot infested Pig Skin samples (Gram)	1.8503	4.4173

Paired Samples Test

		t	df	Sig. (2-tailed)
Pair 1	Weight of Non-infested sample Pig skin (Gram) - Weight of Maggot infested Pig Skin samples (Gram)	4.851	89	.000

Oneway

ANOVA

		Sum of Squares	df	Mean Square
Weight of Non-infested samples of Pig skin (Gram)	Between Groups	841.787	8	105.223
	Within Groups	1954.478	81	24.129
	Total	2796.265	89	
Weight of Maggot infested Pig Skin (Gram)	Between Groups	667.410	8	83.426
	Within Groups	1746.258	81	21.559
	Total	2413.668	89	

ANOVA

		F	Sig.
Weight of Non-infested samples of Pig skin (Gram)	Between Groups	4.361	.000
	Within Groups		
	Total		
Weight of Maggot infested Pig Skin (Gram)	Between Groups	3.870	.001
	Within Groups		
	Total		

General Linear Model

Between-Subjects Factors

		N
Time of measurement of Pig skin weight (Hours)	0	10
	48	10
	96	10
	144	10
	192	10
	240	10
	288	10
	336	10
	384	10

Multivariate Tests[c,d]

Effect		Value	F	Hypothesis df
Intercept	Pillai's Trace	.984	2448.346[a]	2.000
	Wilks' Lambda	.016	2448.346[a]	2.000
	Hotelling's Trace	61.209	2448.346[a]	2.000
	Roy's Largest Root	61.209	2448.346[a]	2.000
TIME	Pillai's Trace	.395	2.492	16.000
	Wilks' Lambda	.622	2.675[a]	16.000
	Hotelling's Trace	.579	2.856	16.000
	Roy's Largest Root	.525	5.315[b]	8.000

Multivariate Tests[c,d]

Effect		Error df	Sig.
Intercept	Pillai's Trace	80.000	.000
	Wilks' Lambda	80.000	.000
	Hotelling's Trace	80.000	.000
	Roy's Largest Root	80.000	.000
TIME	Pillai's Trace	162.000	.002
	Wilks' Lambda	160.000	.001
	Hotelling's Trace	158.000	.000
	Roy's Largest Root	81.000	.000

a. Exact statistic

b. The statistic is an upper bound on F that yields a lower bound on the significance level.

c. Design: Intercept+TIME

d. Weighted Least Squares Regression - Weighted by Replicate samples of Non-infested pig Skin

Tests of Between-Subjects Effects[c]

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square
Corrected Model	Weight of Non-infested sample Pig skin (Gram)	5092.994[a]	8	636.624
	Weight of Maggot infested Pig Skin (Gram)	3385.847[b]	8	423.231
Intercept	Weight of Non-infested sample Pig skin (Gram)	545051.846	1	545051.846
	Weight of Maggot infested Pig Skin (Gram)	417974.050	1	417974.050
TIME	Weight of Non-infested sample Pig skin (Gram)	5092.994	8	636.624
	Weight of Maggot infested Pig Skin (Gram)	3385.847	8	423.231
Error	Weight of Non-infested sample Pig skin (Gram)	10945.015	81	135.124
	Weight of Maggot infested Pig Skin (Gram)	11097.424	81	137.005
Total	Weight of Non-infested sample Pig skin (Gram)	561089.855	90	
	Weight of Maggot infested Pig Skin (Gram)	432457.321	90	
Corrected Total	Weight of Non-infested sample Pig skin (Gram)	16038.009	89	
	Weight of Maggot infested Pig Skin (Gram)	14483.271	89	

Tests of Between-Subjects Effects[c]

Source	Dependent Variable	F	Sig.
Corrected Model	Weight of Non-infested sample Pig skin (Gram)	4.711	.000
	Weight of Maggot infested Pig Skin (Gram)	3.089	.004
Intercept	Weight of Non-infested sample Pig skin (Gram)	4033.727	.000
	Weight of Maggot infested Pig Skin (Gram)	3050.789	.000
TIME	Weight of Non-infested sample Pig skin (Gram)	4.711	.000
	Weight of Maggot infested Pig Skin (Gram)	3.089	.004
Error	Weight of Non-infested sample Pig skin (Gram)		
	Weight of Maggot infested Pig Skin (Gram)		
Total	Weight of Non-infested sample Pig skin (Gram)		
	Weight of Maggot infested Pig Skin (Gram)		
Corrected Total	Weight of Non-infested sample Pig skin (Gram)		
	Weight of Maggot infested Pig Skin (Gram)		

a. R Squared = .318 (Adjusted R Squared = .250)

b. R Squared = .234 (Adjusted R Squared = .158)

c. Weighted Least Squares Regression - Weighted by Replicate samples of Non-infested pig Skin

Correlations

Correlations

		Weight of Non-infested sample Pig skin (Gram)	Weight of Maggot infested Pig Skin (Gram)
Weight of Non-infested sample Pig skin (Gram)	Pearson Correlation	1.000	.359**
	Sig. (2-tailed)	.	.001
	N	90	90
Weight of Maggot infested Pig Skin (Gram)	Pearson Correlation	.359**	1.000
	Sig. (2-tailed)	.001	.
	N	90	90

**. Correlation is significant at the 0.01 level (2-tailed).

Summarize

Case Processing Summary[a]

	Cases	
	Included	
	N	Percent
Weight of Maggot infested sample Pig Skin (Gram) * Sample of Maggot infested Pig Skin	90	100.0%

Case Processing Summary[a]

	Cases			
	Excluded		Total	
	N	Percent	N	Percent
Weight of Maggot infested sample Pig Skin (Gram) * Sample of Maggot infested Pig Skin	0	.0%	90	100.0%

a. Limited to first 100 cases.

