

Efficiency of Using Some New Insecticides against Cotton Leaf Worm (*Spodopteralittoralis*) Based on Biochemical and Molecular Markers

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ABSTRACT

Bioassays were carried out for evaluating the toxicity of different insecticides against the susceptible strain of cotton Leafworm, *Spodoptera littoralis* (Boisd.). Toxicity data based on LC₅₀ values indicated that the proclam[®] is more toxic insecticide against the laboratory strain, while, the OP syclon[®] was less toxic. Esterase isozymes exhibited a wide range of variability among the pupae and larvae. Two common bands were detected among all samples in pupae and larvae. Proclam showed one band in pupae (Es.C₂) compared with other treatments and in larvae detected one band in (Es.C₃) and detecte the highest band number (6 bands) comparing with other insecticides. The results of biochemical analysis showed that there is arelationship between the resistance to insecticide and the increase of enzyme activity such as esterase enzyme. Five out of ten oligonucleotide primers (50%) used in the RAPD analysis gave unique markers. Out of the five primers, two detected single unique markers (OPA-02 and OPA-15), two gave two markers (OPA-05 and opc-02), and one yielded three unique markers (OPC-12). Additionally, compared with the control worms, cotton leaf worm exposed to insecticides recorded the emergence of 7 new fragments from 98 total fragments. So, the total number of fragments which disappeared in response to both treatments was 98 fragments. Exposure to insecticides at all concentrations revealed the increase or decrease the number of amplified polymorphic DNA and increased gene expression in comparison to control cotton leaf worm.

Key words: Cotton leaf worm, *Spodoptera littoralis*, bioassay, esterase Isozymes and RAPD

INTRODUCTION

The cotton leafworm *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) is a prolific and highly polyphagous insect. It is considered to be a major pest of great economic importance in many countries since it attacks a multitude of host plants (~73). The damage is mainly defoliation since the larvae (caterpillars) are principally leaf feeders and those larvae also have the habit of boring into and feeding in the interior of fruits such as tomato, young melon and pepper which near to or rest on soil (Kandil *et al.*, 2003). The use of insecticides is still having an important role in

controlling such insect-pest. Foliar applied insecticides are necessary when the economic threshold is exceeded. Therefore, insecticides are currently the principle method for controlling the Egyptian cotton leafworm in Egypt and will likely continue to be used until more biologically based management systems could be developed (Alotaibi, 2013).

Isozymes defined as multiple forms of enzymes in the same organism and having similar or identical catalytic activity. It is important to notice that enzyme analysis by electrophoresis offers very well defined and effective tools for detection of genetic differences among individuals (Shaw and Kaen, 1967). Genotype identification, based on morphological characteristics of insects, and plants, is often subjected to errors caused by variable environmental conditions. These errors are increased if the genotypes are of a similar origin. Electrophoresis and other techniques have been widely used to group and identify genotypes. Isozymes loci are excellent biochemical markers since they are usually co dominantly inherited, do show pleiotropic effects, rarely exhibit epistasis and are not affected by the environment. Isozymes have been used very successsly in certain aspects of plant breeding and genetics as nearly neutral genetic markers (Tanksley, *et al.*, 1989). Esterase, proxidase are a ubiquitous enzyme found in plants, mammals, fungi and prokaryotes. It catalyzes the oxidation of cellular component. Esterase from many insects' sources has been shown to consist of isozymes, which existed in both anionic and cationic iso- esterase. Esterase isozymes have been reported to occur in both soluble and bound forms, the bind enzymes are associated with the cell walls, bounded by ionic interaction to the cell wall in the undamaged cell with respect to physiological functions (Brewbaker, 1966).

Random Amplified Polymorphic DNA (RAPD) is a PCR-based technique for DNA fingerprinting. This assay, unlike the PCR, does not require knowledge of the target DNA sequence, and a single arbitrary primer will support DNA amplification from a genomic template if binding sites on opposite strands of the

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template exist within a distance that can be traversed by the thermo stable equation usually random oligonucleotides (or 10 bases) used as primer to amplify discrete fragments of genomic DNA. Random amplified polymorphic DNA (RAPD) markers can be used to detect DNA polymorphism without the need for predetermined genetic data. Each product is derived from a region of the genome that contains two short segments in inverted orientations, on opposite strands that are complimentary to the primer and sufficiently close together for the amplification to work. (William *et al.*, 1990; Welsh and McClelland, 1990). RAPD is one of the widely used molecular markers, where it was applied in: determination of paternity and kinship relationships, gene mapping, identification of markers linked to traits of interest without the necessity for mapping the entire genome, plant and animal breeding, to understand the complexity of the transmission cycles of insects vectors and population and evolutionary genetics (Marcili *et al.*, 2009 and Sharma *et al.*, 2009). Random amplified polymorphic DNA (RAPD) analysis has proved useful for estimating genetic diversity particularly to assist in the conservation of rare species and plant genetic resources (Anderson and Fairbanks, 1990).

