Studies of the genetic and physiological alterations in cadmium-resistant *Biomphalaria alexandrina*: Implication of susceptibility to molluscicides and *Schistosoma mansoni* infection.

"A Thesis Submitted in Partial Fulfillment of the Requirements for the Master Degree of Science"

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By

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Declaration

I declare that this thesis has been composed by me, and that the work of which is a record has done by me. It has not been submitted for a degree at this or any other university.

Sincerely

Dina Gamal Tawfik

Dedication

To my dear parents and brothers

To my beloved husband, daughter and son

To my all family and friends

To my all professors and teachers

I dedicate this to you and I hope you will be proud of me.

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Acknowledgement

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Abstract

Abstract

Biomphalaria alexandrina snails were examined for their tolerance to cadmium (Cd) toxicity through three generations produced by self-fertilization by exposing snails to serial concentrations of Cd and selection for the most tolerant ones through the successive generations. Results showed a gradual increase in the LC_{50} and LC_{90} values through successive generations indicating tolerance upgrading phenotype. Also, the selected Cd-tolerant third generation snails (G3), non-selected laboratory breeding snails and field snails were examined for the toxicity of chemical and plant molluscicides, bayluscide and *Anagallis arvensis*, respectively and other heavy metals, zinc (Zn), copper (Cu) and lead (Pb). The selected Cd-tolerant third generation (G3) showed higher LC_{50} and LC_{90} values for *A. arvensis* and Cu than each of non-selected laboratory breeding and field groups.

The selected and non-selected snails were examined for their susceptibility to *Schistosoma mansoni* infection and physiological criteria (haematological and biochemical). The selected snails showed refractory tendency and a significant increase in haemocytes; granulocytes; which indicated that their defence mechanism was stronger than non-selected ones. Biochemical criteria indicated that selected Cd-tolerant group had stable liver enzymes and protein levels while had increase in the levels of urea (significant) and creatinine (non-significant) indicating that the liver cells were still intact while renal cells were affected. On the other hand, field group was the most affected followed by laboratory one.

Dendrogram based on similarity matrices of both protein fractions separated by SDS-PAGE and ISSR-PCR banding patterns resulted with ten random primers, showed that the selected Cd-tolerant 3rd generation was the most divergent group among the other examined snail groups (1st generation, 2nd generation, parent and laboratory breeding groups). So, the present mating system and selection succeeded in the evolution of Cd-tolerant snail isolate that characterized genetically, physiologically, and showed refractory tendency to *S. mansoni* infection.

Keywords:

Biomphalaria alexandrina, toxicity curve, cadmium-tolerance, genetic selection, isolates, bayluscide, *Anagallis arvensis*, heavy metals, biochemical response, haemocytes, *Schistosoma mansoni*, infection, SDS-PAGE, ISSR-PCR, similarity, dendrogram.

List of abbreviations

ALB	Albumin
ALP	Alkaline Phosphatase
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BCM	Billion Cubic Meters
bp	Base pair
Cd	Cadmium
$CuSO_4$	Copper sulphate
Cr	Chromium
dl	dice litter
G1	First generation
G2	Second generation
G3	Third generation
Hg	Mercury
IDS	Internal Defence System
ISSR-PCR	Inter simple sequence repeat polymerase chain reaction
IU	International Unit
kDa	Kilo Dalton
LC	Lethal Concentration
ml	Mille litter
mm	Mille meter
Mn	Manganese

NaPCP	Sodium penta chlorophenate
Ni	Nickel
Nm	Nano meter
Pb	Lead
PCR	Polymerase chain reaction
PPb	Part per billion
ppm	Part per million
r.p.m.	Random per minute
RAPD-PCR	Random amplification of polymorphic DNA polymerase chain reaction.
S	Similarity coefficient
SBSC	Schistosome Biological Supply Centre
SDS-PAGE	Sodium dodecyl sulphate-polyacrylamide gel electrophoresis
TBRI	Theodor Bilharz Research Institute
TP	Total protein
UV	Ultra Violet
Zn	Zinc
µg	Micro gram
µl	Micro litter
µm	Micro meter

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