Case Summaries[a]

				Weight of Maggot infested sample Pig Skin (Gram)
Sample of Maggot infested Pig Skin	Sample Pig Skin 1	1		39.40
		2		39.60
		3		36.50
		4		37.59
		5		35.78
		6		33.82
		7		32.71
		8		30.04
		9		29.19
		Total	N	9
	Sample Pig Skin 2	1		33.90
		2		35.00
		3		31.65
		4		32.00
		5		32.00
		6		31.31
		7		30.06
		8		29.29
		9		27.32
		Total	N	9

Case Summaries[a]

				Weight of Maggot infested sample Pig Skin (Gram)
Sample of Maggot infested Pig Skin	Sample Pig Skin 3	1		32.20
		2		34.00
		3		31.61
		4		32.39
		5		32.22
		6		29.81
		7		29.23
		8		24.72
		9		22.63
		Total	N	9
	Sample Pig Skin 4	1		30.40
		2		32.48
		3		29.59
		4		30.03
		5		30.05
		6		28.11
		7		24.99
		8		20.11
		9		19.18
		Total	N	9
	Sample Pig Skin 5	1		23.40
		2		26.30
		3		23.64
		4		25.25
		5		24.88
		6		24.29
		7		23.39
		8		21.79
		9		20.91
		Total	N	9
	Sample Pig Skin 6	1		34.70
		2		36.30
		3		32.68
		4		33.32
		5		33.08
		6		32.08
		7		31.93
		8		31.00
		9		29.89
		Total	N	9
	Sample Pig Skin 7	1		27.70
		2		29.45
		3		25.70
		4		27.19
		5		27.17
		6		26.53
		7		27.18
		8		24.62
		9		22.47
		Total	N	9

Case Summaries[a]

				Weight of Maggot infested sample Pig Skin (Gram)
Sample of Maggot infested Pig Skin	Sample Pig Skin 8	1		39.60
		2		38.44
		3		35.00
		4		35.78
		5		34.87
		6		32.22
		7		31.80
		8		29.60
		9		28.23
		Total	N	9
	Sample Pig Skin 9	1		34.10
		2		38.10
		3		35.66
		4		36.03
		5		36.28
		6		34.44
		7		35.30
		8		29.09
		9		24.08
		Total	N	9
	Sample Pig Skin 10	1		22.50
		2		25.45
		3		22.60
		4		23.86
		5		23.71
		6		23.05
		7		23.12
		8		21.12
		9		18.67
		Total	N	9
	Total	N		90

a. Limited to first 100 cases.

Report

```
                      Page      1

                       Weight
                           of
                       Maggot
                     infested
                       sample
Sample of Maggot     Pig Skin
infested Pig Skin      (Gram)
_________________    ________

Sample Pig Skin 1
```

```
Sample Pig Skin 2
Sample Pig Skin 3
Sample Pig Skin 4
Sample Pig Skin 5
Sample Pig Skin 6
Sample Pig Skin 7
Sample Pig Skin 8
Sample Pig Skin 9
Sample Pig Skin 10
Grand Total

Mean              29.67
Minimum           18.67
Maximum           39.60
StdDev             5.21
Kurtosis           -.84
Variance          27.12
Skewness           -.12
> 75                .0%
< 25              25.6%
In 25 to 75       74.4%
```

Frequencies

Statistics

Weight of Maggot infested sample Pig Skin (Gram)

N	Valid	90
	Missing	0
Mean		29.6717
Std. Error of Mean		.5489
Std. Deviation		5.2077
Variance		27.1199
Skewness		-.121
Std. Error of Skewness		.254
Kurtosis		-.844
Std. Error of Kurtosis		.503
Range		20.93

Weight of Maggot infested sample Pig Skin (Gram)

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	18.67	1	1.1	1.1	1.1
	19.18	1	1.1	1.1	2.2
	20.11	1	1.1	1.1	3.3
	20.91	1	1.1	1.1	4.4
	21.12	1	1.1	1.1	5.6
	21.79	1	1.1	1.1	6.7
	22.47	1	1.1	1.1	7.8
	22.50	1	1.1	1.1	8.9
	22.60	1	1.1	1.1	10.0
	22.63	1	1.1	1.1	11.1
	23.05	1	1.1	1.1	12.2
	23.12	1	1.1	1.1	13.3
	23.39	1	1.1	1.1	14.4
	23.40	1	1.1	1.1	15.6
	23.64	1	1.1	1.1	16.7
	23.71	1	1.1	1.1	17.8
	23.86	1	1.1	1.1	18.9
	24.08	1	1.1	1.1	20.0
	24.29	1	1.1	1.1	21.1
	24.62	1	1.1	1.1	22.2
	24.72	1	1.1	1.1	23.3
	24.88	1	1.1	1.1	24.4
	24.99	1	1.1	1.1	25.6
	25.25	1	1.1	1.1	26.7
	25.45	1	1.1	1.1	27.8
	25.70	1	1.1	1.1	28.9
	26.30	1	1.1	1.1	30.0
	26.53	1	1.1	1.1	31.1
	27.17	1	1.1	1.1	32.2
	27.18	1	1.1	1.1	33.3
	27.19	1	1.1	1.1	34.4
	27.32	1	1.1	1.1	35.6
	27.70	1	1.1	1.1	36.7
	28.11	1	1.1	1.1	37.8
	28.23	1	1.1	1.1	38.9
	29.09	1	1.1	1.1	40.0
	29.19	1	1.1	1.1	41.1
	29.23	1	1.1	1.1	42.2
	29.29	1	1.1	1.1	43.3
	29.45	1	1.1	1.1	44.4
	29.59	1	1.1	1.1	45.6
	29.60	1	1.1	1.1	46.7
	29.81	1	1.1	1.1	47.8
	29.89	1	1.1	1.1	48.9
	30.03	1	1.1	1.1	50.0
	30.04	1	1.1	1.1	51.1
	30.05	1	1.1	1.1	52.2
	30.06	1	1.1	1.1	53.3
	30.40	1	1.1	1.1	54.4
	31.00	1	1.1	1.1	55.6
	31.31	1	1.1	1.1	56.7
	31.61	1	1.1	1.1	57.8
	31.65	1	1.1	1.1	58.9
	31.80	1	1.1	1.1	60.0
	31.93	1	1.1	1.1	61.1

Weight of Maggot infested sample Pig Skin (Gram)