Molecular techniques help researchers not only to identify the genotypes, but also in assessing and exploiting the genetic variability through molecular markers (Whitkuset *al.*, 1994). Genetic markers are of great value in genetic research and practical breeding programs, since they reflect the genetic variation among individuals. Morphological markers, or mutations in morphological markers, or mutations in genes with visible consequences, have been used in genetic studies since early in the twentieth century (Hussiumet *al.*, 2000). The molecular biology tools provide detailed information about the genetic structure of natural population, which was not available in the past. Such studies help to find new and useful genes, and also provide information about the phylogenetic relationship. Secondly, in case of the large size germplasm it becomes a critical step for selection of parental genotypes from the germplasm (Beckman *et al.*, 1998). The effects of four insecticides on feeding and growth of cotton leafworm, *Spodoptera littoralis* (Boisd.), larvae were investigated during the fourth-instar larvae. Biochemical and molecular studies were done to detect the efficiency of using some new insecticides against Cotton Leaf Worm (*Spodoptera littoralis*) based on Biochemical and Molecular markers.

MATERIALS AND METHODS

The current study was carried out in the Agricultural

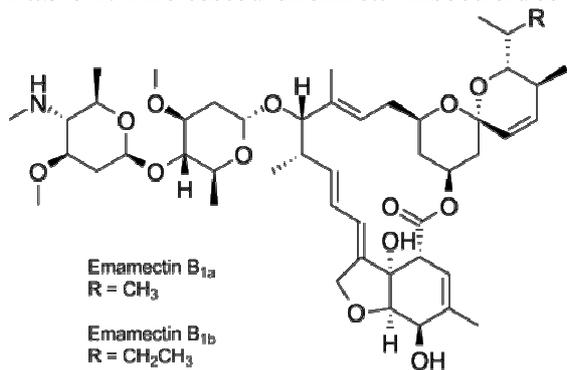
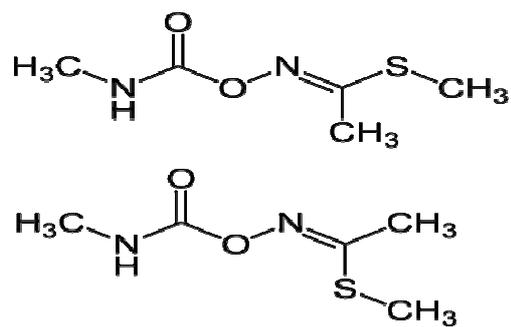
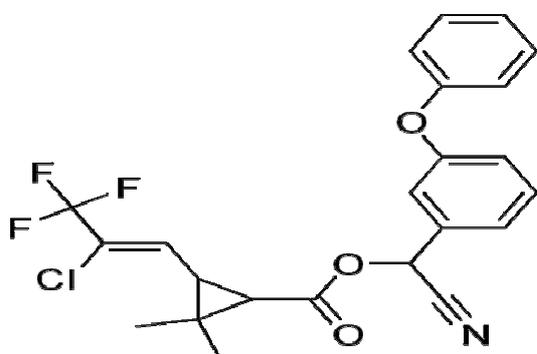
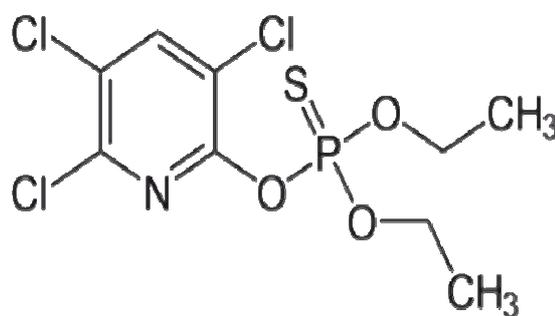
Botany Department, Faculty of Agriculture (Saba Basha), Alexandria University, Alexandria, Egypt and Plant Protection Department Faculty of Agriculture (Saba Basha), Alexandria University, Alexandria, Egypt during the period of 2014-2015 in three different experiments to detect the efficiency of using some new insecticides against Cotton Leaf Worm (*Spodoptera littoralis*) based on biochemical and molecular markers.

