Development and Characterization of Resistance to Some Biocides in Cotton Leaf Worm

Spodoptera littoralis

A thesis submitted in partial fulfillment of the requirement

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Master of Science

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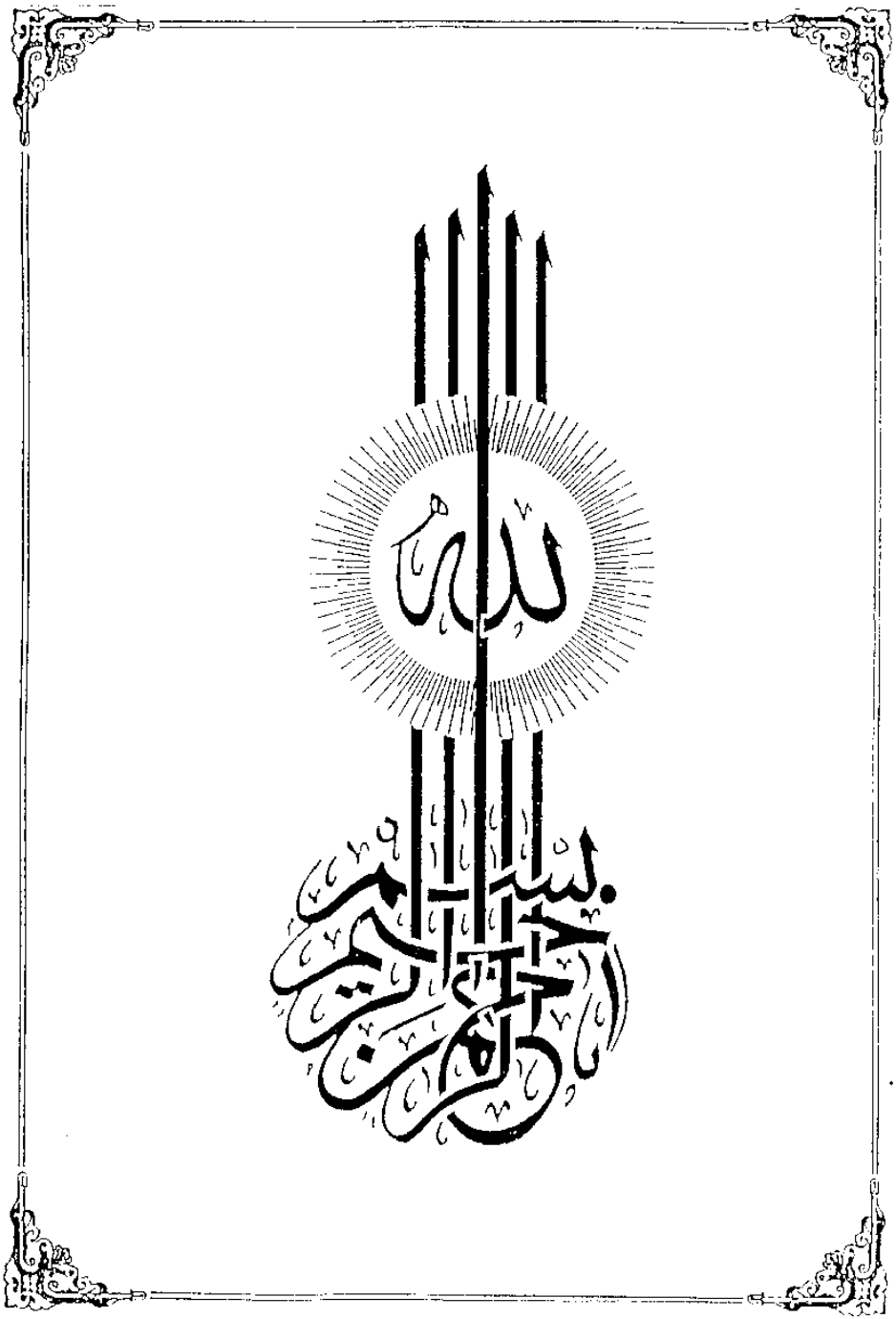

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Abbreviation

BBMV	: Brush border membrane vesicle
Cry	: Crystal protein
D OV	: Degenerating ova
DG Y	: Degenerated yolk
Ent	: entomocidus
FE	: Follicular epithelium
Fin	: fintimus
ICPs	: Insecticidal crystal proteins
ICS	: Intracellular space
KDa	: Kilo-dalton
LB	: Luria- Bertani medium
OC	: Oocyte
OES	: Outer epithelial sheath
OV	: Ova
SDS-PAGE	:Sodium dodecyl sulfate-polyacrylamide gel electrophorasis
T	: Trophocyte
TC	: Trophocyte cytoplasm
TN	: Trophocyte nucleus
V	: Vacuole
Y	: Yolk

