EFFECT OF SOME INSECTICIDES ON GENOMIC DNA OF LABORATORY AND RESISTANT STRAINS OF *SPODOPTERA LITTORALIS*

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Abstract

The RAPD technique was used as a tool for setting up a convenient and standard protocol to determine the interspecific variation between the two strains (laboratory and resistant) of *Spodoptera littoralis* and estimate the intraspecific variation within each strain after treatment.

Five (OPA-7, 10, 16, OPB-15 and 20) out of ten primers gave clearly differences among the two strains on the basis of the amplified product patterns, These primers generated 296 fragments

The comparison between the two strains of *S. littoralis* showed differences in the sizes and numbers of the amplified fragments per primer for each strain and each treatment, indicating a high degree of variability between them. The primers OPA-7,16, OPB-15 and 20 gave band patterns which revealed that, there was some degree of divergence between the genome of untreated and treated larvae, higher than primer OPA-10.

INTRODUCTION

Resistance management requires more effective techniques for detecting resistance in its early stages of development. It is in the early stages of resistance development that heterozygotes are abundant. The more or less intermediate response of those heterozygotes to the dose-mortality tests make them difficult to detect. Molecular diagnostics have been postulated to increase accuracy and to reduce the variability associated with insecticide bioassay that result from both intrinsic (genetic structure) and extrinsic (bioassay conditions, sample size, etc.) factors (Ffrench-Constant and Roush, 1990). Dowdy and McGaughey (1996) used random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) to identify genetic markers in insect populations, and may be useful for differentiating populations of *P. interpunctealla*, also RAPD-PCR was used to differentiate six *P. interpunctealla* populations, and the genetic similarity was examined within and among populations. All individual insects were differentiated into correct population groups using only two 10-mer primers. So, this study was carried out to assess genetic variability between two strains of *Spodoptera littoralis* under insecticides pressure.

MATERIALS AND METHODS

Fifth instar larvae of laboratory and Profenofos-resistant strains of *S. littoralis* were treated with LC_{50} values of Cyanofos (2130.15 and 12387.83ppm), Beta-cyfluthrin (5.78 and 148ppm) and Lufenuron (2.96 and 12.18ppm), respectively. The untreated and treated larvae for the two strains were grinded in liquid nitrogen. The larval genome was isolated and purified by phenol extraction and ethanol precipitation of DNA Method (Sambrook *et al.*, 1989).

The concentration of DNA was measured according to the following equation:

$$DNA\ conc.\ (\mu g\ /\ \mu l) = O.D260 \times dilution \times 0.05$$

The purity of DNA is determined from the ratio:

$$Pure\ DNA = O.D\ 260\ /\ O.D280 = 1.7\text{-}\ 2.0$$

Preparation of PCR Reactions:

Random amplification of DNA from samples was carried out according to the procedure given by Williams *et al.,* (1990) with some modifications. Amplification reaction was carried out in a volume of 50 µl containing 250 ng of genomic DNA as a template.

Primers used in RAPD Analysis:

Subsets of 10 random primers were used in the detection of polymorphism among the all samples.

The sequence of ten primers used for RAPD analysis were shown in the following table:

No.	Primer	Sequence (5---3)
1	OPA-6	GGTCCCTGAC
2	OPA-7	GAAACGGGTG
3	OPA-9	GGGTAACGCC
4	OPA-10	GTGATCGCAG
5	OPA-13	CAGCACCCAC
6	OPA-16	AGCCAGCGAA
7	OPA-17	GACCGCTTGT
8	OPA-18	AGGTGACCGT
9	OPB-15	GGAGGGTGTT
10	OPB-20	GGACCCTTAC

The PCR mixture per genotype consisted the following:

Component	Amount for one PCR reaction
2 mM dNTPs	5µl
50mM MgCl$_2$	2µl
10x PCR buffer	5µl
10µM primer	4 µl
5U/µl Taq.polymerase	1 µl
250ng/ µl template DNA	6 µl
H2O (d.w)	27 µl
Total volume	50 µl

PCR Program and temperature profile:

Amplification of the DNA was performed by placing the tubes containing the reactions in a Perkin Elmer thermal cycler 2400.

