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Genetic variation in different field strains of fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae) larvae resistance treated with two insecticides

Khidr, A. A.; Hend, A. A. Al-Ashry; Sayed, M.; Faten, A. A. Badr and Dalia, A. Abdel-Salam

Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, Egypt.

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Abstract

Discriminating concentrations of the tested products, namely the diakoks (Emamectin benzoate) as well as the tracer (Spinosad), were used to determine the resistance in different strains collected from three governorates, namely Qalubia, Assiut, and Sharkia. It was revealed that diakoks insecticide was more toxic against the 4th instar larvae of the tested pest than the tracer insecticide. It was clear that the discriminating concentration (LC_{99}) of diakoks and tracer insecticides against the baseline laboratory strain caused 82.35 and 44.12; 73.53 and 29.41 and 88.24 and 58.32% mortality in the 4th instar larvae collected from Qalubia, Assiut, and Sharkia Governorates, respectively, whereas the corresponding resistance percentages were 16.82 and 55.43; 25.73 and 70.29 and 10.87 and 40.09%; respectively. Genetic diversity in the field colony of 4th larval instars of *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) collected from three different Egyptian Governorates compared with the baseline laboratory strain was investigated. Primer OP-A3 generated 16 fragments in the three fields colony as well as the baseline laboratory strain of *S. frugiperda* 4th instar larvae. Primer OP-A3 detected 16 fragments in the three-field colony as well as the baseline laboratory strain. Primer OP-A5 generated 18 fragments in the three fields colony and laboratory strain. Primer OP-B3 generated 13 fragments in the three fields colony and laboratory strain of *S. frugiperda* 4th larval instars. Primer OP-C15 generated 16 fragments in the three fields colony as well as the laboratory strain of the tested insect. Primer OP-D1 generated the highest number of fragments, which were 29 fragments in the three fields colony as well as the baseline laboratory insect.

Introduction

Spodoptera frugiperda (J.E. Smith) (Lepidoptera: Noctuidae) caused great damage and qualitative as well as quantitative yield losses in Africa (Matova *et al.*, 2020). Fall armyworm entered Egypt recently during 2019 causing great damage to the maize crop. Kumela *et al.* (2019) reported that *S. frugiperda* is considered the most damaging pest for maize crops. Harrison *et al*. (2019) mentioned that *S. frugiperda* is polyphagous. Excessive use of insecticidal applications created different problems, such as insect resistance. The RAPD-PCR technique can be used for measuring genomic DNA structure and sequences as well as mutagenic effects resulting from the wide use of insecticides. This technique can be utilized to investigate the genetics and insect resistance associated with chemical insecticides (Nei, 1987; Abdel-Baset, 2009; Sayed, 2019 and Allam, 2022).

The investigation aimed to determine resistance rates related to using Random Amplified Polymorphic DNA (RAPD-PCR) of three field populations of *S. frugiperda.*

Materials and methods

1. Insect rearing:

1.1. The baseline laboratory strain of fall armyworm**,** *S. frugiperda*, was reared on castor leaves free from any insecticidal contamination in the Bollworms Research Department on the basis reported by El-Defrawi *et al*. (1964).

1.2. Field strains:

Three field populations were isolated from the natural population in the maize fields of Assiut, Qalubia, and Sharkia Governorates at the end of maize plantation in 2023. The selected plots were applied periodically with insecticidal treatments by the producers to control the pest.

2. Insecticides used:

2.1. Diakoks 5.7% WG

Common name: Emamectin benzoate

Trade name: Diakoks 5.7% WG, at rate of 60 gm/feddan.

Source: Al-Badr for agricultural serves.

2.2. Tracer, 24% SC

Common name: Spinosad

Trade name: Tracer, 24% SC, at rate of 50 ml/feddan.

Empirical formula: Spinosyn A; C_{14} H_{65} NO_{10}

Spinosyn D: C_4 H_{67} NO_{10}

Source: Dow Chemical Company

3. Methods of application:

3.1. Toxicity of two tested insecticides applied against the baseline laboratory strain of fall armyworm *Spodoptera frugiperda* 4th larval instars:

The 4th instar larvae of the laboratory strain of the fall armyworm *S. frugiperda* were fed on castor bean leaves treated with the tested insecticidal solution dissolved in water. For each insecticide, 5 concentrations around the LC_{50} values were used against the 4th instar larvae of the pest for the establishment of toxicity lines. Untreated larvae were exposed to castor leaves dipped in water only.4th instar larvae were tested in 4 replicates containing 20 larvae/replicate placed individually in glass tubes (3 cm in diameter x 7.5 cm in height) to avoid cannibalism among the tested larvae. Mortality counts were made 24 hrs. after each treatment. Mortality percentages were corrected for the natural response according to Abbott (1925). The dosage mortality results were produced by the probit method (Finney, 1971).

