

Genetic Diversity in Adults of Cowpea Aphid, *Aphis craccivora* (Koch) Produced from Treatments of Photosensitizing Compounds

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Abstract

The present investigation aimed to evaluate the efficacy of four photosensitizing compounds, namely, rose Bengal, rhodamine B, methyl violet, and methylene blue, against the adults of the cowpea aphid, *Aphis craccivora* (Koch), under laboratory conditions. The obtained results indicated that rose Bengal was the most effective photosensitizer compound. The corresponding LC₅₀ and LC₉₀ values against the tested adults of cowpea aphid, *Aphis craccivora* were 0.024 and 0.143 %; followed by methyl violet, where the LC₅₀ and LC₉₀ levels recorded 0.125 and 0.620 %, on the other hand rhodamine B exhibited least efficient against tested adult of the pest, the corresponding LC₅₀ and LC₉₀ values recorded 1.49 and 4.50 %; LC₅₀ and LC₉₀ values for methylene blue were 0.140 and 0.660 %; respectively. The molecular changes, after treatment with photosensitizers were tracked using RAPD-PCR with five arbitrary DNA primers. The obtained results showed that the fingerprints generated in adults resulted from treating the aphid adults with each primer, OP-B3, OP-A3, OP-A5, OP-C3, and OP-C9 recorded polymorphic, monomorphic, and unique profiles for the pest exposed to photosensitizing compounds. It was found that DNA of good quality is a prerequisite to have reproducible results from the RAP-PCR technique. The highest number of amplified fragments was 35 generated from primer Op-B3, whereas the lowest number of amplified fragments was 20 detected from primer OP-A5.

The RAPD-PCR patterns resulted from amplification of DNA of untreated adults as well as adults of *A. craccivora* revealed the lowest value of mean of similarity index was noticed in case of treatment with rose Bengal (0.70), the highest toxicity compound, which reflects the highest degree of change in DNA structure and sequence.

Keywords: photosensitizing compounds, cowpea aphid, DNA, *Aphis craccivora*.

1. Introduction

Aphids are economically important insects, causing severe damage to a number of crop plants. Both nymphs and adults suck plant sap and cause serious damage right from the seeding to pod bearing stage (Ascher *et al.*, 1992). Its damage is due to direct feeding and its ability to transmit virus diseases to different plants. Cowpea aphid, *Aphis craccivora* (Koch), is a serious legume pest in Egypt (Abd- El-Aziz, S., 2021).

Controlling aphids in crops is important to increase the quality as well as the quantity of the crop yield. Chemical control is the most commonly used method by producers. The wide use of conventional insecticides created some problems, such as resistance levels in the pests and toxicity against the natural enemies. The use of photosensitizing compounds implemented as tools for controlling the population of several types of insects has been conducted by several investigators on the house fly, *Musca domestica* (Attia, 2016), and fall armyworm, *Spodoptera frugiperda* (Khidr *et al.*, 2022).

The photosensitizing compounds accumulate within the insects and, following exposure to visible light, induce damage to their cuticle, mid-gut wall, followed by feeding inhibition and eventual death (Amor *et al.*, 1998; Khidr *et al.*, 2022). These compounds affect the biochemical contents (Attia, 2016; El-Ghobary *et al.*, 2018).

Polymerase chain reaction (PCR) is a useful tool in many applications in our lives. Random Amplified Polymorphic DNA has been used to differentiate various animal species and their resistance strains. Also, this technique is applied to identify different populations within a species. The technique is quick and easy, and this detects nucleotide sequence polymorphisms using a single primer of arbitrary nucleotide sequence (Williams *et al.*, 1990).

This investigation aims to evaluate the toxicity as well as the lethal time of four photosensitizing compounds against the nymphs of the pest in relation to some biochemical contents, as well as molecular studies.