RAPD PCR performed in 50 µl reaction volumes for 30 cycles. After the reaction mixture was mixed with DNA loading buffer and electrophoresed on 1% Agarose gel.

Temperature profile:

Step 0:	96°C	15 min	(for Hot start Taq only)
Step 1:	96 °C	40 sec	Denaturation
Step 2:	36 °C	40 sec	Annealing
Step 3:	72 °C	2 min	Extension
Step 4:	72 °C	7 min	Extension
Step 5:	4 °C	Hold	

The amplification products were resolved by electrophoresis in a 1.5% agrose gel at 80 V for about 2 h with 1X TAE buffer. PCR products were visualized by staining gel in ethidium bromide and photographed under UV light using a Polaroid camera. Amplified products were visually examined and the presence or absence of each size class was scored as 0 and 1, respectively.

RAPD Analysis:

The banding patterns generated by RAPD-PCR analysis were compared to determine the genetic relatedness of all samples. Bands of the same mobility were scored as identical. The similarity coefficient (F) between two strains and their treatments was defined by the formula of Nei and Li (1979).

Formula: $F = 2 N_{XY} / (N_X + N_Y)$

Where:

N_{XY} = the number of common bands to individual X and Y.

N_X and N_Y = the total number of bands in individual X and Y, respectively.

RESULTS AND DISCUSSION

Random amplified polymorphic DNA (RAPD) analysis was used to assess genetic variability between two strains (laboratory and resistant) of *S. littoralis*.

In Random Amplified Polymorphic DNA (RAPDs) banding profiles are created using small oligonucleotide primers (around 10 bp in length) of arbitrary sequence. These primers bind to homologous sequence along the genome, and PCR amplification only occurs where opposing primer sites are about 3000 bp apart. Within a population sample, mutations influence the base sequence of primer binding sites, allowing polymorphisms to be detected (Williams *et al.,* 1990).

In this study genomic DNA of untreated and treated larvae of laboratory and resistant strains of *S. littoralis* produced more reliable banding patterns when the primers were used at concentration 10μM. Virk *et al.* (1995) reported that the banding pattern of any genomic DNA depends mainly on the frequency of annealing sites for the primers used and on the effective concentration of such primers in the reaction tube

Five (OPA-7, 10, 16, OPB-15 and 20) out of ten primers gave clearly differences among the two strains of *S. littoralis* on the basis of the amplified product patterns, but five (OPA-6, 9, 13, 17 and 18) did not amplify. A low level of variation for RAPD bands scored was observed between the two strains for 5 primers.

The comparison between the two strains of *S. littoralis* showed differences in the sizes and numbers of the amplified fragments per primer for each strain and each treatment, indicating a high degree of variability between them as shown in Tables (1 and 2) and Figs (1-5)

The number and size of RAPD markers depend on the complementary of sequence of particular primer and the template DNA (Williams *et al.,* 1993), so in this study RAPD-PCR produced a series of discrete 296 fragments, which typically vary in intensity and in size, where results in Table (1) show that, these primers generated 296 fragments distributed as 34, 35, 37, 45, 35, 41, 33 and 36 in laboratory strain (L-S), resistant strain (R-S) and their treatments (L-Cy, R-Cy, L-Beta, R-Beta, L-Lu and R-Lu), respectively.

Primer (OPA-7) produced a total number of 50 polymorphic bands in all samples (8 samples). The minimum number was 4 bands in R-S, and the maximum was 10 bands in L-Cy, whereas it produced 8 bands in R-Cy, 7 in R-Beta, 6 in L-S and 5 bands in

L-Beta, L-Lu and R-Lu. The smallest size of the amplified products was 301.5 bp in L-Cy and R-Cy. The largest size was 1820.6 in all samples except in R-S (Fig.1).