3.2. Distinguished concentration procedure:

A distinguished concentration procedure was implemented for quickly measuring insecticidal resistance in a field colony of *S. frugiperda* 4th instar larva. Roush and Miller (1986) revealed that measuring resistance levels in insect pests via using a distinguished concentration procedure is better than determining dose-response regression lines measuring resistance levels. The diagnostic concentration is a single concentration that can discriminate between susceptible and resistant individuals. Gunning *et al*. (1998) demonstrated that a distinguished dose can be used as LD_{99} for susceptible insects. The LC_{99} of the laboratory *S. frugiperda* 4th instar larvae were estimated previously from the treated 4th instar larvae and could be chosen for estimating a diagnostic concentration for the two evaluated insecticides (Roush and Miller, 1986). To estimate distinguished concentration against the *S. frugiperda* 4th instar larvae laboratory strain, four ml of diakoks and tracer solution was dipped and pipetted into a Petri dish (25 cm in diameter) and air dried (Vertically) at room temperature for 3 hours. Twenty randomly selected 4th instar larvae were collected from Qalubia, Assiut, and Sharkia governorates, which received 5-7 applications of insecticides. The 4th treated larvae were replicated four times and placed in each Petri dish pretreated with the discriminated concentration of the two tested insecticides. Percentages of dead larvae in a field colony comparable with the baseline laboratory strain were recorded after one day. Mortality percentage was calculated for the field colony and compared with the laboratory strain. Resistance percentages in the field colony could be determined according to the method of McCutchen *et al*. (1989).

Resistance percentages = 100 – (MF/MSX100)

Where MF = % mortality at discriminating concentration in a field strain

MS = % mortality at discriminating concentration in the susceptible strain.

3.3. Isolation and extraction of genomic DNA were carried out according to the methods described by Williams *et al*. (1990) (Table 1) :

3.3.1. Data analysis:

The similarity matrices were done using Gel works in ID advanced software UVP-England Program.

3.3.2. Similarity index:

The similarity index was used to identify the extent of band sharing and calculated as:

$2N_{ab} / (N_a + N_b)$

Where N_{ab} is the number of common bands to the individuals a, b.

N_a and N_b are the total number of bands in a and b, respectively (Nei and Li, 1979).

Table (1): Nucleotide sequences of five primers used in the study RAPD-PCR procedure.

Primers	Sequences
OP-A3	CAG CAC CCA C
OP-A5	CCTTGACGCA
OP-B3	CAT CCC CCT G
OP-C15	GAC GGA TCA G
OP-D1	ACC GCG AAG G

Results and discussion

1. Monitoring insecticidal resistance in the 4th instar larvae of the field colony strain treated with different toxicants by using the discriminating technique:

Discriminating concentrations of the tested products was used to measure resistance levels in different strains to toxicity of insecticides used. The obtained data are summarized in Tables (2 and 3) and illustrated graphically in Figure (1). It is clear that the discriminating concentration (LC_{99}) of the two insecticides, diakoks and tracer were, 54.351 and 136.446 ppm, respectively, against the baseline laboratory strain, which caused 73.53 and 29.41, 82.35 and 44.12 and and 88.24 and 58.32% mortality in the field colony of the 4th instar larvae, *S. frugiperda* collected from Assiut, Qalubia, and Sharkia Governorates, respectively. The corresponding resistance levels were 25.73 and 70.29, 16.82 and 55.43 and 10.87 and 40.09% in the field colony of the 4th instar larvae, *S. frugiperda*, collected from Assiut, Qalubia, and Sharkia Governorates, respectively. It was obvious that the biocide tracer exhibited the highest in the field colony of the 4th instar larvae, *S. frugiperda*, collected from Assiut, Qalubia, and Sharkia Governorates, respectively. 1st level of resistance in the pest.