2. Materials and Methods

Table 1: Photoactive compounds used

Common name	Rose Bengal	Rhodamine B	Methylene blue	Methyl violet
Trade name	Rosets	Rhodamine 610	Urolene blue	Crystal violet
Chemical formula	C ₂₀ H ₄ Cl ₄ I ₄ O ₅	C ₂₈ H ₃₁ Cl N ₂ O ₃	C ₁₆ H ₁₈ N ₃₅ Cl	C ₂₅ N ₃ H ₃₀ Cl
Molar mass	973.67 g/mole	479 g/mole	319.85 g/mole	408 g/mole
Quantal yield	0.76	0.65	0.52	0.69

2.1 Bioassays:

The four new photosensitizing compounds were diluted with water to prepare stock solutions, and then serial concentrations were prepared freshly before treatments. Preliminary bioassay was carried out by immersing faba bean leaves in the serial concentration solutions for 20 seconds, then the treated faba bean leaves were left to dry at room temperature. Individual groups of ten wingless adults of aphids were placed in the plastic petri-dish (9 cm diameter, 1 cm depth) containing one plant leaf of faba bean, immersed for 10 seconds in the different serial concentrations of each photosensitizer. Bioassays included an untreated check in which leaves were immersed in water only, and then the tested leaves were air-dried at room temperature. Each treatment was replicated four times, and 250 adults per replicate were tested.

After feeding one day in the dark, the petri dishes were taken outdoors and exposed to sunlight. Inspection every 15 min. was recorded till 1:15 hours. after treatments. The average of mortality percentages

was corrected using **Abbott's formula (1925)**. The corrected mortality percentages were statistically computed according to **Finney (1971)**. The slope, LC₅₀, and LC₉₀ values were estimated for each compound.

2.2 Molecular biology:

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

2.3 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 is calculated as:

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

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

N_a and **N_b** are the total number of bands in a&b, respectively. The value produced by this index ranges from zero, respecting no bands sharing, to 1, respecting complete identity (**Nei and Li, 1979**).

Table 2: List of primer names and their nucleotide used in the study for the RAPD-PCR Procedure.

Primer Name	Sequences
OP-A3	CAG CAC CCA C
OP-A5	CCTTGACGCA
OP-B3	CAT CCC CCT G
OP-C9	CTCACCGTCC
OP-C3	GGGGGTCCAG

3. Results

3.1 Toxicological bioassays:

Laboratory bioassays were implemented to study the toxicity of four photosensitizing compounds, namely

rose Bengal, rhodamine B, methylene blue, and methyl violet, on the adult Cowpea aphid, *Aphis craccivora*.

The toxicity of different concentrations of rose Bengal, rhodamine B, methylene blue, and methyl violet against the adults of the cowpea aphid, *A. craccivora*

after 1:15 hours of exposure to sunlight was evaluated. The obtained results are summarized in Table 3 and illustrated graphically in Fig. 1. It is clear that the photosensitizers exhibited toxic effects on the adults of the cowpea aphid. It was obvious that the LC_{50} values ranged between 0.024 and 0.99%. The order of toxicity according to each of the LC_{50} and L_{90} values could be discerningly arranged as follows: rose Bengal, methyl violet, methylene blue and rhodamine B. The corresponding LC_{50} values were 0.024, 0.125, 0.140, and 0.990 %, respectively. The corresponding LC_{90} values were 0.143, 0.620, 0.660, and 11.548%, respectively.

The slope values and LC_{90}/LC_{50} ratios of the tested compounds against the adults of the tested pest after 1:15 hours from exposure to sunlight were calculated. The obtained results are presented in Table 3. Data

showed that the steepest toxicity line was noticed in the case of treatment with methylene blue. The corresponding slope of the toxicity line was 1.951, whereas the flattest one was observed in the case of treatment with rhodamine B, where the slope of the toxicity line was 1.200. The slope value of the toxicity lines of the other two photosensitizing compounds, rose Bengal B and methyl violet, occupied the middle situation among the two products mentioned previously, where their values recorded 1.673 and 1.845, respectively.

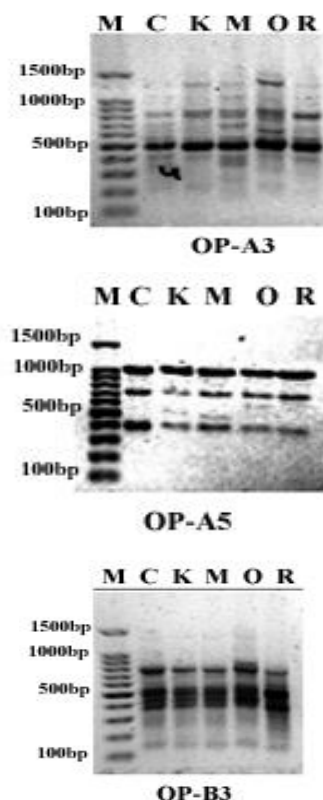
The previous conclusion is correct, whether it is the slope values or the LC_{90}/LC_{50} ratios, since the latter method simply expresses the steepness of the LC-P lines in a reverse way to the slope values. Therefore, an increase in the slope value is parallel to a decrease in the LC_{90}/LC_{50} ratios.