The similarity index values were 0.55, 0.50, 0.40, 0.40, 0.57, 0.55, 0.40, 0.375, 0.57 and 1 were between (L-S and R-S), (L-S and L-Cy), (L-S and L-Beta), (S and Lu), (R and Cy), (R and Beta), (R-S and R-Lu), (L-Cy and R-Cy), respectively.

Results of RAPD analysis of all samples of the cotton leafworm *S. littoralis* with primer OPA-10 are shown in Fig. (2). The minimum number was 5 bands in R-Lu, and the maximum was 12 bands in R-Cy, whereas, the primer produced 8 bands in L-S, L-Cy and L-Lu, 9 bands in L-Beta, 10 bands in R-S and 11 bands in R-Beta. The largest size of the amplified products was 1573 bp in L-Beta only and the smallest size was unique for R-Beta at 147 bp.

The similarity index showed the lowest value of 0.667 between(R-S and R-Lu) and (L-Lu and R-L-Lu). The highest value of 1 between (L-S and L-Cy) and (R-S and R-Cy).

Using primer OPA-16 resulted in detecting a total number of 66 bands in L-S, R-S, L-Cy, R-Cy, L-Beta, R-Beta, L-Lu and R-Lu, (8, 8, 6, 10, 10, 6, 7 and 11 respectively. Three bands of 1400.38, 1052.5 and 677.63 were found in all samples as shown in (Fig.3).The smallest size of amplified products was 271 bp in R-Cy and L-Beta and the largest size was 1952 bp presented in all samples except in L-Cy. The similarity index value increased from 0.5 between L-S and L- Cy and L-Cy and R-Cy to 1 between L-S and R-S.

Primer OPB-15 revealed some variability between the untreated and treated larvae (Fig. 4). The total number of amplified fragments generated by this primer were 47 bands in L-S, R-S, L-Cy, R-Cy, L-Beta, R-Beta, L-Lu and R-Lu, (4, 8, 7, 6, 3, 8, 6 and 5 bands, respectively). The largest size of the amplified products (5066 bp) was unique for L-Beta, whereas the smallest size of the amplified products was 500 bp in R-S and L-Cy.The similarity index value increased from 0.2 between L-Beta and R-Beta to 0.77 between L-S and R-S.

The PCR patterns resulted from using primer OPB-20 discriminated between the untreated and treated samples and generated somewhat diffused bands (Fig. 5). This primer gave 62 bands for all samples. There were 8, 5, 6, 9, 8, 9, 7 and 10 bands detected in L-S, R-S, L-Cy, R-Cy, L-Beta, R-Beta, L-Lu and R-Lu, respectively. The smallest size of polymorphic products (180bp) was unique for L-Beta, whereas the largest size was 1701.67bp in L-S, L-Cy and L-Beta.The similarity index showed the lowest value of 0.47 between L-S and L-Beta. The highest value of 0.86 was detected between L-S and L- Cy.

The results of different RAPD-PCR patterns showed that the primers (OPA-7, 16, OPB-15 and 20) yielded band patterns which revealed some degree of divergence between the untreated and treated larvae genome, higher than the OPA-10.

The amplicons that were either amplified or those that disappeared in the individuals surviving the effect of Profenofos selection pressure can serve as the potential RAPD markers for the identification of resistance at an early stage and could help in the pest management programmes. Early detection of resistance is helpful in the identification of effective insecticides to manage the pest. RAPD fragments are useful as genetic markers to identify insecticide resistance fragments (Stevens and Wall, 1995). RAPD PCR has also been used for detecting cyclodiene resistance in *Tribolium castaneum* Herbst (Andreev *et al.*, 1994). Primers usually do not have the same amplification efficiency, where Kantanen *et al.* (1995) found that some primers fail to amplify, others produce too complex banding patterns.