Table (2): **Toxicity of diakoks and tracer insecticides against the 4th instar larvae of the *Spodoptera frugiperda* laboratory strain.**

Diakoks		Tracer	
Concentration (ppm)	**Mortality %**	**Concentration (ppm)**	**Mortality %**
40	100	40	85
10	80	20	70
0.2	80	10	60
0.01	45	5	15
0.002	25	-	-
Slope	0.676	Slope	2.171
LC_{50} (ppm)	0.078	LC_{50} (ppm)	11.527
LC_{90}(ppm)	2.194	LC_{90} (ppm)	44.804
LC_{99}(ppm)	54.351	LC_{99} (ppm)	136.446

Table (3): Susceptibility status of the 4th instar larvae of the *Spodoptera frugiperda* field strain to the toxicity of diakoks and tracer by using discriminating concentrations.

Strains	**Diakoks**		**Tracer**	
	Mortality %	**Resistance %**	**Mortality %**	**Resistance %**
Qalubia	82.35^{b}	16.82^{b}	44.12^{c}	55.43^{b}
Assiut	73.53^{c}	25.73^{a}	29.41^{d}	70.29^{a}
Sharkia	88.24^{b}	10.87^{b}	58.32^{b}	40.09^{c}
Laboratory	99^{a}	-	99^{a}	-
F	28.41	24.99	235.67	272.35
L.S. D	6.0299	6.6391	5.7865	5.6491

***Resistance %= 100 - (dead percentages in field strains at LC_{99} / dead percentages in the baseline laboratory strain at LC_{99}) x 100**

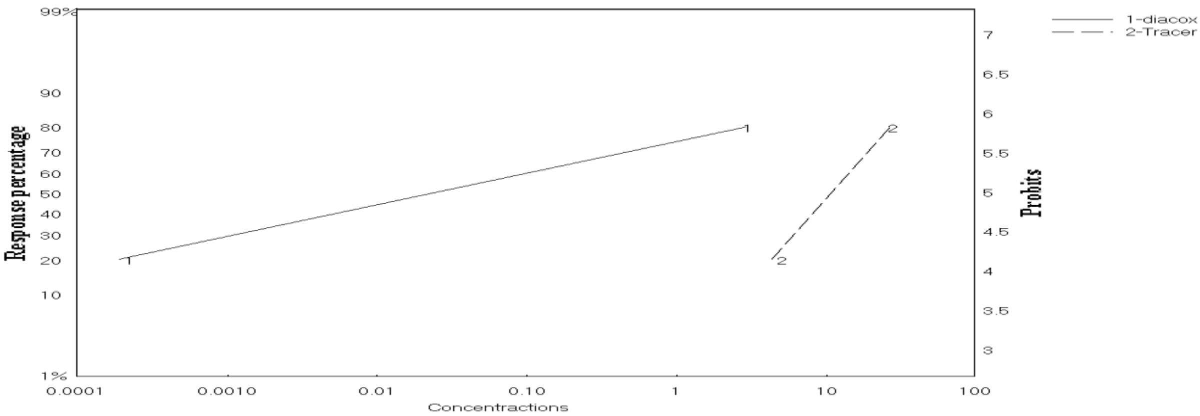

Figure (1): Toxicity regression lines of two tested compounds applied against the 4th instar larvae of the *Spodoptera frugiperda* laboratory strain.

2. Genetic diversity in the field colony of the 4th instar larvae *Spodoptera frugiperda*, collected from different Egyptian Governorate:

In PCR analysis, molecular weight and numbers of detected bands can be varied among tested strains. Identical-sized fragments detected between different populations indicate genetic relatedness or similarity. As illustrated in Table (4) as well as depicted graphically in Figure (2), it is clear that primer OP-A3 used in RAPD-PCR analysis generated 16 fragments in the three fields colony as well as the baseline laboratory strains of *S. frugiperda*. As shown in Table (4), three fragments were detected in the laboratory population; four bands appeared in both fields of Qalubia and Assiut Governorates, whereas five fragments were noticed in the field strain of Sharkia Governorate. Molecular weights of 430 and 340 bp are common in both the laboratory strain and the three field colonies. Amplified one fragment of 580 bp was detected: Qalubia, Assiut, and Sharkia. Molecular weight 500 bp appeared only in Assiut and Sharkia populations. One fragment of 220 bp was detected only by laboratory strain.

