Characterization of the Haemolymph of Honey Bee *Apis mellifera* (L.) Following Experimental Infection with Bacteria

A Thesis submitted to The Department of Entomology, Faculty of Science, Ain Shams University

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The award of the M. Sc. degree in Entomology

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DEDICATION

THIS THESIS IS DEDICATED TO MY FAMILY,
MY FATHER
MY MOTHER
MY SISTER

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ABSTRACT

The immune system of the third, fourth and fifth larval instars of *Apis mellifera* (Linnaeus) was evaluated following injection with a sublethal dose of the bacterium, *Paenibacillus larvae larvae* (White) and in naturally infected larvae at different intensities (low, medium and high). Variable changes in the total body weight, body water content, blood volume and density as well as total haemolymph proteins were recorded at different time intervals post-injection with bacteria in third, fourth and fifth larval instars and in naturally infected larvae of the same stages at different intensities of disease (low, medium and high). Thirteen type of haemocytes were described: prohaemocytes, granulocytes, eosinophil cells, oenocytes, plasmatocytes, spindle shaped cells, micronucleocytes, macronucleocytes, spherulocytes, pycnonucleocytes, basophil cells, adipohaemocytes and neutrophil cells. The plasmatocytes (PLs) represent 60-90% of the total haemocyte count (THC) and the other cells represent 10-40% of the cells. Injection with *P. l. larvae* into the third, fourth and fifth larval instars and naturally infected larvae produced several pathological consequences on the haemocytes including variation in the cell volume, vacuolization in the cytoplasm, distortion of the cell membrane and pycnosis in the nuclei. The phagocytic response against larvae injected with bacteria and naturally infected varies according to the larval age where the phagocytic response percentages increase as the larval age increase. Humoral defense mechanisms are usually integrated with the cellular defense mechanisms by releasing antibacterial substances into the haemolymph as a result of induction of the bacteria. Analysis of the haemolymph proteins by SDS-polyacrylamide gel electrophoresis showed the disappearance of some proteins and the appearance of new immune proteins in the injected fourth and fifth larval instars, the same effects were appeared for the third, fourth and fifth naturally

infected larvae due to the infection. Some of these proteins may function in the immune response. Results indicate that the most susceptible larval instar is the third larval instar and the highly resistant one is the fourth larval instar.

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ABREVIATIONS

ABS	**:**	Absorbency
AFB	**:**	American foul brood
ADS	**:**	Adipohaemocytes
A. mellifera	**:**	*Apis mellifera*
AMPs	**:**	Antimicrobial peptides
Bis	**:**	N, N-methylenebisacrylamide
Bas	**:**	Basophil cells
BP	**:**	"Before Present" means before 1950. The most commonly used convention in radiocarbon dating
BSA	**:**	Bovine serum albumin.
Bt	**:**	*Bacillus thuringinsis*
CBB	**:**	Coomassie brilliant blue
CFU	**:**	Colony formed unit
cm	**:**	Centimeter(s).
DHC	**:**	Differential haemocyte count.
EOs	**:**	Eosinophil cells
EFB		European foul brood
g	**:**	gravity
GRs	**:**	Granulocytes.
hr	**:**	Hour(s).
Ig	**:**	Immunoglobulin.
kDa	**:**	Kilo dalton(s).
L3	**:**	Honey bee third larval instar
L4	**:**	Honey bee fourth larval instar
L5	**:**	Honey bee fifth larval instar
LD	**:**	Lethal dose.
LPS	**:**	Lipopolysaccharidase
LSP	**:**	Larval specific protein
MAs	**:**	Macronuclrocytes
Mis	**:**	Micronuclrocytes
NEs	**:**	Neutrophil cells
µl	**:**	Microliter
ml	**:**	Milliliter
MIs	**:**	Micronucleocytes
min	**:**	Minute(s)
µm	**:**	Micrometer
Mol. wt.	**:**	Molecular weight
N	**:**	Number of test replicates
OEs	**:**	Oenocytoids
PCR	**:**	Polymerase chain reaction

P. l. larvae	**:**	*Paenibacillus larvae larvae*
PLs	**:**	Plasmatocytes
PRs	**:**	Prohaemocytes
pPo	**:**	Prophenoloxidase
PYs		Pycnonucleocytes
rpm	**:**	Round per minute
SE	**:**	Standard error
SPLs	**:**	Spindle Plasmatocytes
SDS-PAGE	**:**	Sodium duodecyl sulphate-polyacrylamide gel electrophoresis
SPs	**:**	Spherulocytes
ssp.	**:**	Sub species
TEMED	**:**	N, N, N', N'-Tetramethylethylenediamine
THC	**:**	Total haemocyte count