In this study, the genomic DNA of untreated and treated larvae of laboratory and resistant strains of *S. littoralis* (with Cyanophos, Beta-cyfluthrin and Lufenuron) was screened for DNA damage or sequence changes using ten primers of arbitrary sequences. Out of these primers, five only gave fragments. Five primers (OPA- 6, 9, 13, 17 and 18) did not amplify the genome of untreated and treated larvae of *S. littoralis*. Absence of a fragment presumably occurs because amplification can not proceed on DNA strands from either of the homologous in an individual. This can occur through point mutation at one or both primer annealing sites on a DNA strand, inversions surrounding a site of insertion that separate the annealing sites at a greater distance than can be amplified (Rafalski *et al.,* 1991).In this respect, Bardakci and Skibinski (1994) found that the patterns of similarities and differences between populations showed broad agreement across primers but that the overall level of similarity varied between primers. Therefore, the choice of a primer is of major importance for discriminatory power of the technique.

In this study results have proven that the arbitrary primers OPA-7, 16, OPB-15 and 20 are strong tool to investigate changes in the cotton leafworm *S. littoralis* genomic DNA than other primers, probably due to changes in sequences of these primers. The sequence of the primer is usually the key factor of evaluating a primer to be used as a tool for screening DNA or polymorphism. The same findings were reported by Radwan (2001) who found that RAPD-PCR analysis showed some differences in genomic DNA of untreated and treated larvae of *E. insulana*. Kim and Sappington (2004) performed randomly amplified polymorphic DNA (RAPD) analysis to infer the magnitude and pattern of genetic differentiation among boll weevil populations from eighteen locations across eight US states and north-east Mexico. Sixty-seven reproducible bands from six random primers were analyzed for genetic variation

within and between weevil populations. Zhu et al. (2004) studied molecular differences by using polymerase chain reaction (PCR) and DNA sequencing on two strains (Sav and Bam) of the parasitoid *Anisopteromalus calandrae* (Howard) (Hymenoptera:Pteromalidae) which differ in their sensitivity to organophosphate insecticides.

Table 1. The total number of RAPD-PCR fragments generated by a battery of 5 primers and their amplification efficiency in the untreated and treated larvae of laboratory and resistant strains of *S .littoralis*.

Primer	Number of fragments in different samples								Total No. of DNA fragments
	L-S	R-S	L-Cy	R-Cy	L-Beta	R-Beta	L-LU	R-Lu	
OPA-7	6	4	10	8	5	7	5	5	50
OPA-10	8	10	8	12	9	11	8	5	71
OPA-16	8	8	6	10	10 .	6	7	11	66
OPB-15	4	8	7	6	3	8	6	5	47
OPB-20	8	5	6	9	8	9	7	10	62
Total*	34	35	37	45	35	41	33	36	296

Total number. for each larval sample

L-S = Larvae of laboratory strain (untreated larvae).

L-Cy = Larvae of laboratory strain treated with Cyanophos.

L-Beta = Larvae of laboratory strain treated with Beta-cyfluthrin.

R-S= Larvae of resistant strain (untreated larvae).

R-Cy = Larvae of resistant strain treated with Cyanophos.

R-Beta = Larvae of resistant strain treated with Beta-cyfluthrin.

L-Lu = Larvae of laboratory strain treated with Lufenuron.

