TOXICITY OF CERTAIN INSECTICIDES TO *Spodoptera littoralis* (BOISD.) IN RELATION TO GENETIC STRUCTURE

By

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B.Sc. Agric. Sci. (Plant Protection), Fac. Agric., Cairo Univ., 2004.
M.Sc. Agric. Sci. (Pesticides), Fac. Agric., Cairo Univ., 2011.

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APPROVAL SHEET

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ABSTRACT

DNA (RAPD), efficacy of certain insecticides against *S. littoralis* and their effect on total protein, protein SDS-PAGE and the persistence of cypermethrin and chlorpyrifos in and on cotton leaves were investigated.
On the basis of LC_{50} and LC_{90} levels, cypermethrin was the most potent compound against *S. littoralis* after 2- days of treatment, followed by esfenvalerate, chlorpyrifos and then *B. thuringiensis.*

The potential of random amplified polymorphic DNA (RAPD) detected genetic polymorphism among the control and treated *S. littoralis* larvae by tested compounds at LC_{50} levels.. Five primers revealed differences among control and (dead & alive) treated samples with visible changes in DNA fragments number and size. Primer C1 was the most powerful in detecting polymorphism frequency as 63.6%.

Total protein of *S. littoralis* alive larvae increased at the 2nd,the 4th, the 6th, and the 9th day posttreatment as 4th instar in most treatments by LC_{50} of the tested compounds. The electrophoretic analysis of proteins by SDS-PAGE was carried out for untreated normal strain at different days posttreatment with LC_{50} of the above mentioned compounds. 119 bands were separated and their molecular weights ranged between 3.06 and 128.09 KDa.

Cypermethrin was the most effective compound caused thickening of outer cuticle fibrous layer in the integument of *S. littoralis* larvae after 6-days of treatment as 4th instar. In addition, all the treatments caused appearance of fissure and breaking down of muscles into small parts.. Neurosecretory cells of nerve cord had shrunk and dwarfed. Also, nucleus and nerve cells were disappeared partly in most treatments.

The initial deposit of chlorpyrifos on cotton leaves was higher than that of cypermethrin (532.3 and 63.74 ppm, respectively); while, at 14 days posttreatment, the residues were 0.46 and 3.12 ppm, respectively.

Key words: Cotton, insecticides, residue, RAPD, SDS-PAGE, *S. littoralis*

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CONTENTS

INTRODUCTION

Cotton (*Gossypium hirsutum* L.) is a cash crop for more than 20 million farmers in developing countries of Asia and Africa. Cotton termed as "The king of fibers and a crop of prosperity" having a great impact on men and industrial commodity of worldwide importance. Cotton seed contains approximately 18-25% of oil and 20-25% high quality protein (Devesh *et al*., 2011).

Cotton seed oil is rich in tocopherols that inhibits rancidity development and thus contribute to its stability in a longer shelf life for the product. It has also gained importance in food preparation due to its higher smoke point (about 232°C) compared to other cooking oils and it is good for frying food articles (O'Brien *et al*., 2005). The areas under cotton production in the world are estimated at around 30-31 million hectares. India is the largest area under cotton production. China is the largest producer of cotton in the world. Low productivity of cotton may be attributed to both biotic and abiotic stresses. Among the biotic stresses, insect pests are known to cause heavy loss to cotton resulting in drastic reduction in yield.

The cotton leaf worm, *Spodoptera littoralis* (Boisd.), (Lepidoptera: Noctuidae) is considered as an important sporadic pest in the world. Its high reproductive potential, short generation duration, migration ability and wide host spectra has enabled it to assume the status of key pest of many cereal, cash and horticultural crops. Leaf worm is known to attack wide range of succulent crops and weeds of many kinds (Skibbe *et al*., 1995). It attacks 112 cultivated plant species belonging to 44 different families (Mallikarjuna *et al*., 2004). Cotton leaf worm is basically a leaf eater but larvae may also attack flowers, buds and immature bolls. The larvae prefer young to old leaf and always feed gregariously with amazing voracity leaving midrib veins only. Heavy infestations of cotton leaf worm can completely defoliate the plants. Due to heavy defoliation the infested fields give a look of standing sticks (Huque, 1994). This sporadic pest may attack the cotton crop at any stage. In Egypt, it has become serious pest of cotton crop (Ahmad *et al.,* 2008). This notorious pest is difficult to control because of its cryptic habitat and high populations after heavy rain fall (Chen, 1979). Females lay 1000-2000 eggs in masses of 100-300 on underside of the leaf surface of the host plant. Despite important advances in technology, only limited control measures have been devised to manage the population of *S. littoralis*. A common strategy preferred by farmers to protect cotton crop is utilization of chemical pesticides as they often have knock down effect. It is reported that about 90% of the farmers protect crops from this insect pest using chemical insecticides (Prayogo *et al*., 2005). Cotton crop is the largest consumer of insecticides throughout the world (Yan *et al.,* 2004). Chemical insecticides provide only short term solution for the pest control (Farag *et al.,* 2011).