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	32.00	2	2.2	2.2	63.3
	32.08	1	1.1	1.1	64.4
	32.20	1	1.1	1.1	65.6
	32.22	2	2.2	2.2	67.8
	32.39	1	1.1	1.1	68.9
	32.48	1	1.1	1.1	70.0
	32.68	1	1.1	1.1	71.1
	32.71	1	1.1	1.1	72.2
	33.08	1	1.1	1.1	73.3
	33.32	1	1.1	1.1	74.4
	33.82	1	1.1	1.1	75.6
	33.90	1	1.1	1.1	76.7
	34.00	1	1.1	1.1	77.8
	34.10	1	1.1	1.1	78.9
	34.44	1	1.1	1.1	80.0
	34.70	1	1.1	1.1	81.1
	34.87	1	1.1	1.1	82.2
	35.00	2	2.2	2.2	84.4
	35.30	1	1.1	1.1	85.6
	35.66	1	1.1	1.1	86.7
	35.78	2	2.2	2.2	88.9
	36.03	1	1.1	1.1	90.0
	36.28	1	1.1	1.1	91.1
	36.30	1	1.1	1.1	92.2
	36.50	1	1.1	1.1	93.3
	37.59	1	1.1	1.1	94.4
	38.10	1	1.1	1.1	95.6
	38.44	1	1.1	1.1	96.7
	39.40	1	1.1	1.1	97.8
	39.60	2	2.2	2.2	100.0
	Total	90	100.0	100.0	

Descriptives

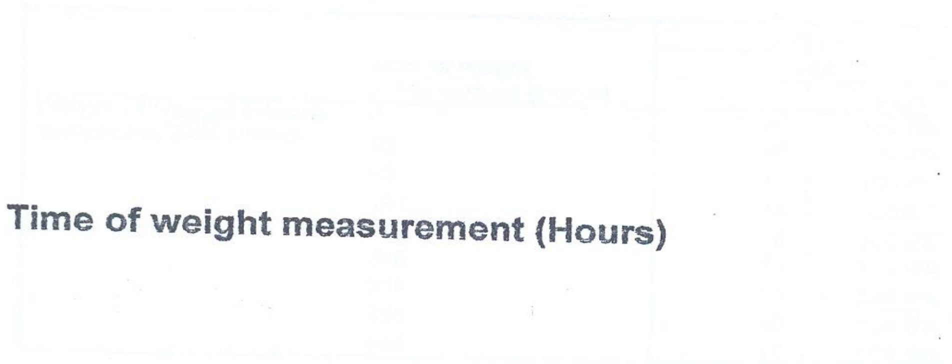
Descriptive Statistics

	N	Minimum	Maximum
	Statistic	Statistic	Statistic
Sample of Maggot infested Pig Skin	90	1	10
Weight of Maggot infested sample Pig Skin (Gram)	90	18.67	39.60
Valid N (listwise)	90		

Descriptive Statistics

	Mean		Std.	Variance
	Statistic	Std. Error	Statistic	Statistic
Sample of Maggot infested Pig Skin	5.50	.30	2.89	8.343
Weight of Maggot infested sample Pig Skin (Gram)	29.6717	.5489	5.2077	27.120
Valid N (listwise)				

Explore



Time of weight measurement (Hours)

Case Processing Summary

	Time of weight measurement (Hours)	Cases	
		Valid	
		N	Percent
Weight of Maggot infested sample Pig Skin (Gram)	0	10	100.0%
	48	10	100.0%
	96	10	100.0%
	144	10	100.0%
	192	10	100.0%
	240	10	100.0%
	288	10	100.0%
	336	10	100.0%
	384	10	100.0%

Case Processing Summary

	Time of weight measurement (Hours)	Cases	
		Total	
		N	Percent
Weight of Maggot infested sample Pig Skin (Gram)	0	10	100.0%
	48	10	100.0%
	96	10	100.0%
	144	10	100.0%
	192	10	100.0%
	240	10	100.0%
	288	10	100.0%
	336	10	100.0%
	384	10	100.0%

Weight of Maggot infested sample Pig Skin (Gram)

Stem-and-Leaf Plots

Weight of Maggot infested sample Pig Skin (Gram) Stem-and-Leaf Plot for TIME= 0

```
 Frequency    Stem &  Leaf

     2.00        2 .  23
     1.00        2 .  7
     5.00        3 .  02344
     2.00        3 .  99

 Stem width:    10.00
 Each leaf:       1 case(s)
```

Weight of Maggot infested sample Pig Skin (Gram) Stem-and-Leaf Plot for TIME= 48

```
 Frequency    Stem &  Leaf

     3.00        2 .  569
     2.00        3 .  24
     5.00        3 .  56889

 Stem width:    10.00
 Each leaf:       1 case(s)
```

Weight of Maggot infested sample Pig Skin (Gram) Stem-and-Leaf Plot for TIME= 96

```
 Frequency    Stem &  Leaf

     2.00        2 .  23
     2.00        2 .  59
```

Weight of Maggot infested sample Pig Skin (Gram) Stem-and-Leaf Plot for TIME= 288

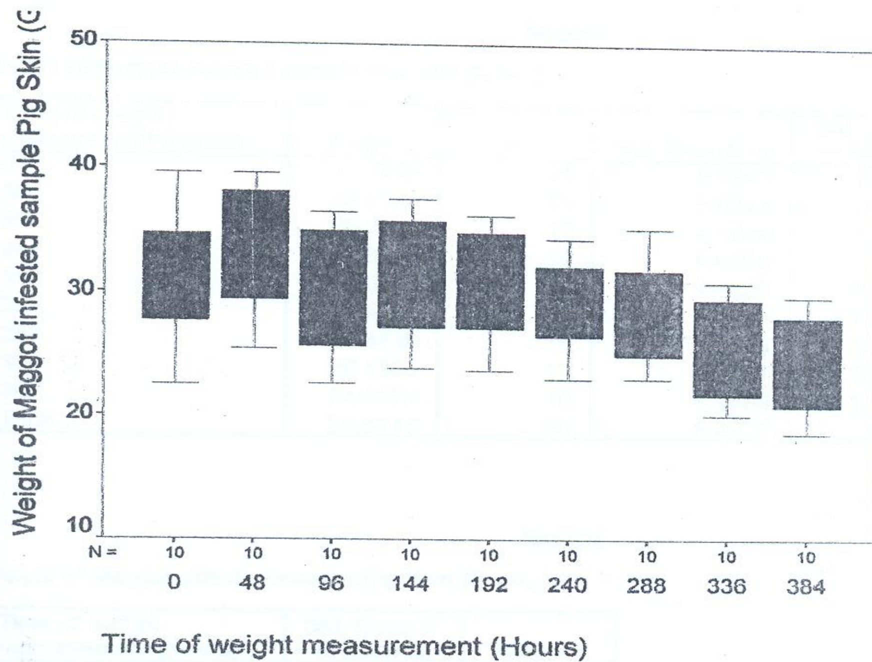
```
 Frequency    Stem &  Leaf

     3.00        2 .  334
     2.00        2 .  79
     4.00        3 .  0112
     1.00        3 .  5

 Stem width:     10.00
 Each leaf:       1 case(s)
```