Table 3: Susceptibility status of the adults of cowpea aphid, *A. craccivora*, to the toxic action of the photoactive compound rose Bengal.

Photosensitizing compounds	Slope	LC_{50} (%)	LC_{90} (%)	LC_{90} / C_{50}
Rose Bengal	1.673	0.024	0.143	5.95
Rhodamine B	1.200	0.990	11.548	11.66
Methylene blue	1.951	0.140	0.660	4.71
Methyl violet	1.845	0.125	0.620	4.96

3.2 Molecular studies:

The RAPD analysis of the generated DNA samples using Primer OP-A3 is depicted in (Table 4) and (Fig. 1). It is noticed that the Primer generated 32 fragments in the four photosensitizing compounds treatment as well as laboratory populations of the pest under investigation. The number of detected fragments was 9, 6, 6, 5, and 9 fragments in the baseline laboratory strain, methyl violet, methylene blue, rose Bengal, and rhodamine B, respectively. One fragment of 715 bp was detected in methylene blue treatment. Also, one band of 650 has appeared only in the pest-treated



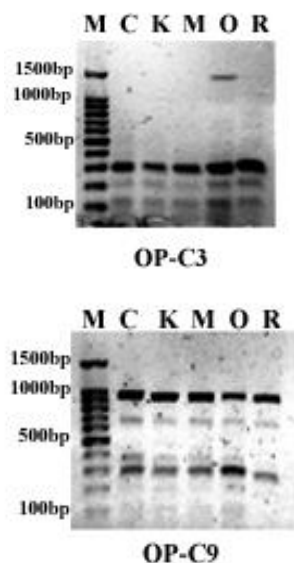


Fig 1: RAPD-PCR produced for Cowpea aphid, *Aphis craccivora* adults using five primers.

Where is M=Marker, 1- C= Control, 2- M=Methyl violet, 3-M= Methylene blue, 4- O= Rose Bengal, and 5- R= Rhodamine B. with rhodamine B. Amplified one

Table 4: Molecular weight of RAPD-PCR fragments generated by arbitrary primers in different photosensitizing compounds against Cowpea aphid, *Aphis craccivora* adults, using primer OP-A3.

MW-bp	Control	Methyl violet	Methylene blue	Rose Bengal	Rhodamine B	Frequency	Polymorphism
1345	1345	1345	1345	1345	1345	1.00	Monomorphic
1135	1135	1135	1135	1135	1135	1.00	Monomorphic
845	845	845	845	845	845	1.00	Monomorphic
715	715	----	715	-----	-----	0.400	Polymorphic
650	650	----	-----	-----	650	0.400	Polymorphic
500	500	500	500	-----	-----	0.600	Polymorphic
410	410	410	-----	410	-----	0.600	Polymorphic
330	330	-----	-----	330	330-	0.600	Polymorphic
225	225	225	225	-----	225	0.800	Polymorphic
Total	9	6	6	5	6	-----	-----

RAPD- PCR analysis of the generated DNA samples by using Primer OP-A5 is summarized in (Tables 5) and illustrated in (Fig. 1). It is clear that Primer OP-A5 detected 20 fragments in the pest treated with four photosensitizing compounds and the untreated adults. The number of observed bands was 3, 4, 5, 4 and fragments in the baseline untreated strain, treatments with methyl violet, methylene blue, rose Bengal, and rhodamine B; respectively. Two fragments of 1050 and 720 bp were common in both the laboratory strain and

fragment of 500 bp was detected in control as well as methyl violet and methylene blue. One fragment of 410 bp appeared in the laboratory strain, as well as methyl violet and rose Bengal. A fragment of 330 bp was noticed in the untreated aphids, rose Bengal, and rhodamine B. A band of 225 bp was found in the adult pest applied with methyl violet, methylene blue, rhodamine B treatment, and untreated adults. Three fragments of 1345, 1135, and 845 bp were common bands detected in all strains. As illustrated in Table 4, polymorphism generated by the primer OP-A3 showed 3 monomorphic and 6 polymorphic profiles in the tissues of the adult of the tested pest.

