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STUDIES ON GENETIC VARIATION OF FIELD COLLECTED PINK BOLLWORM, *PECTINOPHORA GOSSYPIELLA* (SAUNDERS) USING RAPD TECHNIQUE

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ABSTRACT

Five strains of the pink bollworm, *Pectinophoragossypiella* (Sanders)were used in the present study. The laboratory strain was used as a baseline in the molecular biology assays. Four strains were selected from natural populations; fields located in Menoufia, Gharbia, Dakalia and Kafel-Shiekh, Governorates. The molecular studies included the analysis of the plod genomic DNA of the tested strains under this study by using RAPD-PCR method. A battery of five primers was used to evaluate the mutagenic among the sex strains. One primer (C07) generated the highest numbers of fragments, in which the fragments were 25. Four primers (B20, C02, C05 and E07) generated 20, 14, 20 and 17 fragments; respectively. The molecular sizes ranged between.152.891 and 1979.767bp. The RAPD patterns resulted from amplification of DNA of the field colony strains and laboratory strain of the pink bollworm, *P. gossypiella* revealed that the lowest value of similarity index was (0.0%), which reflects the highest degree of change in DNA structure and sequence between the genomes of untreated pink and those exposed to a wide spread of different insecticides used for controlling the pest in the fields. On the other hand, the four primers B20 and C02, recorded similarity index 1.0 between the laboratory strain and Dakahlia&Kafrel-Sheikh field colony strains; respectively. The primer C02 recorded similarity index 1.0 between Menoufia and Gharbia field colony strains. The primer C05 recorded similarity index 1.0 between Gharbia and Dakahlia field colony strains. Also, the primer C07 recorded similarity index 1.0 between Gharbia and Kafrel-Sheikh field colony strains. It is interest to note that the less damaging effect to pink bollworm DNA could be attributed to a good detoxifying mechanism developed by the insect as a result of wide spread and long term exposure of insect larvae in additional to different insecticides used in the fields.

INTRODUCTION

The pink bollworm, *P. gossypiella*is one of the most destructive pests of cotton in Egypt. It has been introduced into Egypt between 1903-1913 from India within badly ginned cotton seeds (Willcooks,1916). In 1913, this pest spread to such degree that it became a real danger to the cotton crop in Egypt. It is capable of causing tremendous financial losses of the cotton yield. RAPD-PCR technique helps in genetic analysis of any new species without any prior knowledge about the particular DNA sequences or the genes of these new species. RAPD-PCR technique is not limited to a single locus but theoretically detects polymorphism across the whole genome, either coding or non coding regions in contradiction to biochemical markers which are limited to few genomic regions, so that this method may be used to any mutagen such as insecticides. Different parts of the genome can evolve at different rats (Nei, 1987). It is possible that the regions amplified by PCR evolve at higher rate. Thus RAPD-PCR finger prints can obtain a higher

variability especially if the amplified regions contain micro or minisatelliteDNA (Baruffi*et al.*, 1995). Amplified fragment length polymorphism was used to detect genetic diversity within and among gypthy moth,*Lymantriadispar*populations (Reineke *et al.*,1999).Alleloenzyme and RAPD analysis were performed to clarify relationship within the *Meligethesviridescens*compex. The M13 RAPD primer is a a diagnostic marker for distinguishing the seven populations of *M.*spp into four species (Audisio *et al.*. 2000). Hundsdoerfer and Wink (2005) examined the variability level of the inter simple sequences repeat in the three Lepidoptera families Pyralidae, Sphingidae and Pieridae. They showed the tetra repeat (GACA) is evidently present in sufficient numbers in these butterflies to provide informative DNA fragments. Studies included the analysis of the plod genomic DNA of different field strains of *P. gossypiella*were carried out by Abdel-Baset (2009) and Abdel-Salam *et al.* (2013) .They concluded that the arbitrary primers OPA-13, OPA-15 OPA-2, OPA_18, OPB-03, OPC-02,)PD-03 and OPE-12 proved to be strong tool to investigate changes in the genomic DNA of the pest. The objective of the present work is to investigate the use of rapid technique to study genetic variation of field collected pink bollworm, *P. gossypiella.*

MATERIALS AND METHODS

1. Test insects:

Laboratory strain:

Laboratory strain of the pink bollworm, *P. gossypiella*was obtained from the Department of Bollworms Research, Plant Protection Research Institute, Agricultural Research Center, Ministry of Agriculture, Dokki, Giza, Egypt, where it had been maintained on artificial diet consisting of kidney beans. The laboratory strain was used as a baseline in the molecular biology assay.