A- Tested insect and Insecticides

Susceptible strain of *S. littoralis* was mass reared in the laboratory under the highthermic conditions of $25 \pm 2^\circ \text{C}$ and $75 \pm 5\%$ R.H. as described by El-Defrawi (1964). The larvae were fed on castor oil-bean leaves (*Ricinus communis* L.) and kept in 1 liter glass jars covered with muslin which fixed tightly by a rubber band. The number of larvae per jar was differed according to the developing instar. After pupation, the resulting pupae were sexed and grouped in groups of 12 pupae at a sex ratio of $2\text{♀} : 1\text{♂}$ and then placed in the glass jar. After the emergence of the moths, they were supplied with a piece of cotton moistened with 10% sugar solution and paper strips to act as sites for egg deposition. The deposited egg masses were daily collected and left till hatching. The newly hatched larvae were transferred to clean jar and supplied with fresh leaves. The different larval instars were differentiated according to Willcocks and Bahgat (1937).

Progressive dilutions of the four tested insecticide were prepared in distilled water. All compounds were tested against the 4th instar larvae of the cotton leaf-worm (*S. littoralis*). Nutritional indices were measured regularly throughout the feeding tests until pupation. The food consumed by larvae in most insecticide-treatments was below that of control. emamectin benzoate (proclam[®] 5% sg), methomyl (newmyl[®] 90%), chlorpyrifos (teraguard[®] 48%) and lambda-cyhalothrin (syclon[®] 5%) as shown in Table, 1. Treatments seemed to be the best feeding deterrents.

The leaf-dipping bioassay method was used to determine the median lethal concentration (LC50) values. Series of concentrations (in water) of the tested formulated compounds were prepared. Castor-bean leaves were dipped for 30 seconds in each concentration then left for one hour to dry. The treated leaves were offered to newly moulted 4th instar larvae of succuptable strain. Mortality percentages were recorded after 24, 48, 72 and 96 hrs and corrected according to (Abbott, 1925). To estimate the LC50 values, the data were analyzed using an adopted computer program based on a standard implementation of the probit analysis recommended by Finney (1971). Estimates of the log dose/mortality % are expressed as the Normal Equivalent Deviate (N.E.D.).

Table 1. The tested chemical insecticides used in the study**Emamectin benzoate procliam® 5 % SG****Methomyl: Newmyl® 90% SP****Lambda-Cyhalothrin Syclon® 5% SC****Chlorpyrifos Teraguard® 48% EC**

* SG= soluble granules, SP= soluble powder, SC= soluble

The median lethal dose (LC₅₀) and its fiducial limits, the interception (a) and slope (b) parameters for the tested compounds were also calculated.

B- Biochemical studies (Isozymes electrophoresis):

Agar-starch-polyvinyl pyrrolidone (PVP) gel electrophoresis was carried out according to the procedures described by Shaw and Kaen (1967) and Andrews (1981). Buffer was prepared by dissolving 92.75 g of 0.3M Boric acid and 12 g sodium hydroxide in 5 liters of distilled water, then the solution was adjusted to pH= 8.3. The used gel buffer was 0.07 M Tris 0.007 M citric acid (pH=8.3). One liter of the gel buffer was prepared by dissolving 9.21 g Tris, 1.05 g citric acid in distilled water, and kept in a refrigerator until experimental use. Agar-starch-polyvinyl pyrrolidone (P.V.P) gel was prepared by dissolving 1.0 g, 0.5 g P.V.P. and 0.5 g of hydrolyzed starch with 10 ml electrode buffer and 90 ml distilled water. The mixture was shaken vigorously and cooked in a boiling water bath until the solution become transparent. The hot liquid gel was poured on glass plates (20 X 30 cm) to

concentrate and EC= emulsifiable concentrate produce a smooth surface layer with thickness of 0.8-0.9 mm and they were kept at 4 °C until usage (El-Metainy *et al.*, 1977). Electrophoresis experiments were conducted in an incubator refrigerator adjusted at 4°C using a 250 volts AC electrical current, with constant voltage throughout the 90 minutes of the running period. Phosphate buffer with 0.1 M at pH=7.0 was used as a staining buffer by adding 39 ml of 0.1 M solution of monobasic sodium phosphate to 61 ml of 0.1 M solution of sodium dibasic phosphate and completed to a final volume of 200 ml with distilled water (Youssef *et al.*, 1989).

C- Molecular studies:

The chemicals were obtained from Sigma Scientific, extraction kit, colored and clear master mix, 10X TBE buffer (pH 8.0), Ethidium bromide, 100bp DNA ladder Agrose were provided from iNtRON Biotechnology, Inc (Germany). Genomic DNA was extracted from tissues of the *Spodoptera littoralis* samples using genomic DNA extraction kit (G-Spin)™ for cell/ tissue extraction (iNtRON Biotechnology, Inc). Kits are designed for