It was obvious that 2 monomorphic, 2 polymorphic, as well as 2 unique profiles were noticed.

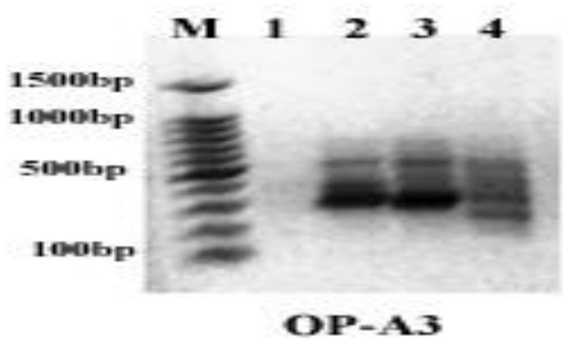

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Figure (2): RAPD-PCR produced for different strains of *Spodoptera frugiperda* 4th instar larvae using primer OP-A3.
Where is 1=Laboratory, 2=Qalubia, 3=Assiut, and 4=Sharkia

Table (4): Molecular weights of bands detected for RAPD-PCR procedures in field and laboratory strains of *Spodoptera frugiperda* with using OP-A3

MW-bp	Laboratory	Qalubia	Assiut	Sharkia	Frequency	Polymorphism
580	……	580	580	580	0.750	Polymorphic
500	……	…….	500	500	0.250	Polymorphic
430	430	430	430	430	1.000	Monomorphic
340	340	340	340	340	1.000	Monomorphic
275	……	……	…….	275	0.250	Unique
220	220	…….	…….	…….	0.250	Unique
Total	3	4	4	5	………..	……….

Values of similarity index of the three-field colony (table 5), Qalubia, Assiut, and Sharkia, in comparison with baseline laboratory strains were 0.57, 0.57, and 0.50, concluding resistance levels in these field strains to conventional insecticidal applications under field conditions. In this respect, the highest resistance could be noticed in Sharkia, followed by Qalubia as well as Assiut field populations. Similarity index values noticed among the three field colony populations ranged between 0.67 and 0.89, indicating genetic variation in the genomic DNA and sequences as a result of heavily insecticidal application under field conditions.

Table (5): Comparative estimated similarity index values associated four strains of *Spodoptera frugiperda*, 4th instar larvae using primer OP-A3.

Strains	Laboratory	Qalubia	Assiut	Sharkia
Laboratory	……..	0.57	0.57	0.50
Qalubia	-----	-----	0.75	0.67
Assiut	-----	-----	-----	0.89
Sharkia	-----	-----	-----	-----

Data shown in Table (6) and depicted graphically in Figure (3) cleared that primer OP-A5 generated 18 fragments in the three field colonies as well as the baseline laboratory strain, including four fragments for the laboratory strains: six, four and four bands in Sharkia, Qalubia, and Assiut Governorates, respectively. Three fragments of 680, 500, and 345 bp were common in both the laboratory strain and the three field colonies. Band weighed 815 bp detected in the laboratory and both Qalubia and Sharkia field populations. Also, one fragment of 530 bp was shared in both Assiut and Sharkia field populations. One fragment of 200 bp was detected only in the Sharkia field population.

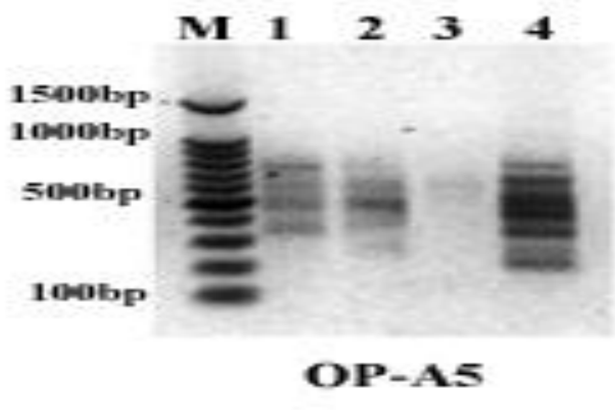

Figure (3): RAPD-PCR produced for different strains of *Spodoptera frugiperda* 4th instar larvae using primer OP-A5.
Where is 1=Laboratory, 2=Qalubia, 3=Assiut and 4=Sharkia

As summarized in Table (6), polymorphism generated by the primer OP-A5 showed 3 monomorphic, 2 polymorphic, and 1 unique profile in the tissues of the 4th instar larvae of the tested pest.