the four photosensitizing compounds. Amplified one fragment of 600 bp was detected in treatments with methylene blue, rose Bengal, and rhodamine B. One fragment of 510 bp appeared in the methyl violet, methylene blue, and rose Bengal applications. One fragment of 400 bp was detected in untreated adults, adults treated with methyl violet, methylene blue, and rhodamine B. As noted in (Table 5), polymorphism generated by the primer OP-A3 showed 2

monomorphic and 3 polymorphic profiles in the tissues of the adult of the tested pest.

Table 5: Molecular weight of RAPD-PCR fragments generated by arbitrary primers in different photosensitizing compounds against Cowpea aphid, *Aphis craccivora* adults, using primer OP-A5.

MW-bp	Control	Methyl violet	Methylene blue	Rose Bengal	Rhodamine B	Frequency	Polymorphism
1050	1050	1050	1050	1050	1050	1.00	Monomorphic
720	720	720	720	720	720	1.00	Monomorphic
600	-----	-----	600	600	600	0.600	Polymorphic
510	-----	510	510	510	-----	0.600	Polymorphic
400	400	400	-----	-----	400	0.600	Polymorphic
Total	3	4	5	4	4	-----	-----

Amplifying the RAPD analysis of the generated DNA samples induced Primer OP-B3 is presented in (Table 6) and depicted in (Fig. 1). It is obvious that Primer OP-B3 generated the highest number of fragments, which being 35 fragments detected as 7 fragments in adults of the pest under the study treated with each of the four photosensitizing and the control. In this respect, five fragments of 800, 530, 385, 265, and 165 bp were common in both untreated adults and adults treated with the four photosensitizing compounds mentioned previously. Amplified one fragment of 840 bp was

detected only in methyl violet as well as rose Bengal treatments. One fragment of 460 bp appeared in the control as well as both methyl violet and methylene blue. One fragment of 300 bp was detected in untreated adults, methylene blue, and rhodamine B. Also, one fragment of 235 bp was detected only in rhodamine B treatments. As noted in Table 6, polymorphism generated by the primer OP-B3 showed 5 monomorphic, 1 unique, and 3 polymorphic profiles in the tissues of the adult of the tested pest.

Table 6: Molecular weight of RAPD-PCR fragments generated by arbitrary primers in different photosensitizing compounds against Cowpea aphid, *Aphis craccivora* adults, using primer OP-B3.

MW-bp	Control	Methyl violet	Methylene blue	Rose Bengal	Rhodamine B	Frequency	Polymorphism
840	-----	840	-----	840	-----	0.400	Polymorphic
800	800	800	800	800	800	1.00	Monomorphic
530	530	530	530	530	530	1.00	Monomorphic
460	460	460	460	-----	-----	0.600	Polymorphic
385	385	385	385	385	385	1.00	Monomorphic
300	300	-----	300	-----	300	0.600	Polymorphic
265	265	265	265	265	265	1.00	Monomorphic
235	-----	-----	-----	-----	235	0.600	Unique
165	165	165	165	165	165	1.00	Monomorphic
Total	7	7	7	7	7	-----	-----

Amplifying the RAPD analysis of the generated DNA samples induced by Primer OP-C9 is summarized in (Tables 7) and illustrated in (Fig. 1). It is noticed that

Primer OP-C9 generated 21 fragments, detected as 5 fragments in untreated adults as well as adults treated with the photosensitizing compound, rhodamine B. It

was obvious that fragments of 970 and 700 bp were common in both untreated adults and adults treated with the four photosensitizing compounds. Amplified one fragment of 386 bp was detected in untreated adults as well as adults treated with methyl violet, rose Bengal, and rhodamine B. One fragment of 300 bp has appeared in the control as well as both methylene blue

and rhodamine B treatments. One fragment of 200 bp was detected in untreated adults as well as adults treated with methyl violet, methylene blue, and rhodamine B. As illustrated in (Table 7), polymorphism generated by the primer OP-C9 showed two monomorphic and 3 polymorphic profiles in the tissues of the adult of the tested pest.

Table 7: Molecular weight of RAPD-PCR fragments generated by arbitrary primers in different photosensitizing compounds against Cowpea aphid, *Aphis craccivora* adults, using primer OP-C9.