Field strains:

Five strains of the pink bollworm, *P. gossypiella*were isolated from Menoufia, Gharbia, Dakahlia and Kafrel- Sheikh Governorates at the late season of 2013 cultivated in different areas as well as temperature conditions and exposed regularly to the recommended insecticidal applications for cotton pests control according to routine schedule program set annually by the Central Administration for Pest Control, Ministry of Agriculture. The cotton fields were treated previously with 4 applications of the recommended insecticides for controlling bollworms representing different groups. These applications are chitin synthesis inhibitors, organophosphates, pyrethroids and organophosphates. These strains were used for molecular biology assay.

Rearing technique:

The rearing procedure used in this study was based on artificial diet of kidney bean similar to that described by Abdel-Hafez *et al.* (1982) with some minor modifications, ten pairs of the newly emerged moths were transferred into glass cages (750 ml volume, 14 cm high, 18 cm upper diameter and 23 cm lower diameter) locally named chimney glass No. 5 and stored in an incubator at temperature of 27±1 Co, photoperiod of 14:10 (light :

dark) and relative humidity of 80 ±5 %. The moths in every cage were fed on 10 % sugar solution that diffused though cotton plugs. The cages were covered with muslin secured with rubber bands and their bottom was covered with screening mesh for stimulating the eggs laying response in the females. The eggs were deposited through the screening mesh on a coarse white papers laced under the cage. The egg sheets were stored under the incubation conditions for 3-4 days until hatching. The newly hatched larvae were transferred using a fine brush to glass tubes (2X7.5 cm) filled to one third with the larval artificial diet, each vial covered with absorbed cotton and held in the same conditions until pupation. The resulted pupae were held in glass tubes (4X10 cm) covered with muslin and kept at the same incubation conditions until the adult emergence.

1.1.4. Diet preparation:

Artificial diet was kidney bean diet prepared according to Abdel-Hafez *et al.* (1982).

Molecular genetic studies:

DNA extraction:

DNA isolation (**Junhans and metzlatt, 1990**)

Buffers

Extraction buffer (pH 8.0)	
Tris –HCl (50 mM)	10 ml
NaCl (100 mM)	10 ml
EDTA (50 mM)	10 ml
SDS (0.5%)	10 ml
Mercapto ethanol (100 ul /100 ml solution) was added under hood	
H_2O up to	100 ml
TE buffer	
Tris (10 Mm, PH 8.0)	0.121 g
EDTA (1 Mm, PH 8.0)	0.029 g
H_2O up to	100 ml

Procedure:

AGATGCAGCC **Polymerase chain reaction (PCR) conditions**

PCR- RAPD was conducted using 5 arbitrary 10-mer primers (Operon Technologies, Inc.), The reaction conditions were optimized and mixtures were prepared (25-µl total volumes) consisting of the following:

dNTPs (8 mM)	2.5 µl
Taq DNA polymerase (5 U/ µl)	0.2 µl
10X buffer with 15 mM MgCl2	2.5 µ
Primer (10 mM)	1.0 µl
Template DNA (10-20 ng/ µl)	1.0 µl
H_2O (dd)	19.3 µl

Amplification was carried out in StrategeneRobocycler Gradient 96, which was programmed for 40 cycles as follows:

Denaturation (one cycle) 94^{o}C for 4 min, (40 cycles) of the following order 94^{o}C for 1.5 min, 36^{o}C for 1.5 min, 72^{o}C for 2.5 min then extension 72^{o}C (one cycle) for 7 min.

Table (1): List of RAPD primers and their nucleotide sequences.