Table (6): Molecular weights of bands detected for RAPD-PCR P-A5 procedures in field and laboratory strains of *Spodoptera frugiperda* using OP-A5.

MW-bp	Laboratory	Qalubia	Assiut	Sharkia	Frequency	Polymorphism
815	815	815	……	815	0.750	Polymorphic
680	680	680	680	680	1.000	Monomorphic
530	……	……	530	530	0.500	Polymorphic
500	500	500	500	500	1.000	Monomorphic
345	345	345	345	345	1.000	Polymorphic
200	…….	…….	…….	200	0.250	Monomorphic
Total	4	4	4	6	………..	……….

The three field colony populations exhibited similarity index values that ranged from 0.80 to 1.00 compared with the baseline laboratory strain.

Similarity index values between the three field populations were 0.75 and 0.80 (Table 7).

Table (7): Estimated similarity index analysis between four strains of *Spodoptera frugiperda*, 4th instar larvae using primer OP-A5.

Strains	Laboratory	Qalubia	Assiut	Sharkia
Laboratory	……..	1.00	0.75	0.80
Qalubia	-----	-----	0.75	0.80
Assiut	-----	-----	-----	0.80
Sharkia	-----	-----	-----	-----

As presented in Table (8) and depicted in Figure (4), it was clear that using primer OP-B3 generated 13 fragments in the three-field colony as well as the laboratory strain. One fragment of 670 bp was common only in the three fields colony. The amplified molecular weight band of 540 bp was recorded only in Qalubia field colony and absent in the other strains. Also, the molecular weight fragment of 415 bp was detected only in Assiut Governorate. On the other hand, two fragments of 485 and 275 bp were common in the laboratory strain as well as the three field populations.

Using primer OP-B3 showed 2 monomorphic, 1 polymorphic, and 2 unique profiles in the tissues of the 4th instar larvae of the tested pest were noticed in case of using primer OP-B3 (Table 8).

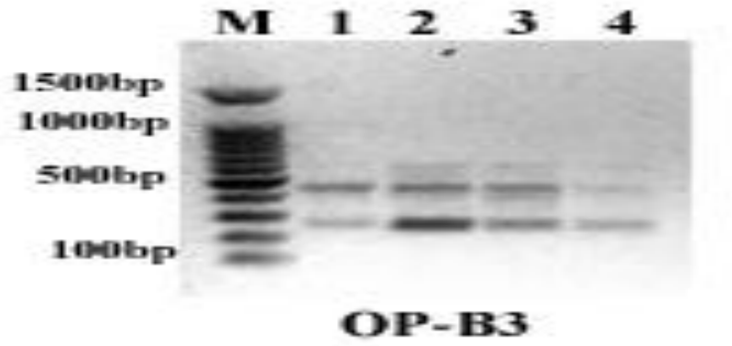

Figure (4): RAPD-PCR produced for different strains of *Spodoptera frugiperda* 4th instar larvae using primer OP-B3.
Where is 1=Laboratory, 2=Qalubia, 3=Assiut, and 4=Sharkia

Table (8): Molecular weights of bands detected for RAPD-PCR procedures in field and laboratory strains of *Spodoptera frugiperda* using OP-B3.

MW-bp	Laboratory	Qalubia	Assiut	Sharkia	Frequency	Polymorphism
670	……	670	670	670	0.750	Polymorphic
540	……	540	……	……	0.250	Unique
485	485	485	485	485	1.000	Monomorphic
415	…..	……	415	……	0.250	Unique
275	275	275	275	275	1.000	Monomorphic
Total	2	4	4	3		

The three field colony populations, which ranged from 0.40 to 0.67 compared with the baseline laboratory strain. Similarity index values between the three field populations were 0.75 and 0.85 (Table 9).

Table (9): Comparative estimated similarity index values associated with four strains of *Spodoptera frugiperda*, 4th instar larvae, using primer OP-B3.