rapid isolation of genomic DNA from various sample sources included fresh or frozen animal cells/tissues (for cell/tissue). RAPD analysis was carried out using 10 oligonucleotide primers (Table 2) were selected from the Operon Kit (Operon Technologies Inc., Alabameda, CA). The polymerase chain reaction mixture (25 μ l) consisted of 0.8 U of Taq DNA polymerase; 25 pmoldNTPs; 25 pmol of primer and 50 ng of genomic DNA. PCR amplification was performed in a Biometra T1 gradient thermalcycler for 40 cycles after initial denaturation for 3 min at 94°C. Each cycle consisted of denaturation at 94°C for 1 min; annealing at 36°C for 1 min; extension at 72°C for 1 min and final extension at 72°C for 10 min (Soliman, *et al.*, 2003). Amplification products were separated on 1.5% agarose gels at 80 volts for 1.30 hrs with 1X TBE buffer. To detect ethidium bromide/DNA complex, agarose gels were examined on ultraviolet transilluminator (302 nm wavelength) and photographed. Using 100pb DNA ladder, the lengths of the different DNA fragments were determined. RAPD fragments were scored as present/absent band using gel analyzer program. Fragment scoring and lane matching were performed automatically on digital images of the gels, using Phoretix 1D advanced Version 4.00 (Phoretix International, Newcastle upon Tyne, UK). Clustering methods and similarity coefficients are described as given by Rohlf, (2000).

Table 2. RAPD primers sequences used for analysis

Primer	Sequences
OPA-02	5' CAT CCC CCT G 3'
OPA-03	5' TTC GAG CCA G 3'
OPA-04	5' GTG AGG CGT C 3'
OPA-05	5' AGG GGT CTT G 3'
OPA-15	5' TTC CGA ACC C 3'
OPC-01	5' TGA TCC CTG G 3'
OPC-02	5' CAT CCC CCT G 3'
OPC-03	5' GGG GGT CTT T 3'
OPC-12	5' TGT CAT CCC C 3'
OPB-01	5' GTT TCG CTC C 3'

RESULTS AND DISCUSSION

A- Evaluation of pesticides against the cotton leafworm *Spodoptera littoralis* (Boisd.)

The estimated values of the Normal Equivalent Deviation (N.E.D) (response/log dose regression) and Lc_{50} of the four different insecticidal tested against the 4th larval instar of the susceptible strain of cotton leafworm. In the present studies Table (3) shows that the susceptibility of the four tested compounds used in the present work caused variable toxic effects against

the 4th larvae of *Spodoptera littoralis* for susceptible strain.

The first tested compound teraguard[®] (Chlorpyrifos) is active by contact, ingestion, and vapor action. It inhibits an enzyme of the nervous system (acetylcholine esterase); this causes convulsions and paralysis. The second tested compound include newmyl[®] (Methomyl) similar to other carbamate insecticides, methomyl inhibits acetylcholinesterase (AChE), which is contained within synaptic junctions between neurons (Kuhr and Dorrough 1976). The third tested compounds have syclon[®] (Lambda-Cyhalothrin). Pyrethroids are axonic poisons that affect the nerve fiber by binding to a protein that regulates the voltage-gated sodium channel. Normally, this gate opens to cause stimulation of the nerve and closes to terminate the nerve signal. The channels are pathways through which ions are permitted to enter the axon and cause excitation. Finally, the last tested compound has the proclam[®] (Emamectin benzoate) which is a novel semi-synthetic derivative of the natural product abamectin in the avermectin family of 16-membered macrocyclic lactones. Avermectins are a family of 16-membered macrocyclic lactone natural product homologues produced by the soil microorganisms, *Streptomyces avermitilis*. Isolation of the crude fermentation product of *S. avermitilis* yielded a complex of eight closely related avermectin homologues (A1a, A1b, A2a, A2b, B1a, B1b, B2a, and B2b), of which avermectins B1 (a and b) were the major components. The anthelmintic properties of the avermectins are due predominantly to potentiation and/or direct opening of glutamate-gated chloride channels, whereas in insects, it is likely that avermectins bind to multiple sites (including glutamate and GABA) in insect chloride channels. In general, the chloride ion flux produced by the opening of the channel into neuronal cells results in loss of cell function and disruption of nerve impulses.

The LC_{50} values were 0.0178, 6.08, 6.75 and 10.77 ppm for proclam[®], teraguard[®], teraguard[®] and syclon[®] after 96 hrs post-treatment, respectively. Comparing the tested compounds based on their Lc_{50} values, it was found that (proclam[®]) was more toxic than other tested compounds. Whereas, the less toxic compound achieved by syclon[®] (Lc_{50} = 10.77 ppm) when determined LC_{50} after 96 hrs post-treatment (Table 3).