Weight of Maggot infested sample Pig Skin (Gram) Stem-and-Leaf Plot for TIME= 336

```
 Frequency    Stem &  Leaf

     5.00        2 .  01144
     3.00        2 .  999
     2.00        3 .  01

 Stem width:     10.00
 Each leaf:       1 case(s)
```

Weight of Maggot infested sample Pig Skin (Gram) Stem-and-Leaf Plot for TIME= 384

```
 Frequency    Stem &  Leaf

     2.00        1 .  89
     4.00        2 .  0224
     4.00        2 .  7899

 Stem width:     10.00
 Each leaf:       1 case(s)
```



Means

Case Processing Summary

	Cases	
	Included	
	N	Percent
Weight of Maggot infested sample Pig Skin (Gram) * Time of weight measurement (Hours)	90	100.0%

Case Processing Summary

	Cases			
	Excluded		Total	
	N	Percent	N	Percent
Weight of Maggot infested sample Pig Skin (Gram) * Time of weight measurement (Hours)	0	.0%	90	100.0%

Report

Weight of Maggot infested sample Pig Skin (Gram)

Time of weight measurement (Hours)	Mean	N	Std. Deviation	Std. Error of Mean
0	31.7900	10	5.8943	1.8640
48	33.5120	10	5.0244	1.5888
96	30.4630	10	4.9834	1.5759
144	31.3440	10	4.6929	1.4840
192	31.0040	10	4.4558	1.4091
240	29.5660	10	3.9433	1.2470
288	28.9710	10	4.1698	1.3186
336	26.1380	10	4.1397	1.3091
384	24.2570	10	4.1539	1.3136
Total	29.6717	90	5.2077	.5489

Report

Weight of Maggot infested sample Pig Skin (Gram)

Time of weight measurement (Hours)	Std. Error of Skewness	Variance
0	.687	34.743
48	.687	25.244
96	.687	24.834
144	.687	22.023
192	.687	19.854
240	.687	15.550
288	.687	17.387
336	.687	17.137
384	.687	17.255
Total	.254	27.120

ANOVA Table

			Sum of Squares	df
Weight of Maggot infested sample Pig Skin (Gram) * Time of weight measurement (Hours)	Between Groups	(Combined)	667.410	8
		Linearity	541.804	1
		Deviation from Linearity	125.606	7
	Within Groups		1746.258	81
	Total		2413.668	89

ANOVA Table

			Mean Square
Weight of Maggot infested sample Pig Skin (Gram) * Time of weight measurement (Hours)	Between Groups	(Combined)	83.426
		Linearity	541.804
		Deviation from Linearity	17.944
	Within Groups		21.559
	Total		

ANOVA Table

			F	Sig.
Weight of Maggot infested sample Pig Skin (Gram) * Time of weight measurement (Hours)	Between Groups	(Combined)	3.870	.001
		Linearity	25.132	.000
		Deviation from Linearity	.832	.564
	Within Groups			
	Total			

Measures of Association

	R	R Squared	Eta	Eta Squared
Weight of Maggot infested sample Pig Skin (Gram) * Time of weight measurement (Hours)	-.474	.224	.526	.277

T-Test

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
Weight of Maggot infested sample Pig Skin (Gram)	90	29.6717	5.2077	.5489
Time of weight measurement (Hours)	90	192.00	124.63	13.14

One-Sample Test

	Test Value = 0			
	t	df	Sig. (2-tailed)	Mean Difference
Weight of Maggot infested sample Pig Skin (Gram)	54.053	89	.000	29.6717
Time of weight measurement (Hours)	14.615	89	.000	192.00

One-Sample Test

	Test Value = 0	
	95% Confidence Interval of the Difference	
	Lower	Upper
Weight of Maggot infested sample Pig Skin (Gram)	28.5809	30.7624
Time of weight measurement (Hours)	165.90	218.10

Correlations

		Weight of Maggot infested sample Pig Skin (Gram)	Time of weight measurement (Hours)
Weight of Maggot infested sample Pig Skin (Gram)	Pearson Correlation	1.000	-.474**
	Sig. (2-tailed)	.	.000
	N	90	90
Time of weight measurement (Hours)	Pearson Correlation	-.474**	1.000
	Sig. (2-tailed)	.000	.
	N	90	90

**. Correlation is significant at the 0.01 level (2-tailed).

Regression

Variables Entered/Removed[b]

Model	Variables Entered	Variables Removed	Method
1	Time of weight measurement (Hours)[a]	.	Enter

a. All requested variables entered.

b. Dependent Variable: Weight of Maggot infested sample Pig Skin (Gram)

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.474[a]	.224	.216	4.6121

a. Predictors: (Constant), Time of weight measurement (Hours)

ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	541.804	1	541.804	25.471	.000[a]
	Residual	1871.864	88	21.271		
	Total	2413.668	89			

a. Predictors: (Constant), Time of weight measurement (Hours)