LIST OF TABLES

		Page
Table 1 :	Susceptibility of third larval instar of *A. mellifera* to *P. l. larvae* injected into body cavity ……	76
Table 2 :	Susceptibility of fourth larval instar of *A. mellifera* to *P. l. larvae* injected into body cavity .	77
Table 3 :	Susceptibility of fifth larval instar of *A. mellifera* to *P. l. larvae* injected into body cavity ……	78
Table 4 :	Total body weight and water content of third larval instar of *A. mellifera* determined at different time intervals post-injection with *P. l. larvae* ….....	82
Table 5 :	Total body weight and water content of fourth larval instar of *A. mellifera* determined at different time intervals post-injection with *P. l. larvae* ….....	84
Table 6 :	Total body weight and water content of fifth larval instar of *A. mellifera* determined at different time intervals post-injection with *P. l. larvae* ….....	86
Table 7 :	Haemolymph volume (µl/larva) of third larval instar of *A. mellifera* determined at different time intervals post-injection with *P. l. larvae* using Amaranth dye method ……...	90
Table 8 :	Haemolymph volume (µl/larva) of fourth larval instar of *A. mellifera* determined at different time intervals post-injection with *P. l. larvae* using Amaranth dye method ……...	91
Table 9 :	Haemolymph volume (µl/larva) of fifth larval instar of *A. mellifera* determined at different time intervals post-injection with *P. l. larvae* using Amaranth dye method ……...	82
Table 10:	Haemolymph density (mg/µl) of third larval instar of *A. mellifera* determined at different time intervals post-injection with *P. l. larvae*……	95
Table 11:	Haemolymph density (mg/µl) of fourth larval instar of *A. mellifera* determined at different time intervals post-injection with *P. l. larvae*……	96
Table 12:	Haemolymph density (mg/µl) of fifth larval instar of *A. mellifera* determined at different time intervals post-injection with *P. l. larvae*……	97

Table 13: Haemolymph density (mg/µl) of different (third, fourth and fifth) larval instars of *A. mellifera* determined at different intensities of infection (low, medium and high)……. 98

Table 14: Total haemolymph proteins (mg/ml) of third larval instar of *A. mellifera* determined at different time intervals post-injection with *P. l. larvae* …..... 101

Table 15: Total haemolymph proteins (mg/ml) of fourth larval instar of *A. mellifera* determined at different time intervals post-injection with *P. l. larvae* …..... 102

Table 16: Total haemolymph proteins (mg/ml) of fifth larval instar of *A. mellifera* determined at different time intervals post-injection with *P. l. larvae* …..... 103

Table 17: Total haemolymph proteins (mg/ml) of different (third, fourth and fifth) larval instars of *A. mellifera* determined at different intensities of infection (low, medium and high)…… 104

Table 18: Characteristics of normal haemocytes of *A. mellifera*……. 108

Table 19: Differential haemocyte counts (DHCs) of third larval instar of *A. mellifera* determined at different time intervals post- injection with *P. l. larvae* ……. 120

Table 20: Differential haemocyte counts (DHCs) of fourth larval instar of *A. mellifera* determined at different time intervals post- injection with *P. l. larvae* ……. 122

Table 21: Differential haemocyte counts (DHCs) of fifth larval instar of *A. mellifera* determined at different time intervals post- injection with *P. l. larvae* …… 124

Table 22: Differential haemocyte counts (DHCs) of different (third, fourth and fifth) larval instars of *A. mellifera* determined at different intensities of infection (low, medium and high)…….. 126

Table 23: Total haemocyte counts (THCs) (cells/mm^3) of third larval instar of *A. mellifera* determined at different time intervals post-injection with *P. l. larvae*……..……..…….. 130

Table 24: Total haemocyte counts (THCs) (cells/mm^3) of 131

fourth larval instar of *A. mellifera* determined at different time intervals post-injection with *P. l. larvae*……..……..……..