The obtained results agreed with (Muthusamy *et al.*, 2011) who found dichlorvos was be more toxic as compared to temephos and lambda-cyhalothrin. But, the obtained results differ with (El-Sheikh, 2012) who found the pyrethroid Cyper (Cypermethrin 10% EC) is more toxic insecticide against the laboratory strain of *Spodoptera littoralis*, while, the OP Dursban H- 48%

EC (Chlorpyrifos) was less toxic. Whereas, Bengochea *et al.*, (2014) found that emamectin benzoate is not only a useful insecticide when ingested by beet armyworm larvae but it also has ovolarvicidal and adult activity. Finally, Vojoudi *et al.*, (2011) showed that chlorpyrifos and spinosad were more effective insecticides against 3rd instar larvae of cotton bollworm compared to abamectin based on formulated materials. On the other hand Gamil *et al.*, (2011) studied the efficacy of Avaunt® (Indoxacarb 15% EC) on newly ecdysed 2nd and 4th instar *Spodoptera littoralis* (Boisd.) Whereas, the bioassay showed that 2nd instars were more susceptible than the 4th instars as the LC₅₀ and LC₉₀ values were 0.63 and 3.1 ppm for 2nd instar larvae and 2.0 and 18.75 ppm for 4th instar larvae, respectively. Finally, <http://www.sciencedirect.com/science/article/pii/S0261219414003342> - cor1EI-Sheikh (2015) investigated the comparative effectiveness of some new insecticides including emamectin benzoate, lufenuron, and spinosad against 3rd and 5th larval instars of *Spodopteralittoralis*. Also, determined Time–mortality relationships, effects on larval weight gain, feeding damage, and larval development of *S. littoralis*.

B- Biochemical studies:

Biochemical methods provide more specific information about the resistance potential or the development of resistance in field populations of living organisms. Esterase iso-enzyme assay was applied as the most appropriate technique for the evaluation some insecticide on cotton leafworm *Spodoptera littoralis*. As conventional symbols in electrophoresis analysis, a pattern was first described in terms of Anodal (A) or Cathodal (C) zones according to their direction of mobility in the electrophoresis field. Each zone is assigned for a locus coding for aesteraseisozymes. The locus with the least migration is designated the first; the next one is the second, and so on. In contrast, as shown in Figure 1, esterase isozymes exhibited a wide range of variability among the pupae and larvae. Two common bands were detected among all samples in pupae and larvae (Es.C₁ and Es.A₁). Proclam showed one band in pupae (Es.C₂) compares with other treatments and in larvae detected one band in (Es.C₃) and detecte the highest band number (6 bands) comparing with other insecticides. The results of biochemical analysis showed that there is relationship between the resistance to insecticide and the increase of enzyme activity such as esterase enzyme as showed in the previous figure. Esterase enzymes play an important role in conferring or contributing to insecticide resistance in insects.

Table 3. Toxicity of some insecticides against the 4th instar larvae of the cotton leafworm *Spodoptera littoralis*.

Tested compound	Bioassay Time (hrs)	N.E.D. (response)/ log(dose) regression	LC ₅₀	95% fiducial limits	
				Lower	Upper
Newmyl	96	Y= - 1.339 + 1.638x	6.75 ppm	9.86	4.46
Teraguard	96	Y= - 0.728 + 0.929x	6.08 ppm	31.48	2.22
Syclon	96	Y= - 7.053 + 6.832x	10.77 ppm	12.08	9.61
Proclam	96	Y= 11.589+ 6.631x	0.0178 ppm	0.0196	0.0163

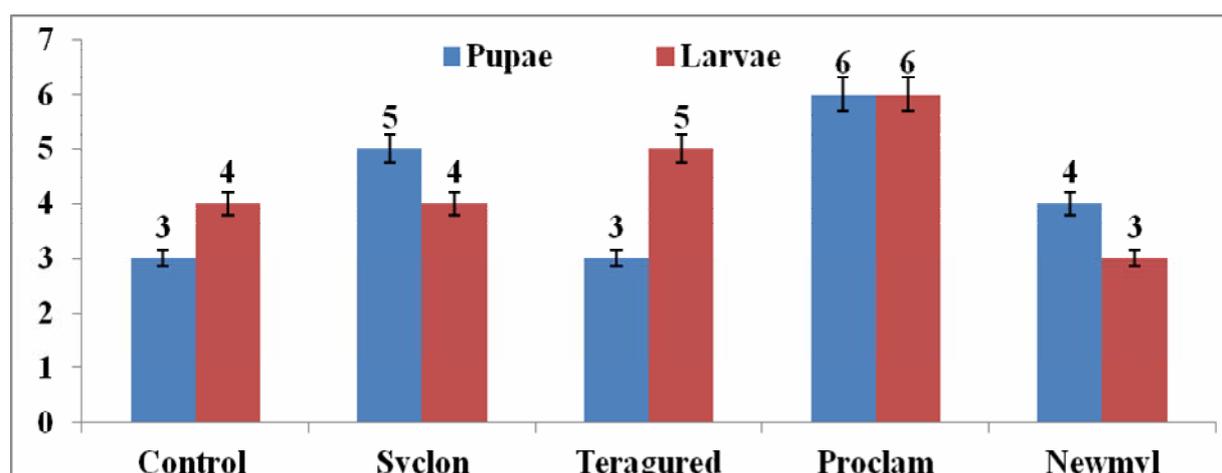


Figure 1. Esterase activity of cotton leafworm *Spodoptera littoralis* under different insecticide treatments in both pupae and larvae