Organophosphates are some of the most widely used pesticides in the world. There are more than 40 different organophosphate pesticides on the market today (Hodgson *et al.,* 2004).

Synthetic pyrethroids are presently used extensively in most countries and have become the most significant class of agricultural insecticides since their introduction in the early 1980s. As a class, they possess a number of significant advantages over most organophosphorus and organochlorine insecticides. These advantages include low toxicity to mammals and birds, rapid breakdown in the environment and rapid elimination from animals (Kidd and James 1991). The compounds of cypermethrin and esfenvalerate belonging to synthetic pyrethroid group, act as a contact and stomach poison. It gives wide spectrum activity against lepidoptera, coleoptera and hemiptera pests and various workers have studied residues of pyrethroids in cotton as mentioned by Battu *et al*. (1999), Sinha and Gopal (2002), Wang and Liu (2000), Ranga *et al*. (1993) and Mandal *et al.* (2010).

Entomopathogenic bacteria represent one of the main microbial control agents that have been applied for about 130 years to control insects and are also still under investigations for their utilization purposes. They are considered potential biological agents for pest control due to their specificity, mode of action and ease of application. Microbial plant protection with entomopathogenic bacteria, *Bacillus thuringiensis* (*B.t.*) reduces use of chemical insecticides (Lacey and Goettel 1995).

B.t. has potential to control resistant species of insects (Ignoffo and Roush 1986). Bacterial biocontrol agents were among the first organisms to be used for pest control before chemical pesticides were developed (Rombach and Gillespie 1988).

The Polymerase Chain Reaction (PCR) that described by Saiki *et al.* (1985) still widely used for a variety of purposes. Classical PCR assay simply requires a target DNA sequence and two synthetic oligonucleotide primers complementary to opposite strands of the target DNA. Indeed, mutations may affect the annealing of the primers whereas DNA damage may interfere with the DNA polymerase activity, thus altering the number of newly synthesized amplicons. (RAPD) assay proved to be a sensitive method to detect genotoxin-induced DNA damage and mutations mentioned by Williams *et al.* (1990), Fritsch and Riesberg (1996), Newton and Graham (1997), Atienzar *et al.* (2001), Atienzar *et al.* (2002 a,b) and DeWolf *et al.* (2004).

Generally, RAPD reactions are performed with a single 10 base pair (bp) primer and multiple amplifiable fragments (from different loci) are usually present for each set of primers across each entire genome (Lynch and Milligan 1994).

The reactions generate a number of amplicons of variable lengths (*e.g.* between 100 and 4000 bp) (Atienzar *et al.*, 2002b). Number of loci that can be examined is essentially unlimited (Lynch and Milligan, 1994). RAPD-PCR is a versatile and inexpensive tool. Its main advantages lie in its rapidity and applicability to any organism (since no information on the nucleotide sequence, cell cycle, or chromosome complement is required) (Atienzar *et al*., 2001 and Lushai *et al.*, 2000).

A variety of bacteria, fungi, and insect species were successfully DNA typed, and closely related species were distinguished by the RAPD-PCR assay mentioned by Lushai *et al*. (2000), Wilkerson *et al.* (1995), Benecke, (1998), Amer, *et al.* (2008), Hamouda, (2008), Karam *et al.* (2008), Kwon *et al.* (2009), Ibrahim *et al.* (2010) and Mohamed *et al.* (2010).

DNA is associated with the transfer of genetic information from cell to cell, while RNA is associated with their transfer within the cell and with the protein synthesis. Therefore, these genetic tool is considered as controlling factors from the normal way effect on the fecundity and fertility of the insects (La Chance *et al*.,1985). RAPD profiles generated by these primers revealed differences between control and treated samples with visible changes in number and size of amplified DNA fragments (Bakr *et al*., 2012).