Description	Sequence
C02	5'- GTG AGG CGT C-3'
C05	5'- GAT GAC CGC C-3'
C07	5'- GTC CCG ACG A-3'
B20	5'- GGA CCC TTA C-3'
E07	5'- AGA TGC AGC C-3'

Gel electrophoresis (Sambrook*et al*., 1989)

Agarose (1.2%) ultra pure (GIBCOBRL) was used for resolving the PCR products One Kb plus DNA ladder (750 ng /3 µl) (GeNetBio) was used which was separated into fourteen bands with molecular weights of 100, 200, 300, 400, 500, 650, 850, 1000, 1650, 2000, 3000, 4000, 5000 and 12000 bp.

TAE buffer (50X)	
Tris	242 g
Glacial acetic Acid	57.1 ml
EDTA	37.2 g
dd H_2O	up to 1 L

TBE buffer (10X)	
Tris	108.00 g
Boric acrid	55.00g
EDTA	7.44 g
d H_2O	up to 1 L

Gel preparation	
Agarose	1.2 g
TAE buffer (1X)	100 ml
Ethidium bromide (10 µg /µl)	1.5 µl
	Loading buffer (6X)
Bromophenol blue	0.25 g
Xylene cyanol	0.25 g
Glycerol (30%)	100 ml
	Sample preparation
PCR- product	20 µl
Loading buffer (6X)	5 µl

The run was performed for 1h at 100 V using Biometra gel electrophoresis submarine (20 cm x 10 cm). Bands were detected on UV-transilluminator and photographed by Gel documentation system (Biometra Bio Doc Analyze 2000).

Analysis of gel images

All DNA electrophoretic patterns were analyzed by *EgyGeneGelAnalyzer version three* software to determine relative mobility (RF), molecular size by base pairs (bp) and presence (1) or absence (0) of each fragment (*http://www.geocities.com/egygene*).

Similarity index:

The similarity index was used to compare patterns within as well as between populations. This index reflects the extent of band sharing and calculated as:

2Nab/ (Na+Nb)

Where, **Nab** is number of bands common to individuals a, b.

Na and **Nb** are total number of bands in a and b, respectively.

RESULTS AND DISCUSSION

After PCR amplification, the number and size of fragments may differ between strains. The specific patterns of the DNA observed represent the genetic make-up of each strain. Identical sized bands observed between different strains indicate genetic relatedness or similarity. The 4th instar larvae of the laboratory strain as well as field colony strains of the pink bollworm, *Pectinophoragossypiella* showed differences in RAPD-PCR patterns of amplified genomic DNA.

Random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) of five colony strains of *P. gossypiella* collected from differenGovernorates:

Five primers of arbitrary sequences were used to screen pooled genomic DNA of the different five strains.

In the 4th instar larvae of *P. gossypiella,* the fingerprints generated by both B20 and C05 revealed monomorphic, unique and polymorphic profiles for the pest strains; whereas the fingerprints generated by E07 revealed unique and polymorphic for the pest strains in terms in number and position of RAPD fragments. The fingerprint generated by C02 revealed polymorphic for the pest strains in terms in number and position of RAPD fragments. On the other hand, the fingerprint generated by C07 revealed polymorphic and monomorphic for the pest strains in terms in number and positionof RAPD fragments. These primers gave good amplification with distinct fragments.

Primer C07 showed the largest number of RAPD fragments where 25 bands were produced in the five strains of the pest under investigation. (Table 2 and Fig. 1). There were 7, 4, 3, 6 and 5 bands in the laboratory strain, Menoufia, Gharbia, Dakahlia and Kafrel-Sheikh strains respectively. The two fragments of 316.607 and 202.479 bp were shared all strains under investigation. Also, the fragment of 431.091 bp was shared in three strains of four colony strains in addition to the laboratory strain. Two amplified fragments of the laboratory strainas well as the four field colony strains were detected in the 4th instar larvae homogenatesas.

Two fragments of 1902.799 and 1249.708 bp were shared between the laboratory strain and both Dakahlia and Kafrel-Sheikh field colony strains.

A fragment of 692.254 bp was shared in the laboratory strain, and Kafrel-Sheikh field colony strains.