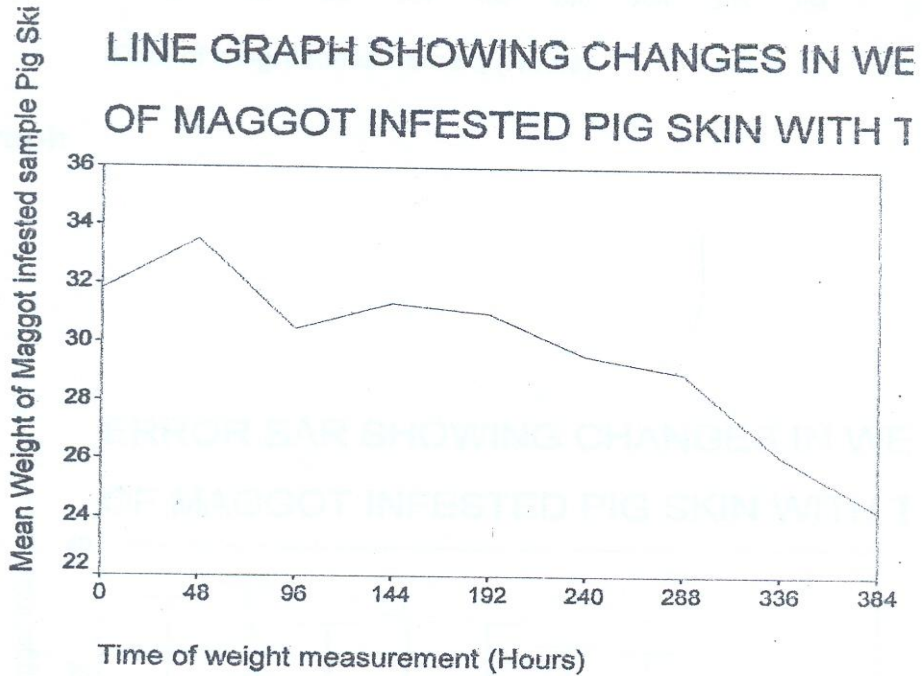
b. Dependent Variable: Weight of Maggot infested sample Pig Skin (Gram)

Coefficients[a]

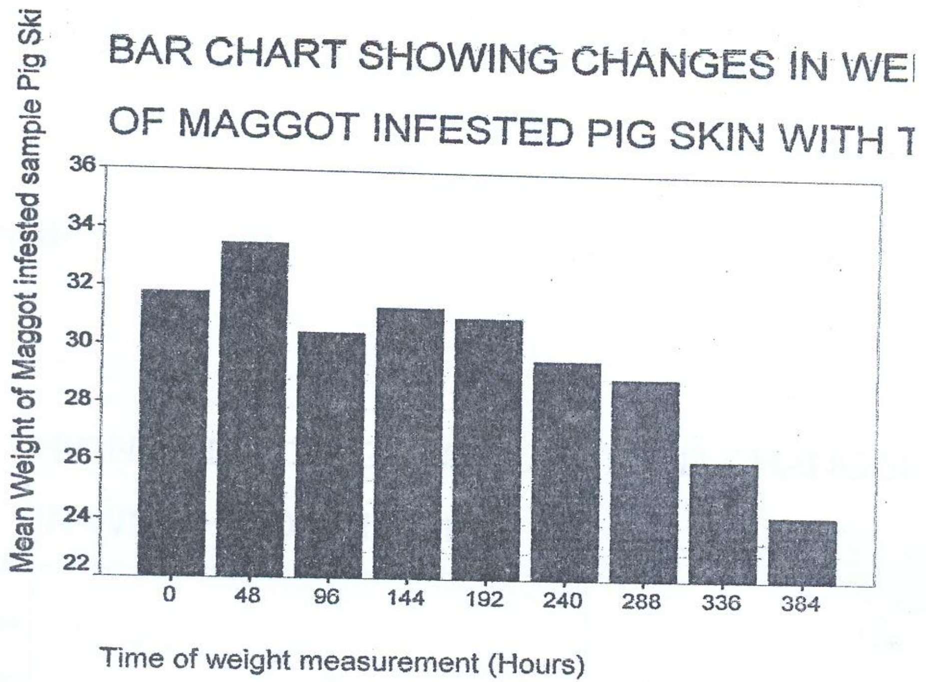
Model		t	Sig.
1	(Constant)	37.340	.000
	Time of weight measurement (Hours)	-5.047	.000

a. Dependent Variable: Weight of Maggot infested sample Pig Skin (Gram)

