

Induced Immune Response against *Bacillus*thuringiensis in the Developmental Stages of the Greater Wax Moth, *Galleria mellonella*(Lepidoptera: Pyralidae)

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BY

Marwa Hamdy Ibrahim Radwan

(B. Sc.)

Demonstrator in Entomology Department, Faculty of Science, Ain Shams University

Supervisors

Prof. Dr. Akila Mohamed ElShafei

Professor of Insect Physiology
Faculty of Science
Ain Shams University

Prof. Dr. Emad Mahmoud Said Barakat

Professor of Insect Physiology
Faculty of Science
Ain Shams University

Dr. Doaa E. Soliman

Lecturer of Entomology
Faculty of Science
Ain Shams University

2015

THESIS EXAMINATION COMMITTEE

NAME	TITLE	SIGNATURE
•••••	•••••	•••••
•••••	•••••	•••••
•••••	•••••	•••••
•••••	•••••	•••••

SUPERVISORS:

Prof. Dr. Akila Mohamed ElShafei

Professor of Insect Physiology, Faculty of Science, Ain Shams University.

Prof. Dr. Emad Mahmoud Said Barakat

Professor of Insect Physiology, Faculty of Science, Ain Shams University.

Dr. Doaa A. Soliman

Lecturer of Entomology, Faculty of Science, Ain Shams University.

BIOGRAPHY

Name: Marwa Hamdy Ibrahim Radwan

Date and place of birth: 9/2/1988, Cairo, Egypt

Degree awarded: B.Sc. (Entomology)

Department: Entomology

Faculty: Science

University: Ain Shams University

Date of graduation: June, 2009

Date of Appointment: February, 2010

Occupation: Demonstrator, Department of Entomology,

Faculty of Science, Ain Shams University

Date of Registration for M.Sc. degree: May, 2011

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DEDICATION

This thesis is dedicated to my dear family; especially my **father's** spirit and my **mother** for love, constant prayers, encouragement and continuous help throughout my whole life.

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ABSTRACT

The present study provides direct tests of Galleria mellonella immune strength across different age groups following injection of *Bacillus thuringiensis kurstaki* (B.t.). Such current tests were quantified using standard immunocompetence assays. All of these assays were performed at different time intervals post injection with sub-lethal concentrations (LC₂₀) of B.t. Preliminary results concerning B.t. toxicity indicated that the pupae are more resistant and the larvae are more susceptible. Results concerning physiological immunity indicated that larvae had more body water content and haemolymph volume than pupae and adults. Bacterial injection decreased the body water content and, in the same time, increased the haemolymph volume in all developmental stages. Sex difference in the mass loss was detected; increasing in males over females.

Injection of *B.t.* produced several pathological events on the haemocytes, in addition to significant changes in both differential haemocyte counts (DHCs) and total haemocyte counts (THCs). Sexual differences in the haemocyte counts were detected, where females were found to have more haemocytes in the circulation.

Quantitative and qualitative estimations of haemolymph proteins revealed differences between the different developmental stages and between both adult sexes, in their responses to bacterial injection. It also approved the transfer of immune proteins of treated insects through metamorphosis and their persistence in the later stages.

Stage-specific differences were shown to phagocytosis and antibacterial immune reactions, as these immune responses were most effective in the pupal, next the larval and then the adult stage. There is no effect of sex on the mounting of immune response. In addition, a transfer of immunological memory through the metamorphosis was observed.

Key words:

Galleria mellonella, haemolymph volume, haemocytes, haemolymph proteins, phagocytosis, antibacterial activity and immunological memory.

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ABREVIATIONS

ABS: Absorbency

AMPs : Antimicrobial peptides
ADs : Adipohaemocytes

AGERIN: Agricultural Genetic Engineering Research

Institute

Bis : N, N-methylenebisacrylamide

BSA : Bovine serum albumin.

B. t. : Bacillus thuringinsis

CBB : Coomassie brilliant blue

CFU : Colony formed unit

CC : Celsius degree

cm : Centimeter(s)
COs : Coagulocytes

DHCs: Differential haemocyte count.

E-coli : Escherichia coli G. mellonella : Galleria mellonella

GRS : Granulocytes.
GWM : Greater wax moth

 \mathbf{h} : Hour(s).

HV : Haemolymph volume
Ig : Immunoglobulin.
JH : Juvenile hormone

g : Gram

kDa : Kilo Dalton(s)

LC: Lethal concentration.
LPS: Lipopolysaccharides

μl : Microlitermg : Milligrammin : minute(s)ml : Milliliter

mm³Millimeter cubicMWMolecular weight

μm
nm
OEs
OD
Micrometer
Nano meter
Oenocytoids
Optical Dinesty

P : Plasma

PAMP: Pathogen-associated molecular pattern

pH : Power of hydrogenPLs : Plasmatocytes

PPO : ProphenoloxidasePO : Phenoloxidase

PRP: Pattern recognition proteins

PRs : Prohaemocytes
Rpm : Round per minute
SE : Standard error

SEM : Scanning electron microscope

SDS-PAGE: Sodium duodecyl sulphate-polyacrylamide gel

electrophoresis

SP : Species

SPs : Spherulocytes

TEMED : N, N, N', N'-Tetramethylethylenediamine

THCsTotal haemocyte countTHPTotal haemolymph protein

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