R-Lu = Larvae of resistant strain treated with Lufenuron.
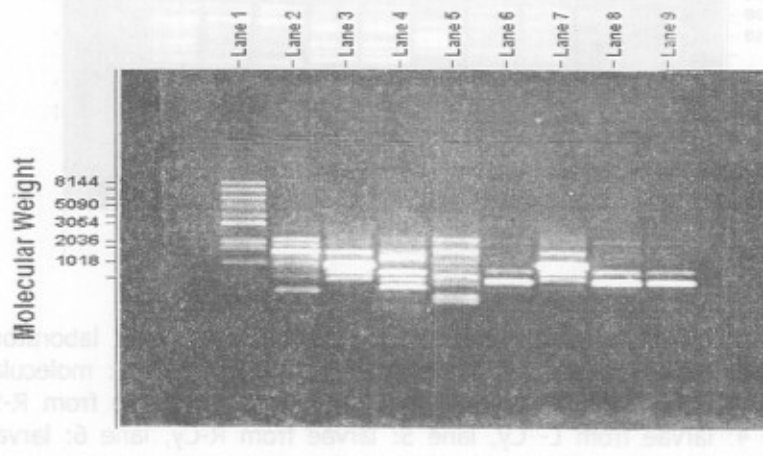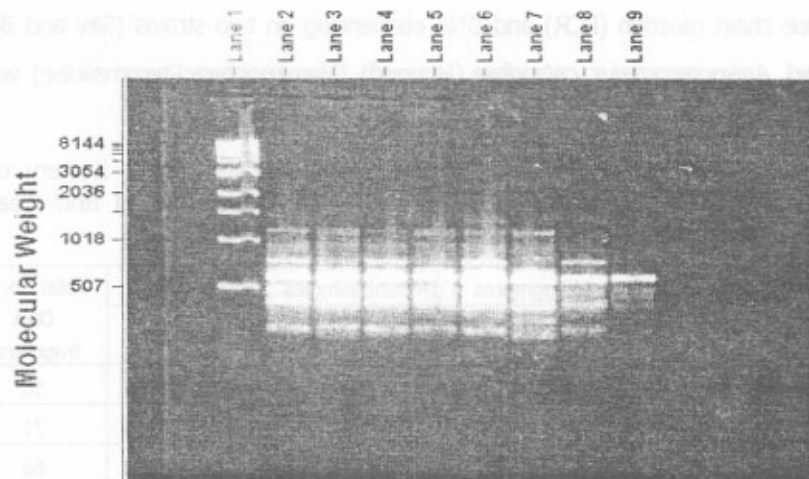

Fig. 1. RAPD-PCR products for untreated and treated larvae of laboratory and resistant strains of *S. littoralis* using OPA-7. Lane 1: molecular size markers. Lane 2: Larvae from L-S, lane 3: larvae from R-S, lane 4: larvae from L- Cy, lane 5: larvae from R- Cy, lane 6: larvae from L-Beta, lane 7: larvae from R- Beta, lane 8:larvae from L- Lu and lane 9: larvae from R-Lu.