This has been shown in aphid *Myzus persicae* (Field and Devonshire, 1998), mosquitoes, *Culex quinquefasciatus* and *C. pipiens* (Guillemaudet *et al.*, 1997), blowfly, *Lucilia cuprina* (Campbell, 1998) and housefly *Musca domestica* (Taskin and Kence, 2004). In insects, the esterase enzyme patterns have shown high rates of intraspecific and interspecific variations (Nascimento and Campos Buicudo, 2002). Possibly, gene duplication followed by divergence of duplicated genes from the ancestral gene is the origin of at least part of this variability (Oakeshott *et al.*, 1993; Nascimento and Campos Bicudo, 2002).

There are several methods that have been developed for detecting resistance in insects. Usually, laboratory bioassays are performed on the progeny of field collected insects for detecting insecticide resistance. Traditional bioassay methods depend on measuring of LD50 or LC50 values, which are based on the criteria of insecticide amount required for killing 50% of a test population. In order to determine the resistance ratio of a field population, these values are divided by the LC50 of a susceptible laboratory population. Although these bioassays are easy to perform, they have several disadvantages. First, they are time-consuming since they need many individuals for testing one insecticide. Second, and most importantly, they do not provide a quantitative measure of potential resistance in individual strains or clones. On the other hand, biochemical methods and molecular biological techniques overcome these disadvantages in many aspects by reducing the sample size and giving more specific information about the resistance potential or the development of resistance in field populations (Kristensen, 2005 and Han *et al.*, 1998). In this study, the biochemical basis of some insecticide resistance and the esterase enzyme patterns were investigated.

C- Randomly amplified polymorphic DNA (RAPD) analysis

Cotton leaf worm (*Spodoptera littoralis*) has been proven to be important insect in experimental laboratory work not only for cytotoxicological and different genetic studies, but also for biochemical and molecular research. The use of Cotton leaf worm cells for the study of genotoxic effects has only recently been explored. RAPD-PCR was performed on cotton leaf worm genome to evaluate the genomic toxicity occurred to cotton leaf worm obtained from treatment with syclon, teraguard, proclaim, and newmyl at LC₅₀. insecticides compared with those obtained from control one. In the current study in Figure 2 and Table 3, five out of ten oligonucleotide primers (50%) used in the RAPD analysis gave unique markers. Out of the five primers, two detected single

unique markers (OPA-02 and OPA-15), two gave two markers (OPA-05 and opc-02), and one yielded three unique markers (OPC-12). Additionally, compared with the control worms, cotton leaf worm exposed to insecticides recorded the emergence of 7 new fragments from 98 total fragments. So, the total number of fragments which disappeared in response to both treatments was 98 fragments. Exposure to insecticides at all concentrations revealed that increase or decrease the number of amplified polymorphic DNA and increased gene expression in comparison to control cotton leaf worm. The results of the present study exposed that proclaminsecticide caused oxidative stress which up regulated gene expression.

According to the cluster analysis in Figure 4 the data divided to two main groups by 72% similarity, the first group includes the insecticide newmyl and the other one divided by two other groups includes the proclamin insecticide as individual one by 77% and the other groups includes control, tetrgrade and syclon by 84%. The similarity between teraguard and syclon was 96%. The data showed that proclaim was in one cluster by general similarity 56% as shown in Table (4).

The present results are in line with Atienzar *et al.*, (2002) assayed and related the random amplified polymorphic DNA (RAPD) to detect genotoxin-induced DNA damage and mutations. The changes occurring in RAPD profiles following genotoxic treatments include variation in band intensity as well as gain or loss of bands. Also, Mironenko *et al.*, (2007) used random amplified polymorphic DNAs (RAPDs) to study the genetic variation of *Pyrenophora tritici-repentis* isolates causing wheat tan spot. Our finding agree with Baurand *et al.*, (2015) showed the study explores the relevance of coupling RAPD and a High-Resolution capillary electrophoresis System (HRS) method for assessing the genotoxic potential of the wide variety commercial formulations of pesticides. The results agree with Ercan, (2015) who detected DNA damage in *Ephesiakuehniella* by RAPD-PCR Abstract. The random amplified polymorphic DNA (RAPD) assay is a useful method for detecting genotoxin-induced DNA damage. Our results is agree with Anderson and Fairbanks, (1990) who reported that Random amplified polymorphic DNA analysis has proved useful for estimating genetic diversity particularly to assist in the conservation of rare species and plant genetic resources. The development of random amplified polymorphic DNA (RAPD) markers, generated by the polymerase chain reaction (PCR), allows the examination of genomic variation without prior knowledge of DNA sequences (Hadrys, 1992). The results proved the same fact with Hadrys *et al.*,