ABSTRACT

Four bacterial isolates of *Bacillus thuringiensis*, designated as DI 29, C 12, Ent and Fin, were used in the present work. They were tested against the larvae of the cotton leaf worm, *Spodoptera littoralis*. The LC50's of the four isolates were estimated graphically from the log probit regression lines. Protein patterns of both vegetative and the sporulating bacteria were analyzed using SDS-PAGE. The two isolates (DI 29 and Ent) were tested for resistance to *B. thuringiensis*. The cross-resistance between the two bacterial isolates in the selected insect was studied. The inheritance of resistance to Ent in the selected insect line was studied also. The biological activities of treated individuals by DI 29 and Ent isolates were determined. They included larval duration, percentage of pupation, sex ratio, pupal duration, adult emergence, fecundity and the hatchability of eggs. The histopathological effect of the bacterial isolates on the female reproductive system was also studied.

Key words : Resistance, selection, biocides and *Spodoptera littoralis*.

Contents

Introduction

INTRODUCTION

The use of synthetic chemicals to control pests of different crops was widespread during the last half of this century. From 1943 to 1985, the world consumption of pesticides jumped from less than 30 thousand metric tons to 3 million metric tons in 1985 (Prokopy 1988). As a consequence of the over-dependence on chemical control, side effects to human health and the environment have been developed. An estimated 25 million agriculture workers in developing countries are poisoned every year by pesticides (Jeyaratnam 1990). Other important effects of the use of pesticides are outbreaks of secondary pests, due to the elimination of natural enemies and the development of pesticides resistance.

According to Wilcox *et al.* (1986), more than $3 billion are spent worldwide each year to control insect pests. Although the use of pesticides worldwide is always increasing, yield losses caused by pests have not decreased since World War II. Even in developed countries, the plant health condition has deteriorated.

Georghieou and Lagunes (1991) stated that the first detection of the resistance phenomenon were in 1914. A bout 504 species of arthropods have shown resistance to different groups of pesticides, 283 of which are agricultural pests. Insect pests of the order Lepidoptera constitute the most important group of all the agricultural pests and most of them are pests created by the overuse of pesticides. The lepidopterans also have the leadership in the number of species resistant to insecticides representing about 26 % of the total.

The use of microbial pathogens as a biological control agent are a new approach for pest control. At present there are a number of viruses, bacteria, and fungi which have been formulated as commercial pesticides. Bacteria are the most promising agents in

this respect. Over 90 species of bacteria which infect insects have been described. Most belong to the families *Pseudomonadaceae*, *Entrobateriaceae*, *Lactobacillaceae*, *Micrococcaseae* and *Bacillaceae*.

One of the most widely used as microbial control agent is *Bacillus thuringiensis*, which is an environmentally sound alternative to the current chemical insecticides in the control of some phytophagous and vector born diseases. This gram-positive bacterium produces crystalline parasporal inclusions containing insecticidal crystal proteins (ICPs). Different strains produce crystal protiens with different insecticidal spectra. Due to their high specificity, ICPs are harmless to non-target organisms. *B. thuringiensis* insecticides have been used for over 30 years but still represent less than 1% of all insecticides in use (Ferre *et al.* 1991).

It is expected that during the coming years the bacterial insecticidal sprays will be applied on a much larger scale. It has been projected to increase to as much as 50 % by the year 2000 (Klausner 1984) and using of *B. thuringiensis* is expected to increase dramatically because of recent advances in genetic engineering including insertion and expression of *B. thuringiensis* toxin genes in several crop plants such as Cotton , Tobacco, and Tomato (Gasser &Fraley 1989) as well as Potato (Ebora *et al.* 1994). Development of new strains of *B. thuringiensis* with higher potency will increase its usefulness (Ferro & Gelernter 1989; and Zehnder &Gelernter 1989).

Despite its use since 1960, only diamondback moth, *Plutella xylosella*, has recorded resistance in the Philippines (Tabashnik *et al.* 1990), Hawaii (Ferre *et al.* 1991) and more recently in Florida, Japan, Malaysia and Thailand (Tabashnik 1994). However, in laboratory selection experiments, various levels of resistance were