The similarity index ranged between 0.60 and 1.00 (Table 3). The Rapd-PCR pattern produced by amplification of the 4th instar larvae DNA with primer C07contained a total of 25 bands (Table 2). The number of DNA fragments ranged from 3-7 bands.

The PCR patterns resulted from using Primer B-20 discriminated between the laboratory and four field colony strains. Most of amplified fragments of the laboratory strain were not detected in all field colony strains. Majority of the fragments was noticed in Gharbia field colony strain, where seven fragments were detected. Using primer B-20 revealed some variability between the five different strains of *P. gossypiella.*

The fingerprints generated by primer B-20 revealed polymorphic profiles for each strain in addition to unique profiles for Gharbia and Dakahlia field colony strains of *P. gossypiella* in terms in number and position of RAPD fragments.

The total number of amplified fragments generated by this primer was 20 bands. There were 4, 2, 7,4 and 3 in the laboratory strain, Menoufia, Gharbia, Dakahlia and Kafrel-Sheikh field colony strains (Table 4 and fig. 1). Only one band of 158.754 pb was shared in laboratory and the field colony strains. Another band of 288.981 pb was shared in the laboratory and the field colony strains except Menoufia field colony strain. Moreover, two bands of 512.625 and 197.204 bp fragments were found in Gharbia field colony strain. Another band of 360.829 bp fragment was shared in the laboratory strain and the field colony strain except Kafrel-Sheikh field colony strain. One band of 421.289 bp fragment was shared in the laboratory, Gharbia and Dakahlia strains.

As illustrated in (Table 5), the values of similarity index were ranged from 0.40 to 1.00. The highest value 1.00 was appeared between the laboratory and Dakahlia strains. The lowest similarity index values 0.40 was appeared between Menoufia and Kafrel-Sheikh field colony strains.

Primer C02 showed the lowest number of fragments where 14 bands were detected in the five strains under this investigation of pink bollworm, *P. gossypiella* larvae(Table 6 and Fig.1). There were 2, 3, 3, 4, and 2 bands in the laboratory strain, Menoufia, Gharbia, Dakahlia and Kafrel-Sheikh strains; respectively.

The finger prints generated by the primer C02 revealed polymorphic profiles for the five strains in terms in number and position of RAPD fragments.

Fragment of 152.891 bp was detected in the different strain except Kafrel-Sheikh field colony strains; whereas the fragment 608.616 was appeared in all field colony strains of the pest. On the other hand, the fragment 879.696 pb was detected in the field colony strain except Kafrel-Sheikh field colony strain. The fragment of 270.980 bp was shared in the laboratory strain as well as Dakahlia and Kafrel-Sheika field colony strains.

F1

2-3-4

5- 6-7

The RAPD-PCR patterns produced by amplification of *P. gossypiella*DNA with primer C02 contained a total of 14 bands (Table 6 and Fig. 1). The number of DNA fragments ranged from 2-4 bands
As shown in Table (7), the similarity index values were ranged between 0.40 and 1.00.

Table(8):Total number and size of RAPD-PCR fragments generated by primer Co5 in different strains of *P. gossypiella* larvae.

Laboratory		menoufia		gharbia		dakahlia		Kafrel-sheikh		Total no. of DNA fragments	Polymorphism
No.	size	No.	size	No.	size	No.	size	No.	Size		
3	-	4	-	5	1979.767	5	1979.767	3	-	20	polymorphic
	-		-		1341.876		1341.867		-		polymorphic
	-		-		867.098		867.098		-		polymorphic
	560.304		560.304		560.304		560.304		560.304		monomorphic
	-		329.075		-		-		-		unique
	279.369		279.369		-		-		279.369		polymorphic
	173.283		173.283		173.283		173.283		173.283		monomorphic

As represented in Table (8) and illustrated in Fig.(1),the fingerprints generated by the primer C05 revealed polymorphic, monomorphic and unique profiles for the five strains in terms in number and position of RAPD fragments.

