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# Evaluation the expression of some insecticidal protein coding genes during seeds storage

A Thesis Submitted for the Degree of Doctor of Philosophy of Science (Botany)

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#### Abstract

Seed considers the basic and crucial input for agricultural production. The most injurious insects which causes a great damage for Vicia faba seeds and Triticum durum grains is Callosobuchus maculatus F. and Rhyzopertha dominica. The present work was designed to evaluate the efficacy of three protectants (deltamethrin as synthetic insecticide; Beauveria bassiana as bioinsecticide and phosphate fertilizer) on these storage pests. This study focused on some defense-related genes as resistant factors under storage conditions. Plants developed different defense mechanisms to reduce insect attack, such as the induction of defensive proteins towards a range of economically important insect pests. Amongst the genes encoding insecticidal proteins are those that encode lectins protein. Quantification the expression of *lectin* gene, and two cell wall-remodeling genes (pectin and pectin-esterase) at the transcriptional level in Vicia faba and pectin-methyl-esterase inhibitor gene in wheat plant were examined. The result obtained showed that the imported V. faba cultivar and the T. durum Beni-Suef5 cultivar was the most susceptible to infestation by C. maculatus and R. dominica respectively, while Noubaria1 and Suhag5 cultivars appeared to be more resistant. The mortality percentage of C. maculatus and R. dominica was increased with increasing the concentrations of the tested materials and the residual activity of  $LC_{95}$  in V. faba and T. durum cultivars treated with deltamethrin or *B. bassiana* indicating high residual effect till three months, while a sharp decline in the efficiency of the fertilizer was recorded at 8<sup>th</sup> week.

Considerable variations in protein banding patterns were observed in both *V. faba* and *T. durum* treated with LC<sub>95</sub> deltamethrin or with *B. bassiana* in which some bands are disappeared and new are appeared. SCoTs analyses showed alteration in *Vicia faba* and *T. durum* genomes and decreasing in the Genomic Template Stability (GTS) index in response to treatments with LC<sub>95</sub> of deltamethrin and LC<sub>95</sub> *B. bassiana* after storage periods were recorded. The expression of three defense related genes (*Vf\_Lectin*, *Vf\_Pectin* and *Vf\_Pectinesterase*) were investigated in *Vicia faba* and only one gene (*Td\_Pectin-methylesterase* inhibitor) was investigated in *T. durum* cultivars. The upregulation of *Vf\_Lectin* genes was recorded in both *Vicia faba* cultivars after deltamethrin treatments while its expression downregulated with the bio-insecticide, compared with control. The expression level of  $Vf\_Pectin$  gene in response to deltamethrin and *B. bassiana* was upregulated in both *Vicia faba* cultivars, but the highest expression level recorded in imported cultivar by 44-fold in response to *B. bassiana*. The expression level of  $Vf\_Pectin-esterase$  gene was upregulation in all samples, except in the cultivar Noubaria1 treated with LC<sub>95</sub> deltamethrin was downregulated. *Td\_Pectin-methyl-esterase Inhibitor (PMEI)* gene was upregulated in both *T. durum* cultivars (Suhag5 and Beni-Suef5) in response to LC<sub>95</sub> of deltamethrin or to LC<sub>95</sub> of *B. bassiana* and the expression level in response to *B. bassiana* has exhibited highest level by 12-fold for Beni-Suef5 compared to non-treated grains.

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# List of abbreviations

Abbreviation	Complete name
ABM	Abamectin
ALA	Amino-levulinic acid
AP-PCR	The arbitrarily primed polymerase chain reaction
APX	Ascorbate peroxidase
αAl-I	Amylase inhibitors
C: I	Chloroform isoamyl
CAT	Catalase
CpTI	Cowpea encoding trypsin inhibitor
CV	Cultivar
DEPC	Diethylpyrocarbonate
DNA	Deoxyribonucleic acid
dsRNA	Double-stranded- Ribonucleic acid
EDTA	Ethylene-diamine-tera-acetic acid
FAO	Food and agriculture organization of the United Nations
F1	First generation
g/ ha	gram per hectare
GR	Glutathione reductase
GTS	Genomic template stability
IPs	Insect pests
IRAP	Inter-retrotransposon amplified polymorphism
ISSR	Inter simple sequence repeat
KD	Kilodalton
mg/kg	milligram per kilogram
ml/L	Milliliter per liter
mM	Millimole
μM	Micrometer
mRNA	messenger ribonucleic acid
NPs	Nanoparticles
PAE	Pectin acetyl-esterase
PE	Pectin-esterase
PIs	protease inhibitors

Abbreviation	Complete name
PME	Pectin methyl esterase
PMEI	Pectin methyl-esterase inhibitor
ppm	Parts per million
qRT-PCR	Quantitative Reverse Transcription PCR
RAPD	Random amplified polymorphic DNA
REMAP	Retrotransposon-microsatellite amplified polymorphism
RNA	Ribonucleic acid
RNAi	RNA interference
RIPs	Ribosome-inactivating proteins
ROS	Reactive oxygen species
SAPs	Stress-associated proteins
(SCoT) marker	Start codon targeted
SDS	Sodium dodecyl sulphate
SOD	Superoxide dismutase
ТЕ	Tris-EDTA buffer
ТХМ	Thiamethoxam
U	Unit
WAKs	Wall-associated kinases
WHO	World Health Organization