Table 25: Total haemocyte counts (THCs) (cells/mm^3) of fifth larval instar of *A. mellifera* determined at different time intervals post-injection with *P. l. larvae*……..……..…….. 132

Table 26: Total haemocyte counts (THCs) (cells/mm^3) of different (third, fourth and fifth) larval instars of *A. mellifera* determined at different intensities of infection (low, medium and high)…….. 133

Table 27: Percentages of phagocytosis in third, fourth and fifth larval instars of *A. mellifera* determined at different time intervals post-injection (3, 6, 12 and 24 hr) with *P. l. larvae*…… 137

Table 28: Percentages of phagocytosis of different (third, fourth and fifth) larval instars of *A. mellifera* determined at different intensities of infection (low, medium and high)……. 138

Table 29: Nodule formation of third larval instar of *A. mellifera* determined at different time intervals post-injection with *P. l. larvae*…… 142

Table 30: Nodule formation of fourth larval instar of *A. mellifera* determined at different time intervals post-injection with with *P. l. larvae*……... 143

Table 31: Nodule formation of fifth larval instar of *A. mellifera* determined at different time intervals post-injection with with *P. l. larvae*……... 144

Table 32: Antibacterial activity of third larval instar of *A. mellifera* determined at different time intervals post-injection with *P. l. larvae*……..... 148

Table 33: Antibacterial activity of fourth larval instar of *A. mellifera* determined at different time intervals post-injection with *P. l. larvae*……. 149

Table 34: Antibacterial activity of fifth larval instar of *A. mellifera* determined at different time intervals post-injection with *P. l. larvae*……..... 150

Table 35: Antibacterial activity of different (third, fourth and fifth) larval instars of *A. mellifera* determined at different intensities of infection (low, medium and high)……. 151

Table 36: Comparative analysis of rate of flow (Rf),

molecular weight (mol.wt.) and relative concentration (band %) of haemolymph protein bands of third larval instar of *A. mellifera.* The bands were separated using SDS-PAGE technique…….. 160

Table 37: Comparative analysis of rate of flow (Rf), molecular weight (mol.wt.) and relative concentration (band %) of haemolymph protein bands of fourth larval instar of *A. mellifera*. The bands were separated using SDS-PAGE technique…… 164

Table 38: Comparative analysis of rate of flow (Rf), molecular weight (mol.wt.) and relative concentration (band %) of haemolymph protein bands of fifth larval instar of *A. mellifera*. The bands were separated using SDS-PAGE technique. 168

Table 39: Comparative analysis of rate of flow (Rf), molecular weight (mol.wt.) and relative concentration (band %) of haemolymph protein bands of different (third, fourth and fifth) larval instars of *A. mellifera* determined at different intensities of infection (low, medium and high). The bands were separated using SDS-PAGE technique. 172

LIST OF FIGURES

		Page
Figure 1 :	Standard calibration curve for the estimation of weight of dye in the sample ……	61
Figure 2 :	Standard calibration curve for the estimation of total protein content determined by the dye binding method ……	63
Figure 3 :	*Paenibacillus larvae larvae* colonies shape……..	74
Figure 4 :	*Paenibacillus larvae larvae* bacterial slant……....	74
Figure 5 :	Paenibacillus larvae larvae vegitative cells……....	74
Figure 6 :	*Paenibacillus larvae larvae* spore cells……	74
Figure 7 :	*Paenibacillus larvae larvae* vegetative and spore cells stained with carbol fuchsin……	74
Figure 8 :	*Paenibacillus larvae larvae* spore cells stained with carbol fuchsin……	74
Figure 9 :	Holst milk Test result……..	74
Figure10:	Catalase production Test result……	74
Figure 11:	Nitrate reduction Test result……	74
Figure 12:	Susceptibility of third larval instar of *A. mellifera* to *P. l. larvae* injected into body cavity ……	76
Figure 13:	Susceptibility of fourth larval instar of *A. mellifera* to *P. l. larvae* injected into body cavity	77
Figure 14:	Susceptibility of fifth larval instar of *A. mellifera* to *P. l. larvae* injected into body cavity …….	78
Figure 15:	Total body weight, dry weight and water content of third larval instar of *A. mellifera* determined at different time intervals post-injection with *P. l. larvae* ….....	83
Figure 16:	Total body weight, dry weight and water content of fourth larval instar of *A. mellifera* determined at different time intervals post-injection with *P. l. larvae* ….....	85
Figure 17:	Total body weight, dry weight and water content of fifth larval instar of *A. mellifera* determined at different time intervals post-injection with *P. l. larvae* ….....	87
Figure 18:	Haemolymph volume (µl/larva) of third larval instar of *A. mellifera* determined at different time intervals post-injection with *P. l. larvae* using Amaranth dye method ……..	90
Figure 19:	Haemolymph volume (µl/larva) of fourth larval instar of *A. mellifera* determined at different time	91