Two fragment of 560.304 and 173.283 bp were appeared in all strains including the laboratory strain as well as the four field colony strains, also fragment of 279.369 bp was detected in the laboratory strain as well as Menoufiaas and Kafrel-Sheikh field colony strains. Only one fragment of 329.075 bp was detected in Menoufia field colony strain. Three fragments of 1979.767, 1341.876 and 867.098 were appeared only inGharbia and Dakahlia field colony strains.

The fingerprints generated by primer C05 revealed polymorphic between Gharbia and Dakahlia field colonystrains and between laboratory, Menoufia and Kafrel-Sheikh strains. Also, the primer C05 generated monomorphic profiles for all strains under investigation and unique profile for Menoufia field colony strain in terms number and position of RAPD fragments.

The RAPD-PCR patterns produced by amplification of *P. gossypiella*DNA with primer C05 contained a total of 20 bands (Table 8 and Fig. 1). The number of DNA fragments ranged from 3-5 bands

The similarity index ranged between 0.44 and 1.00. The lowest value was 0.44 between Menoufia and both Gharbia and Dakahlia field colony strains; whereas the highest value of similarity index was 1.00 between laboratory and Kafrel- Sheikh as well as between Gharbia and Dakahlia field colony strains (Table 9).

The fingerprints in *P. gossypiella* generated by the primer, Eo7 showed differences in RAPD-PCR patterns of amplified genomic DNA, where unique and polymorphic profiles in terms of number and position of RAPD fragments were detected. The primer showed number of RAPD fragments, where 17 bands were produced in the five strains under investigation of *P. gossypiella*(Table 10 and Fig. 1) . There were 3, 3, 4, 1 and 6 bands in the

laboratory strain, Menoufia, Gharbia, Dakahlia and Kafrel-Sheikh field colony strains; respectively.The number of DNA fragments ranged from1-6 bands

Table(9): Estimated similarity index between the 5 strains of *P. gossypiella* larvae using primer Co5.

Strains	Laboratory	Menoufia	Gharbia	Dakahlia	Kafel-sheikh
Laboratory	-	0.86	0.57	0.57	1.00
Menoufia	-	-	0.44	0.44	0.86
Gharbia	-	-	-	1.00	0.50
Dakahlia	-	-	-	-	0.50
Kafel-sheikh	-	-	-	-	-

Three fragments of 672.747 bp were shared in the laboratory, Dakahlia and Kafrel-Sheikh strains. Also, three fragments of 410.449 bp were shared in laboratory, Menoufia and Gharbia strains. Additionally, three fragments of 283.345 bp were detected in Menoufia, Gharbia and Kafrel-Sheikh field colony strains. Two fragments of 335.179 were shred in Gharbia and Kafrel-Sheikh field colony strains. Fragments of 240.713 bp were shred in all strains except Dakahlia field colony strain. Only one fragment of both 187.093 and 164.537 bp was detected in Kafrel-Sheikh field colony strain.

Table(10):Total number and size of RAPD-PCR fragments generated by primer Eo7 in different strains of *P. gossypiella* larvae.

Laboratory		Menoufia		Gharbia		Dakahlia		Kafrel-sheikh		Total no. of DNA fragments	Polymorphism
No.	Size	No.	Size	No.	Size	No.	Size	No.	Size		
3	672.747	3	-	4	-	1	672.747	6	672.747	17	Polymorphic
	410.449		410.449		410.449		-		-		Polymorphic
	-		-		335.179		-		335.179		Polymorphic
	-		283.345		283.345		-		283.345		Polymorphic
	240.713		240.713		240.713		-		240.713		Polymorphic
	-		-		-		-		187.093		Unique
	-		-		-		-		164.537		Unique

No major changes in the banding pattern were observed among samples showing polymorphic and unique patterns (Fig.1).

The fingerprints generated by the primer Eo7 revealed polymorphic profiles for laboratory strain and the four field colony strains & polymorphic profiles in Menoufia and Gharbia as well as in Gharbia and Kafrel-Sheikh field colony strains. Also, polymorphic profiles in Menoufia, Gharbia and Kafrel-Sheikh field colony strains. Unique profile was detected only in Kafrel-Sheikh field colony strain.