MW-bp	Control	Methyl violet	Methylene blue	Rose bengal	Rhodamine B	Frequency	Polymorphism
970	970	970	970	970	970	1.00	Monomorphic
700	700	700	700	700	700	1.00	Monomorphic
385	385	845	-----	385	385	0.800	Polymorphic
300	300	-----	300	-----	300	0.600	Polymorphic
200	200	200	200	-----	200	0.800	Polymorphic
Total	5	4	4	3	5	-----	-----

Amplifying the RAPD analysis of the generated DNA samples induced Primer OP-C3 is summarized in (Tables 8) and illustrated in (Fig. 1). It is cleared that Primer OP-C3 generated the lowest number of 21 fragments, which detected 17 fragments were distributed as 4 fragments in untreated adults as well as adults treated with the photosensitizing compound, rhodamine B. It

was obvious that fragments of 300, 215, and 145 bp were common in both untreated adults and adults exposed to the four photosensitizing compounds. As noticed in (Table 8), polymorphism generated by the primer OP-C3 showed three monomorphic and one polymorphic profile in the tissues of the adult of the tested pest.

Table 8: Molecular weight of RAPD-PCR fragments generated by arbitrary primers in different photosensitizing compounds against Cowpea aphid, *Aphis craccivora* adults, using primer OP-C3.

MW-bp	Control	Methyl violet	Methylene blue	Rose Bengal	Rhodamine B	Frequency	Polymorphism
1500	1500	-----	-----	-----	1500	1.00	Polymorphic
300	300	300	700	300	300	1.00	Monomorphic
215	215	215	215	215	215	0.800	Monomorphic
145	145	145	145	145	145	0.600	Monomorphic
Total	4	3	3	3	4	-----	-----

The data of the mean of based on the toxicity of the photosensitizing similarity index values presented in (Table 9) and depicted in (Figure 1) demonstrated that the untreated as well as treated adults with four photosensitizing compounds were varied to each other approximately, where the similarity index values ranged between 0.70 and 0.89 compounds against the

adult of the pest. It was obvious that rose Bengal recorded the lowest similarity index, which was 0.70 as compared with untreated adults, confirming its highest toxicity against the tested adults, followed by methyl violet, methylene blue, and rhodamine B. where their corresponding mean analysis of similarity index values were 0.85, 0.86, and 0.89, respectively.

Table 9: Estimated similarity index analysis between Cowpea aphid, *A. craccivora*, adults treated with four photosensitizing compounds and control, as a means of using five primers.

Treatments	Control	Methyl violet	Methylene blue	Rose Bengal	Rhodamine B
Control	-----	0.85	0.86	0.70	0.89
Methyl violet	-----	-----	0.87	0.84	0.80
Methylene blue	-----	-----	-----	0.74	0.83
Rose bengal	-----	-----	-----	-----	0.76
Rhodamine B	-----	-----	-----	-----	-----

4. Discussion

Generally, it's widely believed that photosensitizers do not cause any toxicity to people or other animals with non-translucent bodies because the toxicity of those molecules is mainly dependent on photodynamic reactions (Lucatoni *et al.*, 2011). It is interesting to mention that conventional insecticides play an important role in the overall cotton leafworm suppression program. However, the development of the cotton leaf worm resistance to various insecticides used indicated that great efforts are needed to find effective alternative control methods. Several studies indicated that photosensitizer compounds represent a possible alternative to traditional chemical compounds for pest control (Attia 2016). According to Attia (2016), light light-dependent mechanism of xanthene compounds involves the production of singlet oxygen that causes toxicological as well as biochemical effects on insects. The present study is one of the trials contributing to such studies. In this field of study, Lukšiene *et al.* (2007) indicated that the insecticidal mechanism of photosensitizing compounds, as they accumulate within the insect body, causes lethal photochemical reactions after exposure to visible light, causing destruction and finally death of cells. Photosensitizers, typically chemical compounds, absorb light energy and then transfer it to molecular oxygen, initiating the production of reactive oxygen species (ROS) within the insect's cells. These generated ROS, including singlet oxygen, induce oxidative stress, causing damage to essential cellular components such as proteins, lipids, and DNA.

