بسم الله الرحمن الرحيم

MONA MAGHRABY

شبكة المعلومات الجامعية

التوثيق الالكتروني والميكروفيلم

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التوثيق الإلكتروني والميكروفيلم

جامعة عين شمس

التوثيق الإلكتروني والميكروفيلم

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MONA MAGHRABY

Studies on the production and characterization of antimicrobial compounds in the haemolymph of the greater wax moth (*Galleria mellonella*)

A Thesis
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BY

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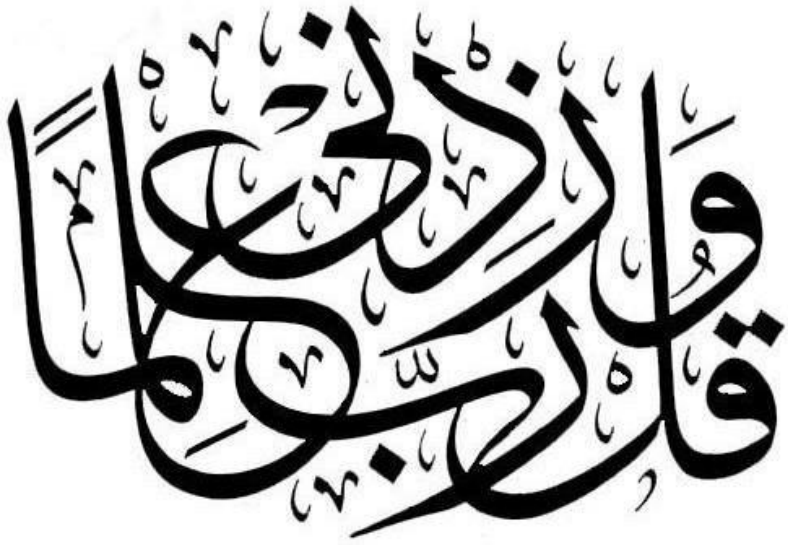
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ABSTRACT

Resistance of bacteria to antibiotics has become one of the main problems in human health. The discovery of new antibiotics is a new way to circumvent such problem and the antimicrobial activity induced in the hemolymph of infected insects may represent a new type of such antibiotics. The present investigation used *Galleria mellonella* larvae as an infection model to describe the regulatory events involved in the induction of antimicrobial activity following injection of a sub-lethal dose of *Escherichia coli* into larval hemocoel. Bacterial injection caused a decrease in fresh body weight and body water content and an increase in the hemolymph volume of the injected larvae. This may be due to the loss of tissue water and gained it into the hemolymph. In the same time, the hemolymph density and pH were immediately decreased following injection, while they restored its original level with time. Bacterial injection also recorded obvious decrease in the hemolymph proteins and lipids. This may be due to their elimination and/or their involvement in immune defense reactions or may be due to the intensive consumption and depletion of nutrition during infection. In contrary, the levels of hemolymph carbohydrates increased due to infection. This increase may be due to the release of stored sugars (trehalose) which is responded strikingly due to bacterial infection causing an increase in the level of glucose and glycogen in the hemolymph. Bacterial injection also caused induction of antibacterial activity in the hemolymph of injected larvae. The role of antimicrobial activity induced in the larval hemolymph after different time intervals post-*E. coli*-injection was examined as natural antibiotics against *E. coli* as Gram-negative bacteria,

Staphylococcus aureus as Gram-positive bacteria, *Candida albicans* and *Aspergillus fumigatus* as fungi. The highest response was found against Gram negative bacteria (*E. coli*), than any other pathogenic microorganism tested, and the highest activity was obtained after 48 h of inoculation of bacteria. The capacity of a drug candidate by rapid screening of antibacterial activity against target pathogens was also evaluated by using the minimum inhibitory concentration (MIC) assay that inhibits bacterial growth and the minimum bactericidal concentration (MBC) assay that used to kill a target bacterium. The MIC assays have confirmed the apparent potency of larval immune plasma against the tested bacteria, while the MBC have confirmed this activity is bacteriostatic and not bactericidal. Additional biochemical test was performed to determine the ability of the tested antimicrobial agent of the immune plasma to alter the cell membrane integrity of the *E. coli* bacterial cell by analysis of proteins. The results confirmed that the immune plasma is capable of inducing leakage of the intracellular protein molecules in the tested bacterial cells. Scanning electron microscopy (SEM) was also used to visualize the morphological effects caused by treating target bacterium with antimicrobial agent generated in the immune plasma. Results indicated that a weak growth and irregular pitted surface of the treated bacterial cells were observed as compared to dense growth and regular smooth surface exhibited in controls. These results may provide a baseline data about the development and production of new means of protection of man and animals against infectious diseases.

Key words: *Galleria mellonella*, *Escherichia coli*, hemolymph volume, density and pH, hemolymph carbohydrates, lipids and proteins, antibacterial activity, MIC, cell membrane integrity, SEM.

DEDICATION

This thesis is dedicated to the sprite of my parents

May God have mercy on them

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ABREVIATIONS

ABS	**:**	Absorbency
AMP	**:**	Antimicrobial peptides
ANOVA	**:**	Analysis of variance
ATCC	**:**	American Type Culture Collection
BSA	**:**	Bovine serum albumin
Bt	**:**	*Bacillus thuringiensis*
°C	**:**	Degrees Celsius
CBB	**:**	Coomassie brilliant blue
CFU	**:**	Colony forming unit
cm	**:**	Centimeter(s)
cm^3	**:**	Centimeter(s) cubic
DMSO	**:**	Dimethyl sulfoxide
DNA	**:**	Deoxyribonucleic acid
E. coli	**:**	*Escherichia coli*
EDTA	**:**	Ethylene-diaminetetraacetic acid
OEs	**:**	Essential oils
g	**:**	Gram
g	**:**	Gravity
G. mellonella	**:**	*Galleria mellonella*
G +ve	**:**	Gram positive
G –ve	**:**	Gram negative
h	**:**	Hour(s)
HDL-p	**:**	High-density lipoprotein
IU/mg	**:**	Integrated unit/milligram.
kDa	**:**	Kilo Dalton(s).
LD	**:**	Lethal dose
LPS	**:**	Lipopolysaccharidase
MIC	**:**	Minimal inhibitory Concentration
min	**:**	Minute(s)
mg	**:**	Milligram
mg/mL	**:**	Milligram per milliliter
mL	**:**	Milliliter(s)
mm	**:**	Millimeter(s)
mM	**:**	Millimolar(s)

mRNA	**:**	Messenger ribonucleic acid
MRSA	**:**	Methicillin-resistant *Staphylococcus aureus*
MSSA	**:**	methicillin-sensitive *S. aureus*
MHA	**:**	Mueller–Hinton agar
MW	**:**	Molecular weight
n	**:**	Number of test replicates
NCCLS	**:**	National Committee for Clinical Laborator Standards
nm	**:**	Nanometer
OD	**:**	Optical Density
p	**:**	Probability
PPS	**:**	Phosphate-buffered saline
pH	**:**	Potential of hydrogen
RNA	**:**	Ribonucleic acid
rpm	**:**	Round per minute
SE	**:**	Standard error
SPSS	**:**	Statistical Package for the social sciences
TEMED	**:**	N, N, N', N'-Tetramethylethylenediamine
uL	**:**	Microliter
µm	**:**	Micrometer
v/v	**:**	Volume per volume

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