Fig. 2. RAPD-PCR products for untreated and treated larvae of laboratory and resistant strains of *S. littoralis* using OPA-10. Lane 1: molecular size markers. Lane 2: Larvae from L-S, lane 3: larvae from R-S, lane 4: larvae from L- Cy, lane 5: larvae from R- Cy, lane 6: larvae from L- Beta, lane 7: larvae from R- Beta, lane 8:larvae from L- Lu and lane 9: larvae from R-Lu.
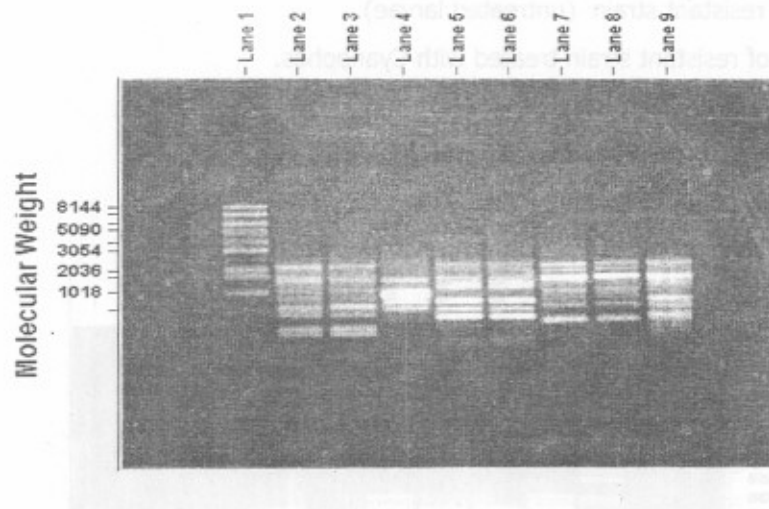
Fig. 3. RAPD-PCR products for untreated and treated larvae of laboratory and resistant strains of *S. littoralis* using OPA-16. Lane 1: molecular size markers. Lane 2: Larvae from L-S, lane 3: larvae from R-S, lane 4: larvae from L- Cy, lane 5: larvae from R-Cy, lane 6: larvae from L- Beta, lane 7: larvae from R- Beta, lane 8:larvae from L- Lu and lane 9: larvae from R-Lu.
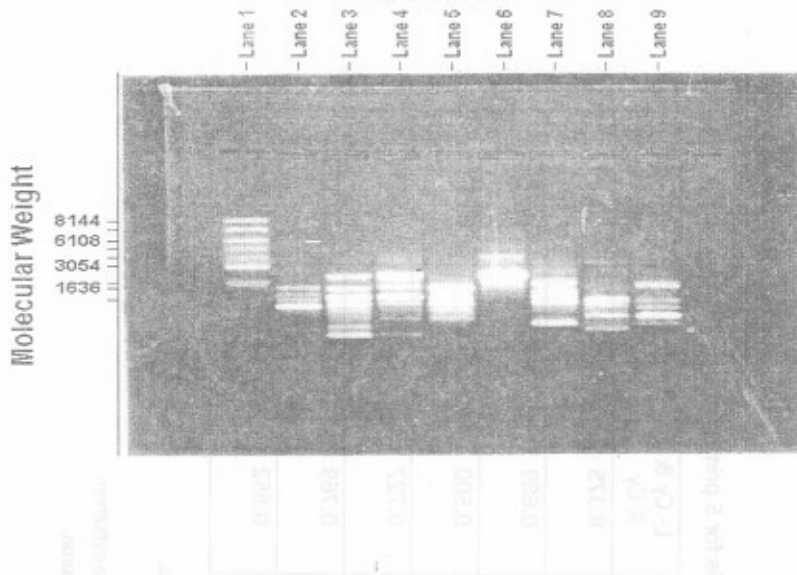
Fig. 4. RAPD-PCR products for untreated and treated larvae of laboratory
   and resistant strains of *S. littoralis* using OPB-15. Lane 1: molecular
   size markers. Lane 2: Larvae from L-S, lane 3: larvae from R-S,
   lane 4: larvae from L- Cy, lane 5: larvae from R-Cy, lane 6: larvae
   from L- Beta, lane 7: larvae from R- Beta, lane 8:larvae from L- Lu
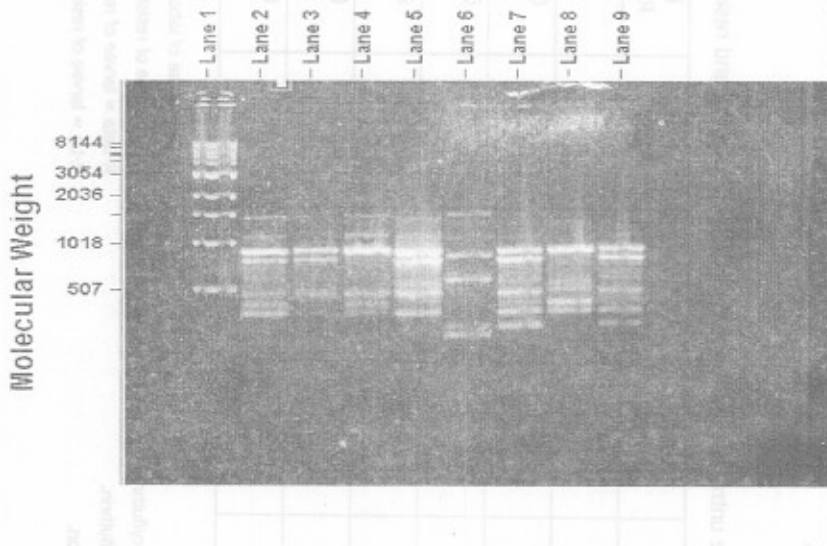   and lane 9: larvae from R-Lu.