(1992) that developed molecular genetic markers into powerful tools to analyse genetic relationships and genetic diversity. Also, Abdel-Wahed, et al., (2013) studied the 6th instar larvae of *Spodopteralittoralis* treated in 2nd instar larvae with LC₅₀ of Match and Protecto at 0.0057 ppm, and 0.1099 gm/ml., respectively, Five random primers were used in this study to generate a fragmenting pattern as a tool to investigate the molecular differences between treated

samples and control. The numbers of unique and common fragments generated by using these primers (OPO1, OPO2, OPO3, OPO4 and OPO5) was recorded. It has been found that primer OPO2 was the most powerful in generating a unique informative fragmenting pattern, it gave 4 specific unique fragments. The primer OPO4 was the poorest one in generating an informative fragmenting pattern.

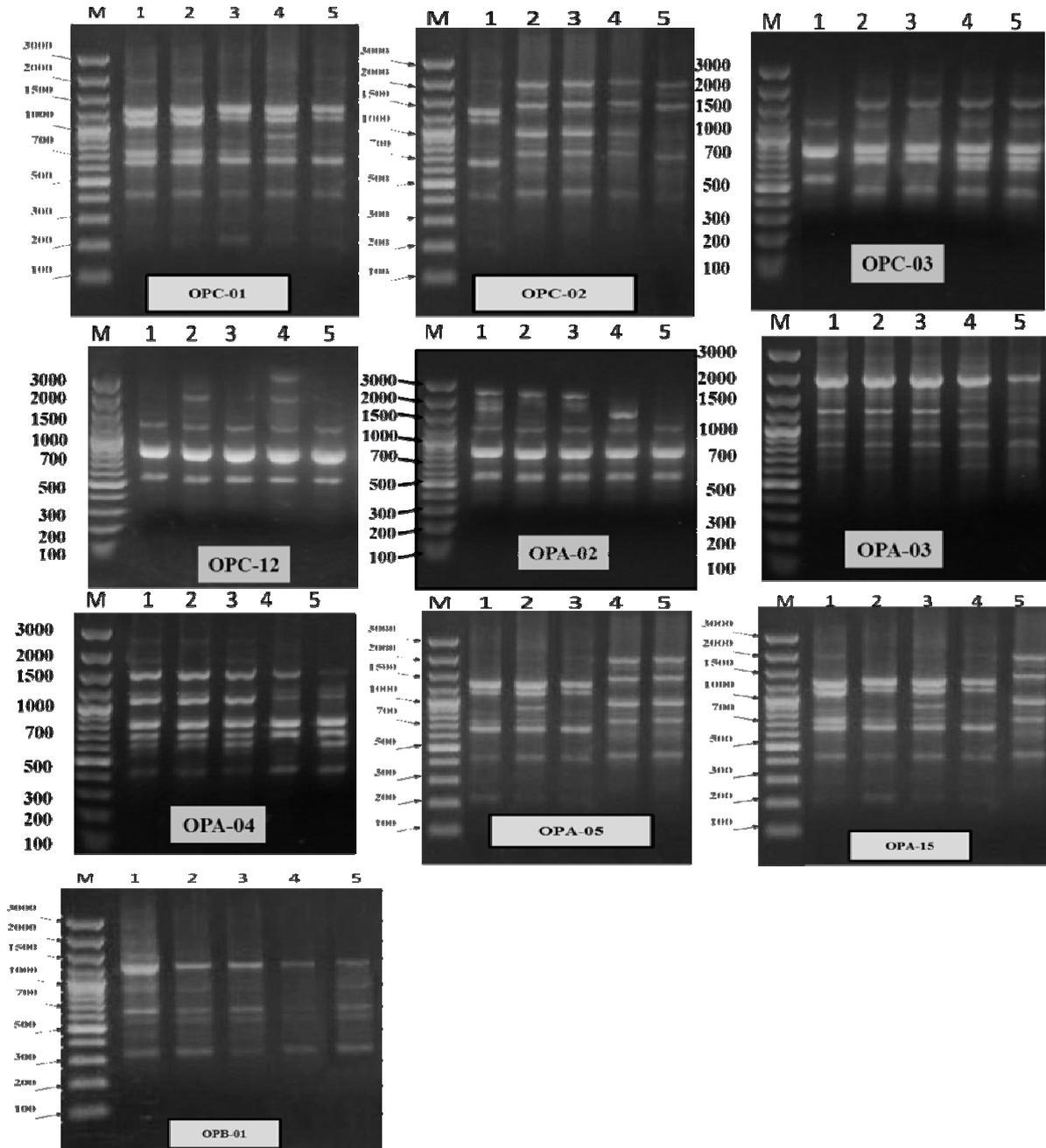
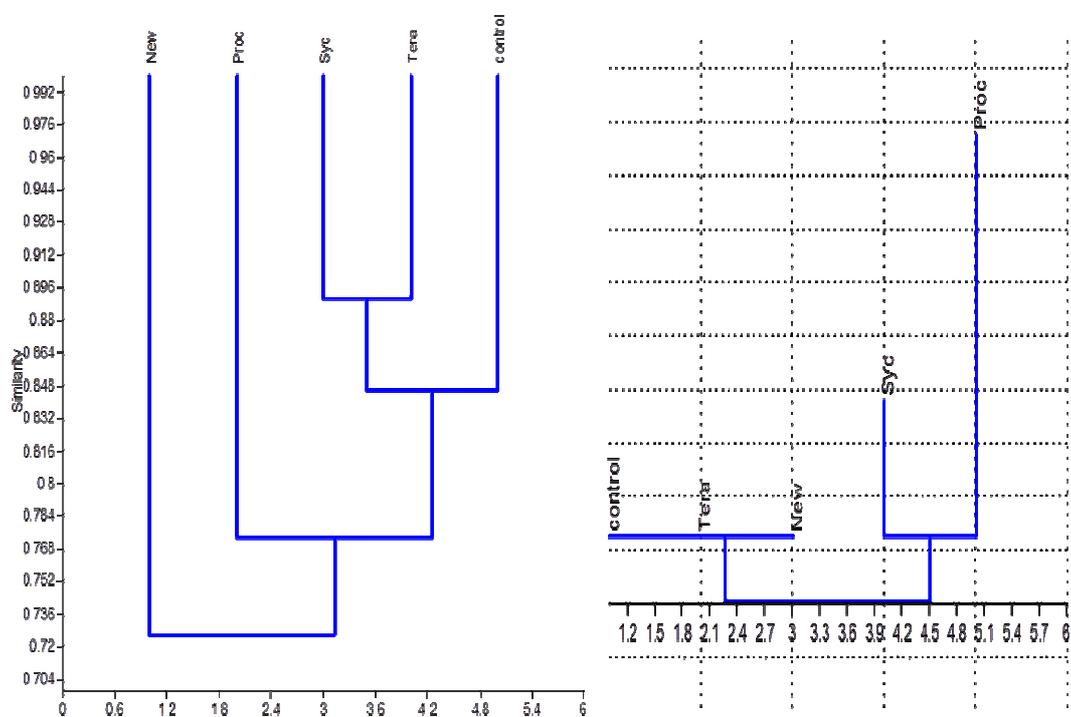