The similarity index ranged between 0.00 and 0.86 (Table 11). The lowest value was 0.00 between Menoufia and Dakahlia field colony strains as well as between Ghabia and Dakahlia field colony strains whereas the highest value was 0.86 between Menoufia and Dakahlia field colony strains.

Table(11):Estimated similarity index between the 5 strains of *P .gossypiella* larvae using primer Eo7.

Strains	Laboratory	Menofia	Gharbia	Dakahlia	Kafrel-sheikh
Laboratory	-	0.67	0.57	0.50	0.44
Menoufia	-	-	0.86	0.00	0.44
Gharbia	-	-	-	0.00	0.60
Dakahlia	-	-	-	-	0.29
Kafel-sheikh	-	-	-	-	-

Reviewing aforementioned results, it could be revealed that the banding patterns are created using short oligonucleotide primers (10bp in length) of arbitrary sequence in Random Amplified polymorphic DNA (RAPD) technique. These arbitrary sequences are not specific for a particular gene or DNA sequence, so they are designed to screen the whole genome in general detecting any changes between two or more genomes under comparison. These primers bind the homologous sequence along the genome and PCR amplification only occurs when opposing primer sites are about bp apart. Within a population sample, mutation caused by any stress (e.g. insecticide treatment or different temperature) change the base sequence of primer binding sites, allowing polymorphism to be detected (Williams *et al.*, 1990). The word “random” in the term RAPD may be somewhat misleading in that the only random component is the sequence of a primer rather than the region amplified.

RAPD-PCR technique has several advantages over other methods used for studying genetic variability. These advantages can be summarized as: It helps in genetic analysis of any new specie without any prior knowledge about the particular DNA sequences for the genes of these new species.

It is not limited to a single locus but theoretically detects polymorphism across the whole genome, either coding or non coding regions in contradiction to biochemical (isozymes and protein) markers which are limited to few genomic regions, so that this method may be used for studying mutations among populations exposed to any mutagen.

Different parts of the genome can evolve at different rates (Nei, 1987). Therefore, it is possible that the regions amplified by PCR evolve at higher rate. Thus RAPD-PCR finger prints can obtain a higher variability especially if the amplified regions contain micro or minisatellite DNA (Baruffi*et al.*, 1995).

Because of the small amount of template used in each reaction, this protocol allows for the simultaneous analysis of several primers in a single run. Furthermore, the RAPD-PCR technique does not require the expensive and hazardous use of radioactive nucleotides as in Restriction Fragment Length Polymorphism (RELP) technique (Cenis and Betita 1994).

PCR cycling may be initiated overnight and the products are loaded into a gel and analyzed the following day (Chen *et al*.,1999).

In RAPD finger printing an oligonucleotide primer amplifies distinct DNA fragments. These fragments are referred to as RAPD markers. The number and size of RAPD markers depend on the complementarily of the

sequence of a particular primer and template DNA which is characteristic to an individual (Hedrick, 1992 and Williams *et al.*, 1993).

In the present work it was found that DNA of a good quality is a prerequisite to have reproducible results from RAPD-PCR technique. The highest numbers of amplified fragments were 25 resulted from primer C07, whereas the lowest number of amplified fragments were 14 resulted from primer C02 in *P. gossypiell*larvaea*.*

Primers usually do not have the same amplification efficiency.Katanen*et al*. (1995) found that some primers fail to amplify; others produce too complex banding patterns.

In the present investigation, the genomic DNA of the laboratory strain and field colony strains was screened for DNA damage or sequences changes using five primers of arbitrary sequences. The absence of a fragment from the RAPD pattern of tested insects may be due to changes in DNA sequence of the insect under investigation. The effect of deletion, insertion or breakage at one or both primer annealing sites on a greater distance than can be amplified (Rafalski*et al.*1991). RAPD-PCR produced a series of discrete DNA fragments, which typically vary in intensity and range in size from 77-2505 bp(Abdel-Baset, 2009).