These results are by those reported by Attia (2016), El-Ghobary *et al.* (2018), Khidr *et al.* (2022), and El-Shennawy *et al.* (2024). As indicated by a lot of studies, compounds functioning as photosensitizers have the

potential to serve as alternatives for traditional chemical pesticides in the future (Pieterse *et al.* 2023). Concerning the toxicity of the four photosensitizing compounds used against the pest under study, the *A. craccivora* adults were allowed to feed on different concentrations of the tested photosensitizer compounds: rose Bengal, rhodamine B, methyl violet, and methylene blue, and for different periods of sunlight exposure. According aforementioned results, the treated adults showed different susceptibility to the tested compounds. The LC₅₀ values ranged between 0.024 and 0.990 %. This agrees with the previously found findings of several authors working with different photosensitizing compounds in both laboratory and field applications (Attia, 2016). As revealed from the obtained results, rose bengal was the most promising compound. On the other hand, methylene blue exerts as extremely low toxic activity against the cotton leaf worm larvae; these results are in agreement with Attia (2016) on house fly adults, El-Ghobary *et al.* (2018) on cotton leaf worm, Khidr *et al.* 2022 on fall armyworm, and El-Shennawy *et al.* (2024) on Egyptian stem borer, *Earias insulana*. Mangan and Moreno (2001) mentioned that the efficiency of the photosensitizers used as pesticides depends on the feeding intensity, sunlight exposure, and ingestion of the target insect species. According to the chemical structure of the tested compounds, rose Bengal has the highest number of halogen atoms, including 4 iodine and 4 chlorine atoms; whereas rhodamine B, methylene blue, and methyl violet have the lowest number, including one chlorine atom. The photosensitizer compounds with the greatest number of halogen atom substituents yield greater toxicity, therefore, the halogen atoms amplify the reactions (Attia, 2016; Khidr *et al.*, 2022). As stated from the previous results, the photosensitizer's effectiveness depends on the concentration of the

tested product as well as the time of exposure to sunlight after treatments. These results are by those reported by **Attia (2016)** and **El-Ghobary et al. (2018)**. The mechanism for photodynamic activity has been described by **Vilensky and Feitelson(1999)**, **Attia (2016)**, **EL-Ghobary et al. (2018)**, and **Khidr et al. (2022)**. Upon absorption of light photons, the excited single state of photosensitizer (1Sens) reaches the excited triple state (3Sens) via intercrossing system. The excited triplet is characterized by a long lifetime, so it can play a major role in the excitation of the triplet ground state of oxygen ($3O_2$) into the excited singlet state ($O^{\cdot-}$), which is donated with a high cytotoxicity.

Reviewing aforementioned results associated to molecular biology, it could be concluded that the banding patterns are created using short oligonucleotide primers (10bp in length) of arbitrary sequence in the 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, the mutation caused by any stress (e.g. insecticide treatment or gamma radiation) changes the base sequence of primer binding sites, allowing polymorphism to be detected (**Williams et al., 1990**).

The number and size of RAPD markers depend on the complementarity of the sequence of a particular primer and template DNA, which is characteristic of an individual (**Williams et al., 1993**). In the present work, it was found that DNA of good quality is a prerequisite to have reproducible results from RAPD-PCR technique. The highest number of amplified fragments was 35, resulting from primer OP-B3, whereas the lowest number of amplified fragments was 20, produced from primer OP-A5 in *S. craccivora* adults. The present results are by those obtained by **Abdel-Baset (2009)**. The author revealed that primers OPA-13, OPA-15, and OPD-5 are strong tools to investigate changes in the pink bollworm, *Pectinophora gossypiella*, and the common house mosquito, *Culex pipiens* genomic DNA. The results are in agreement with those reported by **Salem (2018)**, the author demonstrated that the RAPD patterns resulting from amplification of DNA structure and sequence were different between the genomes of

untreated pink bollworm and those exposed to a wide spread of different insecticide applications used to control the pest under field conditions. **Attia (2020)** stated that genomic DNA extracted from 6th instar larvae of the rice moth, *Corcyra cephalonica* screened from treated and untreated groups, all groups' DNA electrophoresis results appeared as one band of 1050 bp. Three bands were quite sharp, which stands for the control group and the treated groups with cinnamon oil and silica gel. On the other hand, silica nanoparticles and encapsulated cinnamon oil-treated groups showed very faint bands.

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