Strains	Laboratory	Qalubia	Assiut	Sharkia
Laboratory	……..	0.67	0.67	0.40
Qalubia	-----	-----	0.75	0.85
Assiut	-----	-----	-----	0.85
Sharkia	-----	-----	-----	-----

Amplifying RAPD-PCR for detecting DNA fragments by selecting primer OP-C15 was summarized in Table (10) and observed in Figure (5). It is noticed that Primer OP-C15 generated 16 fragments in the three fields colony as well as the baseline laboratory strain. One fragment of 720 bp was detected only in the colony of Sharkia Governorate and absent in the laboratory, Qalubia, and Assiut strains. Amplified one fragment of 530 bp was shared in the three field populations and absent in the laboratory strain. A band of 235 bp was common in the three field populations and had disappeared in the baseline laboratory population. A fragment of 280 bp was absent in the three-field population. One fragment of 180 bp was common in the laboratory strain and the three field populations. The primer OP-C15 showed 1 monomorphic, 3 polymorphic, and 3 unique profiles in the tissues of the 4th instar larvae of the tested pest (Table 10). Based on similarity index values, which are 0.57, 0.57 and 0.25, indicating resistance levels in the three-field colony compared with the baseline laboratory strain (Table 11).

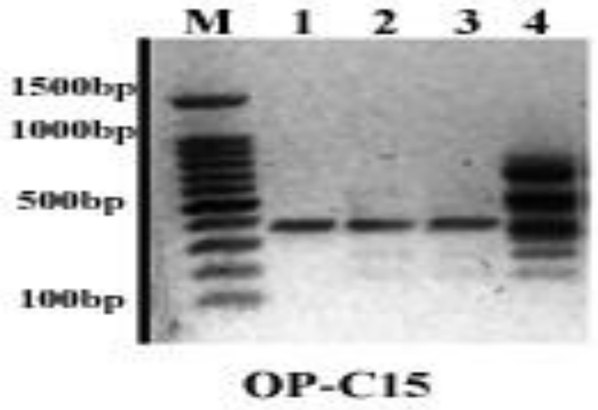

Figure (5): RAPD-PCR produced for different strains of *Spodoptera frugiperda* 4th instar larvae using primer OP-C15.
Where is 1=Laboratory, 2=Qalubia, 3=Assiut, and 4=Sharkia

Table (10): Molecular weights of bands detected for RAPD-PCR procedures in field and laboratory strains of *Spodoptera frugiperda* using OP-C15.

MW-bp	Laboratory	Qalubia	Assiut	Sharkia	Frequency	Polymorphism
720	-----	-----	-----	720	0.250	Unique
530	-----	530	530	530	0.750	Polymorphic
380	380	380	380	-----	0.750	Polymorphic
345	-----	-----	-----	345	0.250	Unique
235	-----	235	235	235	0.750	Polymorphic
280	280	-----	……..	-----	0.250	Unique
180	180	180	180	180	1.000	Monomorphic
Total	3	4	4	5	…….	……

Table (11): Comparative estimated similarity index values associated with four strains of *Spodoptera frugiperda*, 4th instar larvae using primer OP-C15.

Strains	Laboratory	Qalubia	Assiut	Sharkia
Laboratory	……..	0.57	0.57	0.25
Qalubia	-----	-----	1.00	0.67
Assiut	-----	-----	-----	0.67
Sharkia	-----	-----	-----	-----

RAPD-PCR magnification for detecting genetic variation by selecting primer OP-D1, which is summarized in Tables (12 and 13) as well as depicted in Figure (6) noticed that primer OP-D1 generated the highest number of fragments, which were 29 fragments in the three fields colony as well as the baseline laboratory strain. One fragment of 1365 bp was detected in the three fields colonies of Qlubia, Assiut, and Sharkia Governorate and was absent in the laboratory strain.

Amplified two fragments of 1285 and 485 bp were shared in the laboratory strain as well as the Sharkia field colony; where it was absent in Qalubia and Assiut filed colonizing strains. One molecular weight band of 865 bp appeared in the baseline laboratory strain and both populations of Qalubia, and Assiut, whereas it was absent in Sharkia field populations. Four fragments of 780, 625, 600, and 375 bp were common in the baseline laboratory strain as well as the three field populations. Two bands having molecular weights of 270 and 185 bp are generated in the baseline laboratory strain, whereas they are absent in the other field populations.

The primer OP-D1 (Figure 6) showed 4 monomorphic, 4 polymorphic, and 3 unique profiles in the tissues of the 4th instar larvae of the tested pest (Table 12). Measuring the similarity index detected in the different strains via using primer OP-D1 indicated moderate values of the three field populations compared with the baseline laboratory strain.