The RAPD patterns resulted from amplification of DNA of laboratory & field colony strains of *P. gossypiella* revealed the lowest value of similarity index (0.0) which reflects the highest degree of change in DNA structure and sequence was recorded between the genomes of the different strains using primer E7. The same findings were reported by Soliman (1997) who found that primers OPA-1, 2 and 3 exhibited a degree of differences that reached 66.71 to 100 % between the genomic DNA untreated and treated med flies. In this field of study, Hundsdoerfer and Wink (2005) examined the variability level of the inter simple sequences repeat in the three Lepidoptera families Pyralidae, Sphingidae and Pieridae. They showed the tetra repeat (GACA) is evidently present in sufficient numbers in these butterflies to provide informative DNA fragments. The present results are accordance with those obtained by Abdel-Baset (2009). She revealed that primers OPA-13, OPA-15 and OPD-5 are strong tools to investigate changes in the *P. gossypiella* and *Culexpipiens* genomic DNA, probably due to changes in sequences of these primers. The sequence of the primer is known to be the factor for screening DNA or polymorphism. The present study are going in line with those of Lakshmi (2012) who revealed that the 5^{th} instar larvae of silk worm, *Anthereaemylitta* exposed to low temperatures during winter have shown significant variation in the levels of various bimolecular to cope with heat-chock. The obtained results are supported with those published by Abdel-Salam *et al*. (2013). They recorded that the RAPD patterns resulted from amplification of DNA of the field colony strains and laboratory strain of the pink bollworm, *P. gossypiella* fourth instar larvae revealed that the lowest value of similarity index (0.00), which reflects the highest degree of change in DNA structure and sequence between the genomes of untreated pink bollworm larvae and those exposed to a wide spred of different insecticides which used for controlling the pest.

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دراسات علي الاختلافات الجينية بين السلالات الحقليه لدودة اللوز القرنفليه باستخدام وسيلة البلمرة

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اجريت هذه الدراسة على خمس سلالات لدودة اللوز القرنفلية منها اربع سلالات حقلية جمعت من محافظات المنوفية و الغربية والدقهلية وكفر الشيخ وسلاله معمليه تم تربيتها لعدة اجيال دون تعريضها لاى مبيدات كيميائية والتى استخدمت كقاعدة اساسية للاختبارات على الحامض النووى DNA الخاص بالطور اليرقى الرابع لدودة اللوز القرنفلية باستخدام جهاز الفصل الكهربائى على مادة الاجاروز للدراسات الجزيئية على التضخم الحشرى للحامض النووى DNA فى السلالات المختلفة تحت الدراسة بواسطة جهاز البلمرة المتسلسل مع خمس بادئات تفاعل ذات توالى عشوائى هى : B20, Co2, Co5, C7&Eo7 وقد انتجت انماط تفاعل البلمرة المتسلسل مجموعة 96 شريط نووى وتراوح عدد الاشرطة بين 1, 7 لكل عينة. انتج بادئ تفاعل Co7اعلى عدد من الحزم فى يرقات دودة اللوز القرنفلية حيث بلغ 25 حزمة. انتجت الاربع بادئاتB20 , Co2, Co5, Eo7 ، 20 ، 14 ، 20 ، 17 حزمة على التوالى.تراوحت اوزان الاشرطة النووية بين 152.891، 1979.767 زوج قاعدى .وقد ادت بادئات التفاعل اهمية تشخيصية للتفرقة بين السلالات الحقلية لدودة اللوز القرنفلية والسلاله المعملية. وترجع اهمية هذه الدراسة الى امكانية استخدام بادئات التفاعل كدلالات وراثية لاجراء المسح على الحامض النووى DNA نتيجة التعرض للمبيدات الكيميائية. وقد اوضح التضخم العشوائى لقطع الحامض النووى DNA استخدام بادئ التفاعل Eo7 لكل من السلالات الحقلية والمعملية وجود معامل تشابه صفر مما يدل على ان هناك درجة عالية من الاختلاف بين التركيب للحامض النووى DNA للسلاله المعملية غير المعاملة والسلاله الحقلية المعرضة لضغط من المبيدات الحشرية الكيميائية لمكافحة هذه الحشرة. على الجانب الاخر سجلت بادئات التفاعل تحت الدراسة معامل تشابه 1.0 بين السلاله المعملية والسلاله الحقلية وكذلك بين سلالات حقلية وبعضها ويمكن القول ان التأثير الضار للمبيدات الحشرية ضد السلالات الحقلية يرجع الى بعض النظم البيوكيميائية والتى تختص بميكانيكية حيوية هامة فى مقاومة الافه لفعل المبيدات عن طريق تحطيم المبيدات السامة وتحويلها الى نواتج قليله او عديمة السمية.