Quantitative analysis of protein by spectrophotometer showed that amount of proteins was differenced in crystal-spore mixture of the tested *B.t.* strains. Qualitative analysis of proteins by sodium dodecyl sulphate - polyacrylamide gel electrophoresis on the basis of molecular masses of proteins showed that the protein profiles of *B.t.* strains could distinguish into three main protein groups (Haggag and Abou Yousef 2010).

The central nervous system, C.N.S., of the cotton leaf worm, *Spodoptera littoralis* (Boisd.) develops from a median row of embryonic neuroblasts which divide repeatedly to form a double ganglionic chain (Banhawy and Anwar 1983). The two chains fuse together laterally giving rise to the larval C.N.S. which is differentiated into a brain, a frontal, a suboesophageal, three thoracic and seven abdominal ganglia. During the pupation period, the larval C.N.S. undergoes marked changes leading to the formation of the C.N.S. of adult moths. While, Sampsont and Gooday (1998) stated that *Bacillus thuringiensis* subsp. *israelensis* IPS78 and *B. thuringiensis* subsp. *aizawai* HD133 both secreted exochitinase activity when grown in a medium containing chitin. In addition, Osman and Abou-zeid (2015) conducted the organophosphorus insecticide; Profenofos for controlling the 4th instar larvae of cotton leaf worm under the semi field circumstances and follow up histological effects on insect. Histological changes as found in the insect were destruction of cell walls of their body, mid gut and cuticle layers of treated insect.

According to Codex (2009), a pesticide residue means any specified substances in food, agricultural commodities, or animal feed resulting from the use of a pesticide. The term pesticide residue also includes residues from unknown or unavoidable sources (*e.g.* environmental), as well as known uses of the chemical) (Codex Alimentarius Commission, 2009).

The aim of the present investigation is to contribute to the available knowledge on the following:

1. Efficacy of tested insecticides against the 4^{th} instar larvae of *S. littoralis*.
2. Molecular study to estimate the insecticidal activity of sublethal concentration of aforementioned insecticides to evaluate the potential of the RAPD-PCR assay for the detection of genetic polymorphism between control and dead & alive treated *S. littoralis* larvae.
3. Biochemical study to estimate protein changes by sodium dodecyl sulphate poly acrylamide gel electrophoresis (SDS-PAGE) in normal and treated *S. littoralis* larvae treated with $LC_{50,}$s of tested insecticides .
4. Ultra structural effects of insecticides on integument, muscle, fat body, mid gut and nerve core sections of *S. littoralis* alive and dead larvae treated with LC_{50},s of tested insecticides.
5. Persistence of cypermethrin and chlorpyrifos inside and outside cotton leaves at different times (Zero, 1,3,5,7,10,14 and 21 days) post treatment.

REVIEW OF LITERATURE

1. Toxicological studies

Abou El-Ghar (1994) reported that profenofos gave the highest potency on cotton leaf worm larvae at all tested concentrations, because profenofos is an insecticide and recommended for controlling *S. littoralis* and this is considered to be the target effect of it. The reduction of growth rate was due to lower efficiency of conversion for ingested food to body substances for larvae with these treatments.

Abo El-Ftooh (2004) mentioned that pesticide of profenofos was more effective on *S. littoralis* than the isolated bacteria, *B. thuriengiensis* through the experiment period.

Marzouk *et al*. (2012) investigated the Egyptian cotton leaf worm, *Spodoptera littoralis* (Boisd.) 4^{th} instar larvae that fed on castor-bean leaves treated with profenofos at 40, 45, 50, 60, 75 and 90 ppm of profenofos (Silicron 72 % EC) insecticide. The obtained results showed that profenofos, caused significant increases in larval and pupal weights, growth rate, consumption index (C.I.), fecundity level , % of hatchability , % of deformed pupa , % of emergency and % of sterility decreased by the tested pesticides compared with that in control.

Said *et al.* (2012) compared the efficiency of the biocides (protecto) and synthetic insecticides against cotton leaf worm, *Spodoptera littoralis* (Boisd.). Results appeared that protecto was the least efficient compound, as it gave 27.14% reduction only, but about insecticides as lannate (methomyl+ diflubenzuron) and Cord (Profenofos) in eleven days after treatment it has a high efficiency in