Figure 2. DNA polymorphism of the 4th instar larvae of the cotton leaf worm (*Spodoptera littoralis*) treated with insecticides using randomly amplified polymorphic DNA

Table 4. Number of scorable bands, Polymorphic, Monomorphic bands and Percentage of polymorphism for cottonleaf worm (*Spodoptera littoralis*)

Markers	Number of scorable bands	Polymorphic bands	Monomorphic bands	Unique band	Percentage of polymorphism (%)
OPA-02	14	9	5	1 (2168 bp.)	64%
OPA-03	11	10	1		91%
OPA-04	9	7	2		77%
OPA-05	6	5	1	2 (1957 & 1638bp.)	83%
OPA-15	9	4	5	1 (712 bp.)	44%
OPC-01	10	6	4		60%
OPC-02	10	4	6	2 (1318 bp.)	40%
OPC-03	12	9	3		75%
OPC-12	10	9	1	2 (29590 & 2000 bp.) and 1 (2000 bp.)	90%
OPB-01	7	3	4		42%
Total	98	66	32	--	--

**Figure 3. Cluster analysis and similarity for cottonleaf worm (*Spodoptera littoralis*) treated with four insecticides****Table 5. Similarity indices (%) among four insecticides based on 10 RAPD primers**

	Control	Syclon	Teraguard	Proclam	Newmyl
Control	1	0.73171	0.72093	0.58696	0.45238
Syclon	0.73171	1	0.80247	0.61798	0.55844
Teraguard	0.72093	0.80247	1	0.63043	0.51807
Proclam	0.58696	0.61798	0.63043	1	0.56962
Newmyl	0.45238	0.55844	0.51807	0.56962	1

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