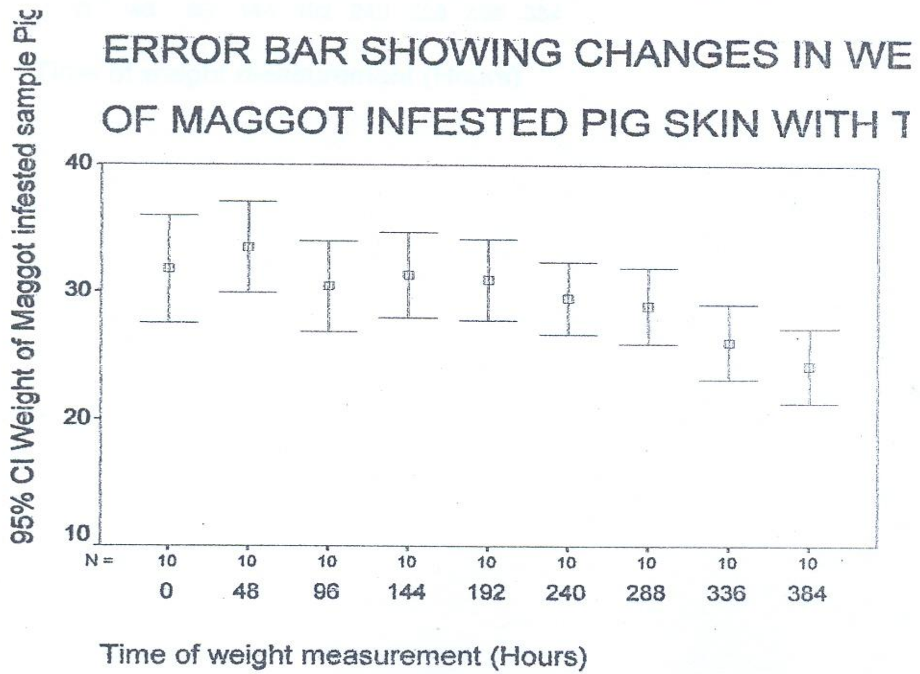
Graph



Graph



Graph



Graph

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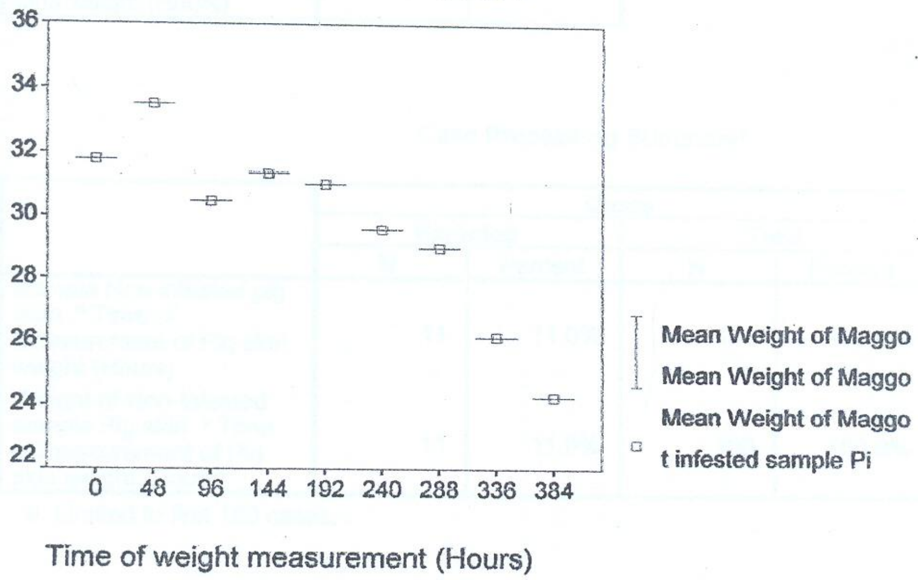

INCREASE PH IN MAGGOT'S MEDIUM (LARVAL SECRETION HARVEST) WITH TIME

TABLE OF MEASURED pH CHANGE OF LARVAL SECRETION WITH TIME

S/NO	TIME MINUTE	MEASURED pH
1.	0	5.93
2	30	7.12
3	60	7.50
4	90	7.60
5	120	7.70
6	150	7.78
7	180	7.82
8	210	7.85

r = + 0.97724

TABLE III

There is positive correlation between time and the pH in maggots' medium. The calculated r value (+ 0.97724) is greater than the tabulated r (0.8233) at 0.001 confidence limit and with degree of freedom (df) = 10.

DECREASE IN CALCULATED POH CHANGE IN MAGGOT'S MEDIUM (LARVAL SECRETION) WITH TIME

TABLE OF CALCULATED POH CHANGE WITH TIME IN THE LARVAL SECRETION

S/No	TIME (MINUTE)	POH
1.	0	8.07
2.	30	6.88
3.	60	6.50
4.	90	6.40
5.	120	6.30
6.	150	6.22
7.	180	6.18
8.	210	6.15

r = -0.97724

TABLE IV

There is negative (-) correlation between time and the POH in maggots' medium. The calculated r value (- 0.97724) is greater than the tabulated r (0.8233) at 0.001 confidence limit and with degree of freedom (df) = 10.

INCREASE IN BASE CONCENTRATION IN MAGGOT'S MEDIUM (LARVAL SECRETION)

TABLE OF CALCULATED BASE CONCENTRATION ([0H] IN MOL/DM^3) CHANGES WITH TIME (MINUTE)

S/NO	TIME (MINUTE)	BASE CONCENTRATION (MOL/DM $^{-3}$)
1	0	1.17×10^{-8}
2	30	7.59×10^{-6}
3	60	3.16×10^{-6}
4	90	2.51×10^{-6}
5	120	1.99×10^{-6}
6	150	1.66×10^{-6}
7	180	1.51×10^{-6}
8	210	1.41×10^{-6}

r= +0.91091

TABLE V

The calculated r (+ 0.9109) is greater than the tabulated r (0.8233) at degree of freedom 10 and 0.001 confidence limit. Base concentration positively and significantly correlated with time.

TIME DEPENDENT INCREASE IN HAEMOGLOBIN CONCENTRATION IN MAGGOTS'S MEDIUM

TABLE OF COLOROMETRIC MEASUREMENTS OF THE TIME DEPENDENT INCREASE IN HAEMOGLOBIN CONCENTRATION, FROM PREWASHED BATH-EFFLUENT OF LARVAE GROWN IN NATURAL ENVIRONMENT

S/NO	TIME (MINUTE)	ABSORBANCE (AMSTRONG)	HAEMOGLOBIN CONCENTRATION (GRAM/DL)
1.	0	0.068	2.846
2.	30	0.091	3.77
3.	60	0.003	4.267
4.	90	0.113	4.689
5.	120	0.122	5.070
6.	150	0.129	5.344
7.	180	0.133	5.51
8	210	0.367	5.663

r= +0.81471

TABLE VII

The calculated r (+ 0.8147) is greater than tabulated r (0.7348) at degree of freedom 10 and confidence limit of 0.001. There is significant and positive correlation between time and increase in Haemoglobin concentration.

MAGGOT FACTOR SIGNIFICANTLY ENHACES THE RATE OF PRTOTEOLYSIS AT ROOM TEMPERATURE

TABLE OF MEAN PLASMA PROTEIN CHANGES IN CONTROL AND TEST SAMPLES OF PLASMA INCUBATED WITH MAGGOT FACTOR AT ROOM TEMPERATURE

S/No	TIME (HOUR)	MEAN PLASMA PROTEIN CONC. IN CONTROL SAMPLES G/DL (G/DL)	MEAN OF PLASMA PROTEIN IN TEST SAMPLE WITH LARVAL EXTRACT. (G/DL)	DEVIATION	$(DEVIATION)^2$ D^2
1.	0	5.60	4.63	0.97	0. 9409
2.	24	5.19	3.82	1.37	1. 876
3.	48	4.86	1.09	3.77	14. 213
4.	72	4.68	0.49	4.19	17. 56
5.	96	3.99	0.38	3.61	13. 03
6.	120	3.49	0.23	3.26	10. 63
		ΣX	Σy	ΣD=17.17	$\Sigma(D^2) = 58.249$
				)=2.86166	

TABLE XII

Where SEM = S.D/ √N (Where Sample Size N = or < 30) or

S.D / √ N-1 (Where Sample Size Is . 30)