Fig. 5. RAPD-PCR products for untreated and treated larvae of laboratory
   and resistant strains of *S. littoralis* using OPB-15. Lane 1: molecular
   size markers. Lane 2: Larvae from L-S, lane 3: larvae from R-S,
   lane 4: larvae from L- Cy, lane 5: larvae from R-Cy, lane 6: larvae
   from L- Beta, lane 7: larvae from R- Beta, lane 8:larvae from L- Lu
   and lane 9: larvae from R-Lu.

Table 2. The similarity index between the untreated and treated larvae of laboratory and resistant strains of S. littoralis for 5 primers

Primer	L-S& R-S	L-S& L-Cy	L-S& L-Beta	L-S& L-Lu	R-S& R-Cy	R-S& R-Beta	R-S& R-Lu	L- Cy & R-Cy	L- Beta & R-Beta	L-LU& R-Lu
OPA-7	0.545	0.500	0.400	0.400	0.545	1.00	0.571	0.375	0.571	1.00
OPA-10	0.889	1.000	0.889	0.933	1.00	0.762	0.667	0.889	0.857	0.667
OPA-16	1.00	0.500	0.750	0.533	0.75	0.533	0.875	0.500	0.667	0.533
OPB-15	0.769	0.615	0	0.364	0.727	0.571	0.500	0.727	0.200	0.600
OPB-20	0.667	0.857	0.471	0.667	0.727	0.615	0.667	0.769	0.556	0.533
Mean value	0.774	0.6944	0.502	0.579	0.750	0.696	0.656	0.652	0.570	0.666

L-S = larvae of laboratory strain (untreated larvae).

L- Beta = larvae of laboratory strain treated with Beta-cyfluthrin.

R-Cy = larvae of laboratory strain treated with Beta-cyfluthrin.

L-Lu = larvae of laboratory strain treated with Lufenuron.

L- Cy= larvae of laboratory strain treated Cyanophos.

R-S = larvae of resistant strain (untreated larvae).

R-Beta = larvae of resistant strain treated with Beta-cyfluthrin.

R-Lu = larvae of resistant strain treated with Lufenuron.

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تأثير بعض المبيدات علي المادة الوراثية للسلالة المعملية والمقاومة لدودة ورق القطن

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1 – *المعمل المركزي للمبيدات – مركز البحوث الزراعية – الدقي*

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استخدمت طريقة التضخيم العشوائي لـ DNA عن طريق جهاز تفاعل البلمــرة المتسلســل RAPD-PCR باستخدام عشرة بادئات عشوائية (كل منها مكون من عشرة قواعد نيتروجينية) لتقيم التغيرات الناتجة في DNA عند التعرض للمبيدات المختبرة في كل من السلالة المعملية والســلالة المقاومة لدودة ورق القطن محل الدراسة وكانت النتائج كالتالي.

1– خمسة بادئات (OPA-6, 9, 13, 17 and 18) لم تعطي نتائج مع DNA يرقات دودة ورق القطن بينمــا خمسة بادئات أخرى (OPA-7, 10, 16 and OPB-15, 20) أنتجت 296 حزمة لكــلا مــن الســلالة المعملية والمقاومة ومعاملتهما بالمبيدات المستخدمة. وقد ظهــرت 34-35-37-45-35-41-33-36 حزمة في يرقات (L-S$_{24}$, L-Cy, L-Beta, R-S$_{24}$, R-Cy, R-Beta, L-Lu and R-Lu) على التوالي.

2– أوضحت النتائج أن البادئات العشوائية (OPA-7, OPB-15 and 20) كانت ذات كفاءة عاليــة في تحديد درجة القرابة أو الاختلاف ما بين السلالتين أكثر من البادئات العشــوائية الأخــرى (OPA-10 and 16).

3– أن المعاملة بالمبيدات أحدثت اختلاف في الدنا الجيني للسلالتين المعملية والمقاومة (OPA-7, 10, 16 and OPB-15, 20)