قام بتحكيم البحث

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Table(2):Total number and size of RAPD-PCR fragments generated by primer Co7 in different strains of *P.gossypiella* larvae.

Laboratory		Menoufia		Gharbia		Dakhlia		Kafrel-sheikh		Total no. of DNA fragments	polymorphism
No.	Size	No.	Size	No.	Size	No.	Size	No.	Size		
7	1902.799	4	-	3	-	6	1902.799	5	1902.799	25	Polymorphic
	1249.708		-		-		1249.708		1249.708		Polymorphic
	842.907		-		-		842.907		-		Polymorphic
	692.254		692.254		-		-		692.254		Polymorphic
	431.091		431.091		431.091		431.091		-		polymorphic
	316.067		316.067		316.607		316.607		316.607		Monomorphic
	202.479		202.479		202.479		202.479		202.479		Monomorphic

Table(3): Estimated similarity index between the 5 strains of *P. gossypiella* larvae using primer Co7.

Strains	Laboratory	Menoufia	Gharbia	Dakahlia	Kafel-sheikh
Laboratory	-	0.73	0.60	0.92	0.83
Menoufia	-	-	0.86	0.60	0.67
Gharbia	-	-	-	0.67	1.00
Dakahlia	-	-	-	-	0.73
Kafel-sheikh	-	-	-	-	-

Table(4): Total number and size of RAPD-PCR fagments generated by primer B20 in different strains of *P.gossypiella* larvae.

Laboratory		Menoufia		Gharbia		Dakahlia		Kafrel-sheikh		Total no. of DNA fragments	polymorphism
No.	Size	No.	size	No.	size	No.	size	No.	Size		
4	-	2	-	7	512.625	4	-	3	-	20	Unique
	421.289		-		421.289		421.289		-		Polymorphic
	360.829		360.829		360.829		360.829		-		Polymorphic
	288.981		-		288.981		288.981		288.981		Polymorphic
	-		-		248.790		-		248.790		Polymorphic
	-		-		197.204		-		-		Unique
	158.754		158.754		158.754		158.754		158.754		monomorphic

Table (5): Estimated similarity index between the 5 strains of *P.gossypiella* larvae using primer B20.

Strains	Laboratory	Menoufia	Gharbia	Dakahlia	Kafrel-sheikh
Laboratory	-	0.67	0.73	1.00	0.57
Menoufia	-	-	0.44	0.67	0.40
Gharbia	-	-	-	0.73	0.60
Dakahlia	-	-	-	-	0.57
Kafrel-shiekh	-	-	-	-	-

Table(6):Total number and size of RAPD-PCR fragments generated by primer Co2 in different strains of *P.gossypiella* larvae.

Laboratory		Menoufia		Gharbia		Dakahlia		Kafrel-sheikh		Total no. of DNA fragments	polymorphism
No.	size	No.	size	No.	size	No.	size	No.	Size		
2	-	3	879.696	3	879.696	4	879.696	2	-	14	Polymorphic
	-		608.616		608.616		608.616		608.616		Polymorphic
	270.980		-		-		270.980		270.980		polymorphic
	152.891		152.891		152.891		152.891		-		polymorphic

Table (7): Estimated similarity index between the 5 strains of *P.gossypiella* larvae using primer Co2.

Strains	Laboratory	Menoufia	Gharbia	Dakahlia	Kafel-sheikh
Laboratory	-	0.40	0.40	0.67	0.50
Menoufia	-	-	1.00	0.86	0.40
Gharbia	-	-	-	0.86	0.40
Dakahlia	-	-	-	-	0.80
Kafel-sheikh	-	-	-	-	-