$D = \frac{\Sigma D}{n}$ $\Sigma\Xi = \frac{17.17}{6}$

$S.d = \delta n\text{-}1 = \sqrt{\frac{\Sigma(D - D)^2}{n\text{-}1}}$

And $\Sigma(D - D^{-})^2 = \Sigma(D2) - \frac{(\Sigma D)2}{n}$

$= 58.249 -- \frac{(17.17)^2}{6}$

$= 58.249 - \frac{294.809}{6}$

$= 58.249 - 49.1348$

$= 9.114$

$\therefore S.d = \sqrt{\frac{9.114}{6-1}} = \frac{3.0189}{5} = 0.603788$

$S.E.M = \frac{S.d}{\sqrt{n}} = \frac{S.d}{\sqrt{6}} = 0.603788 \div 2.449489$

$= 0.246495$

$= 0.2465$

$t = D^{-} \div SEM = 2.86166 \div 0.2465 = 11.6092$

From t - distribution table at df = n –1 = 6 – 1 = 5, t - calculated t (11.6092) is greater than (>) t – tabulated (6.869) at 0.0005 level of significance. This also means that Null hypothesis

(H_o) is rejected.

MAGGOT FACTOR SIGNIFICANTLY ENHANCES THE RATE OF PROTEOLYSIS AT CONTROLLED TEMPERATURE OF 37 ^{O}C.

TABLE OF MEAN TOTAL PLASMA PROTEIN CHANGE IN CONTROL AND PLASMA TEST SAMPLES MIXED WITH CRUDE LARVAL EXTRACT.

S/NO	TIME (HOUR)	MEAN PLASMA PROTEIN CONC. IN CONTROL SAMPLES (G/DL)	MEAN PLASMA PROTEIN CONC. WITH MAGGOT FACTOR IN SERIAL TEST SAMPLES (G/DL)	DEVIATION (D)	(DEVIATION) (D^2)

1.	0	5.6	4.45	1.15	1.32
2.	24	5.4	3.52	1.88	3.53
3.	48	5.20	2.30	2.90	8.41
4.	72	4.96	1.31	3.65	13.32
5.	96	4.61	0.89	3.72	13.84
6.	120	4.54	0.536	4.00	16.00
		ΣX	ΣY	$\Sigma D = 17.30$	$\Sigma D^2 = 56.42$

TABLE XIII

$S.d = \delta n\text{-}1 = \sqrt{\frac{\Sigma(D-D)^2}{n-1}}$

And $\Sigma(D-D^{-})^2 = \Sigma(D2) - \frac{(\Sigma D)\ 2}{n}$

$D = \frac{\Sigma D}{n} = \frac{17.3}{6} = 2.8833$

$S.D = \sqrt{\frac{6.538}{6-1}} = \frac{2.5569}{5}$

$= 0.5114$

$\Sigma(D^2) - \frac{(\Sigma D)^2}{n} = (56.42 - \frac{(17.3)^2}{6}$

$= 56.42 - 49.88$

$= 6.538$

From t – distribution table, at df = n-1 = 5, calculated t (13.808) is greater than t-tabulated (6.869) at 0.0005 level of significance. The Null hypothesis is therefore rejected.

INSIGNIFICANT DIFFERENCE IN DEGREE OF PROTEOLYSIS AT TROPICAL ROOM TEMPERATURE IN JUNE AND CONTROL TEMPERATURE OF 37 ^{O}C.

TABLE OF MAGGOT FACTOR INDUCED MEAN PLASMA PROTEIN CHANGE AT ROOM TEMPERATURE AND CONTROL TEMPERATURE OF 37 ^{O}C.

S/NO	TIME (HOUR)	MEAN PLASMA PROTEIN CHANGE (G/DL) IN TEST SAMPLE SERIES MIXED WITH MAGGOTS' EXTRACT AT ROOM TEMPERATURE.	MEAN PLASMA PROTEIN CHANGE (G/DL) IN TEST SAMPLE SERIES MIXED WITH MAGGOTS' EXTRACT AT TEMPERATURE OF 37°C.
1.	0	4.63	4.45
2.	24	3.82	3.52
3.	48	1.09	2.30
4.	72	0.49	1.31
5.	96	0.38	0.89
6.	120	0.23	0.536

t = 0.078242

TABLE XIV

From t – distribution table, at df = n-1 = 5, calculated t (0.0782) is less than t-tabulated (2.015) at 0.05 level of significance. The Null hypothesis is therefore accepted (there is no difference between the mean of the 2 sample series).

TIME (minute)	Na^+ mMol/L	k^+ mMol/L	Cl^- mMol	HCO_3^- mMol/L	UREA mMol/L	CREAT µMol/L	Total Protein g/dL	Bilirubin µMol/L	Amylase S IU/L
0	39	0	25	<10	0	0	0	0	0
30	42	0.7	29	<10	2.4	0	1.1	0	40
60	45	0.9	34	<10	3.1	5.6	1.8	0	69
90	48	1.3	37	<10	3.5	7.9	1.8	0.7	74
120	60	1.4	43	<10	3.5	9.8	2.1	0.9	120
150	89	1.9	49	<10	3.9	12.9	2.2	1.2	168
180	108	2.1	87	<10	4.1	16.8	2.2	1.4	194
210	117	2.2	106	<10	4.5	18.8	2.3	1.74	216

TIME (minute)	Na^+	Na^+	Na^+	Na^+	Na^+	MEAN Na^+
0	39	39	39	39	39	39
30	42	41	43	45	39	41.4
60	45	49	56	50	41	48.2
90	48	53	89	58	47	59
120	60	68	115	78	51	74.4
150	89	70	126	90	71	89.2
180	108	89	128	93	95	102.6
210	117	130	131	100	111	117.8

TIME (minute)	Cl^-	Cl^-	Cl^-	Cl^-	Cl^-	MEAN Cl^-
0	25	25	25	25	25	25
30	29	40	31	27	29	31.2
60	34	43	50	31	31	37.8
90	37	58	98	38	48	55.8
120	43	64	105	64	55	66.2
150	49	80	118	83	89	83.8
180	87	110	121	95	101	102.8
210	106	127	127	99	109	113.6