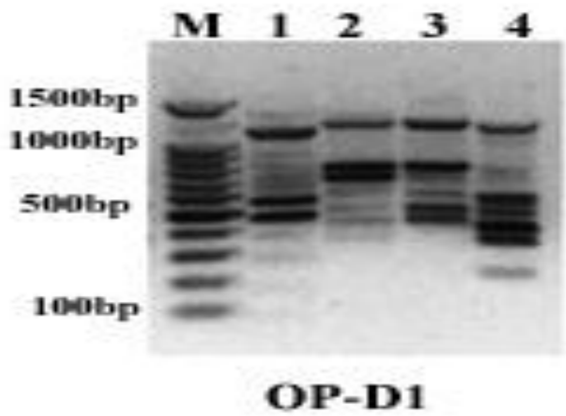

Figure (6): RAPD-PCR produced for different strains of *Spodoptera frugiperda* 4th instar larvae using primer OP-D1.

Where is 1=Laboratory, 2=Qalubia, 3=Assiut, and 4=Sharkia

Table (12): Molecular weights of bands detected for RAPD-PCR procedures in field and laboratory strains of *Spodoptera frugiperda* with OP-D1.

MW-bp	Laboratory	Qalubia	Assiut	Sharkia	Frequency	Polymorphism
1365	-----	1365	1365	1365	0.750	Polymorphic
1285	1285	……..	……..	1285	0.500	Polymorphic
865	865	865	865	-----	0.750	Polymorphic
780	780	780	780	780	1.000	Monomorphic
625	625	625	625	625	1.000	Monomorphic
600	600	600	600	600	1.000	Monomorphic
485	485	……	…….	485	0.500	Polymorphic
375	375	375	375	375	1.000	Monomorphic
270	270	…….	…….	…….	0.250	Unique
245	……	…….	…….	245	0.250	Unique
185	185	……	…….	…….	0.250	Unique
Total	9	6	6	8	…….	……

Table (13): Estimated similarity index analysis between four strains of *Spodoptera frugiperda*, 4th instar larvae using primer OP-D1.

Strains	Laboratory	Qalubia	Assiut	Sharkia
Laboratory	……..	0.67	0.67	0.70
Qalubia	-----	-----	1.00	0.70
Assiut	-----	-----	-----	0.71
Sharkia	-----	-----	-----	-----

The aim of determining resistance levels in different field colonies is to study to predict resistance management in field populations and to implement effective insecticides against insect pests. In this field of investigation, Roush and Miller (1986) reported that laboratory studies could be utilized to determine the resistance levels in different field populations by implementing discriminating concentrations. The present investigation revealed that Assiut population exhibited the highest levels of resistance against the fall armyworm, *S. frugiperda*, via using discriminating concentration (LC_{99}) determined against the laboratory strain. In this field of investigation, Abdel-Baset (2009), Sayed (2019) and Allam (2022) demonstrated that a field colony of pink bollworm, *Pectinophora gossypiella* (Saund.) (Lepidoptera: Gelechiidae), collected from different governorates recorded high resistance levels to the evaluated insecticide by using discriminating concentration (LC_{99}). Khidr *et al.* (2002) mentioned that using discriminating methods were promoted to give high information about resistance levels in pink bollworm.

Concerning fingerprints of molecular biology, Williams *et al.* (1990) revealed that insecticidal applications can affect the genomic structure of DNA and sequences of the tested insects. Cenis and Beitra (1994) reported that Randomized Amplified Polymorphic DNA Polymerase Chain Reaction (RAPD-PCR) is considered an accurate method for amplifying fingerprints and does not need to use radioactive nucleotides for detection fragments. Williams *et al.* (1993) demonstrated that the sequences and molecular weight of RAPD markers depend on the sequence of specific primers used. Present studies are in accordance with those findings by Abdel-Baset (2009). The author demonstrated that primers used could be considered strong tools for investigation of fingerprints of molecular biology in both *P. gossypiella* and *Culex pipiens* L.

(Diptera: Culicidae). The study is in agreement with the results published by Salem (2018), Sayed (2019), and Allam (2022). They revealed that the wide use of insecticidal applications against pink bollworm under field conditions induced diversity in genomic DNA structure and sequences compared with laboratory strains.

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