TIME (minute)	k^+	k^+	k^+	k^+	k^+	MEAN k^+
0	0	0	0	0	0	0
30	0.7	0.5	0	0.4	0	0.32
60	0.9	0.7	0	0.8	0	0.48
90	1.3	0.9	0	1.4	0.2	0.76
120	1.4	1.4	0	1.6	0.4	0.96
150	1.9	1.8	0	1.7	0.4	1.16
180	2.1	2.2	0	1.8	0.5	1.32
210	2.2	2.2	0	1.9	0.5	1.36

TIME (minute)	HCO_3^-	HCO_3^-	HCO_3^-	HCO_3^-	HCO_3^-
0	<10	<10	<10	<10	<10
30	<10	<10	<10	<10	<10
60	<10	<10	<10	<10	<10
90	<10	<10	<10	<10	<10
120	<10	<10	<10	<10	<10
150	<10	<10	<10	<10	<10
180	<10	11	<10	<10	<10
210	<10	12	<10	<10	<10

TIME (minute)	UREA	UREA	UREA	UREA	UREA	
0	0	0	0	0	0	0
30	2.4	3.1	0.5	0.8	0.6	1.48
60	3.1	4.2	1	1.1	0.6	2
90	3.5	3.2	1.6	1.8	0.8	2.18
120	3.5	3.5	1.8	2.1	0.8	2.34
150	3.9	3.6	2.7	2.7	1.1	2.8
180	4.1	3.7	2.7	3.3	1.9	3.14
210	4.5	3.7	2.9	3.3	2	3.28

TIME (minute)	CREAT	CREAT	CREAT	CREAT	CREAT	MEAN CREAT
0	0	0	0	0	0	0
30	0	9	0	0.5	0	1.9
60	5.6	12.1	7.8	7.1	7	7.92
90	7.9	14.2	11.5	9.8	12.1	11.1
120	9.8	17.1	14	16.3	15.3	14.5
150	12.9	18.5	16.3	19.9	19.8	17.48
180	16.8	20.4	17.9	20.2	21.1	19.28
210	18.8	21	18.4	20.3	23.3	20.36

TIME (minute)	TP	TP	TP	TP	TP	MEAN TP g/dL
0	0	0	0	0	0	0
30	1.1	0	0	0	0	0.22
60	1.8	0.4	0.3	0.7	0.5	0.74
90	1.8	0.4	0.4	0.9	0.5	0.8
120	2.1	0.4	0.4	1.5	1.7	1.22
150	2.2	0.5	0.5	1.6	2.8	1.52
180	2.2	0.6	0.5	2.2	3.4	1.78
210	2.3	0.6	0.5	2.2	3.4	1.8

TIME (minute)	Bil	Bil	Bil	Bil	Bil	MEAN Bil
0	0	0	0	0	0	0
30	0	0.71	0	0	0	0.142
60	0	1.21	0.6	0	0	0.362
90	0.7	1.63	0.91	0.6	0	0.768
120	0.9	2.14	1.31	0.8	0.6	1.15
150	1.2	2.39	1.4	0.8	0.8	1.318
180	1.4	2.43	1.57	1.1	1	1.5
210	1.74	2.49	1.68	1.22	1.03	1.632

TIME (minute)	Amylase	Amylase	Amylase	Amylase	Amylase	Mean Amylase IU/L
0	0	0	0	0	0	0
30	40	0	30	0	0	14
60	69	33	53	61	55	54.2
90	74	47	60	90	60	66.2
120	120	110	78	160	99	113.4
150	168	189	90	210	130	157.4
180	194	210	99	233	133	173.8
210	216	212	112	249	143	186.4

TIME (minute)	Na^+	k^+	Cl^-	HCO_3^-	UREA	CREAT	TP	Bil	Amylase
0	39	0	25	<10	0	0	0	0	0
30	39	0	29	<10	0.6	0	0	0	0
60	41	0	31	<10	0.6	7	0.5	0	55
90	47	0.2	48	<10	0.8	12.1	0.5	0	60
120	51	0.4	55	<10	0.8	15.3	1.7	0.6	99
150	71	0.4	89	<10	1.1	19.8	2.8	0.8	130
180	95	0.5	101	<10	1.9	21.1	3.4	1	133
210	111	0.5	109	<10	2	23.3	3.4	1.03	143

TIME (minute)	Na^+	k^+	Cl^-	HCO_3^-	UREA	CREAT	TP	Bil	Amylase
0	39	0	25	<10	0	0	0	0	0
30	41	0.5	40	<10	3.1	9	0	0.71	0
60	49	0.7	43	<10	4.2	12.1	0.4	1.21	33
90	53	0.9	58	<10	3.2	14.2	0.4	1.63	47
120	68	1.4	64	<10	3.5	17.1	0.4	2.14	110
150	70	1.8	80	<10	3.6	18.5	0.5	2.39	189
180	89	2.2	110	11	3.7	20.4	0.6	2.43	210
210	130	2.2	127	12	3.7	21	0.6	2.49	212

TIME (Minute)	Na +	K+	Cl^-	HCO_3^-	UREA	CREATIN INE	TOTAL PROTEIN Gm/DL	BILIRUBIN	AMYLASE IU/L
0	39	0	25	<10	0	0	0	0	0
30	45	0.4	27	<10	0.8	0.5	0	0	0
60	50	0.8	31	<10	1.1	7.1	0.7	0	61
90	58	1.4	38	<10	1.8	9.8	0.9	0.6	90
120	78	1.6	64	<10	2.1	16.3	1.5	0.8	160
150	90	1.7	83	<10	2.7	19.9	1.6	0.8	210
180	93	1.8	95	<10	3.3	20.2	2.2	1.1	233
210	100	1.9	99	<10	3.3	20.3	2.2	1.22	249

TIME (minute)	Na^+	k^+	Cl^-	HCO_3^-	UREA	CREAT	TP	Bil	Amylase
0	39	0	25	<10	0	0	0	0	0
30	43	0	31	<10	0.5	0	0	0	30
60	56	0	50	<10	1	7.8	0.3	0.6	53
90	89	0	98	<10	1.6	11.5	0.4	0.91	60
120	115	0	105	<10	1.8	14	0.4	1.31	78
150	126	0	118	<10	2.7	16.3	0.5	1.4	90
180	128	0	121	<10	2.7	17.9	0.5	1.57	99

210	131	0	127	<10	2.9	18.4	0.5	1.68	112