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Detection of The Effect of Deltamethrin On Gene of Cucumber Plants Using RAPD-PCR

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Abstract: The current study was conducted to find out the effect of some insecticides on the genetic characteristics of the plant that parasitized it, where the study included the cultivation of cucumber plant on previously prepared land in the form of repeaters, and the number of repeaters was 10 times, and in each repeater, ten seedlings of the plant were placed, then each repeater was covered with tulle cloth Transparent through which air and water pass only. The plant was treated with a chemical pesticide (Deltathor) from the American company Ensysetx and with the doses on the cover of the box, with three treatments between each other treatment for 20 days. Samples were taken from the plant after each treatment, DNA was extracted from it, the genetic changes were known and compared with the control sample. Where RAPD-PCR and Sequence technology were used to identify these genetic changes and mutations.

The results showed that the primers of the deltathor pesticide at three concentrations used on the cucumber plant were different bands, as they showed different patterns of bands, as if the total number of bands recognized by the primers on the sample gene was 258 bands, and the total distinct bands resulting from the primers of this pesticide were 157 bands, as if 86 of them were bands. unique and 71 absent bands, and the primer 6 P and p4 were characterized by the highest number of bands produced, which amounted to 66 bands, while the two initiators P5 produced the lowest number of bands, which amounted to 16 bands

Where the results of the primers showed a clear discrepancy, as it ranged between 200-1700 bp, as if the lowest molecular size was 200 bp in the primer 2P and p4, and the highest molecular size was in each of the two primers P3, 6p, as the molecular size reached 1700 bp for each of them. The samples treated with the pesticide were 100% characterized by the highest percentage. There were 58 distinct packages, followed by samples treated with pesticide 50%, which produced 53 packages, followed by samples treated with pesticide 25%, which produced 46 distinct packages.

The results of Sequence technology showed exactly the same results as the results of RAPD-PCR. Where it indicated in the genome of the cucumber plant, it indicated that the effect of deltathor is a strong pesticide.

Keywords: detection, deltamethrin, gene, plants

Introduction

Chemical pesticides are the use of chemicals to affect the vital activities of pests, and we may use some of them to kill, others to expel, or others to prevent eggs or feeding. These chemicals may be called pesticides, and those used in insect control are called insecticides. Including humans,

therefore, should be used with caution to combat pesticides. At the present time, pesticides are the main weapon for humans against pests

The use of pesticides is one of the main factors in increasing the supply of food and clothing in the world. It was found in hypothetical studies that when chemicals are not used in agriculture, this may lead to a 30% reduction in production, which will increase prices by 70-50%. The purchase of pesticides in the United States alone is estimated at $8.7 billion/year or more (Zhu, et al. 2020).

The chemical pesticides that are relied upon are mainly toxic compounds, and they have great, dangerous and devastating damage. If there is no accuracy in choosing their types and full awareness of how to use them, this leads to the emergence of larger strains of pesticide-resistant insect pests (Sahar, 2010).

Therefore, the Plant Protection Department at the Ministry of Agriculture conducted a study on the effect of pesticides used on natural enemies when controlling insects, in order to demonstrate the importance of following the integrated management of pests on natural enemies to reach healthy production and the use of less harmful pesticides with economic feasibility in the medium and long term while preserving the environmental balance and the health of farmers and consumers (Trapp and Croteau 2001). Pesticides with a high concentration have high toxicity on the larva and the adult insect, while pesticides with a low concentration (less than 25%) have little or no specific toxicity on the larva or the adult insect (Balqt and Asiyah, 2020).

Some pesticides that are used on plants have unusual effects as a result of their effect on the physiology and biochemistry of the plant cell, and the harmful effect varies according to the type and nature of the pesticide preparation used and the type of plants being treated. The harmful effect on plants may lead to its complete destruction, which is desirable (Razeeq et al, 2019).

The effect of pesticides on the representation and composition of living plants is very complex, and their effects are not determined by the nature and type of chemical pesticide, but also by the type of preparation, the concentration used, the degree of acidity, the effectiveness of the carrier and wet materials, and even the method of treatment. On the other hand, it depends on the type of plant, the part treated with the pesticide, the age of the plant, the state of growth, the density of cultivation, as well as the prevailing weather conditions such as heat, humidity, and light when treated, as well as the nature of the soil and its content of nutrients, minerals, salts, and succession (Saeed and Awad, 2019).

Chemicals capable of causing mutations are factors that have the ability to change the properties of the hydrogen bonding of the base in the DNA chain, as the properties of hydrogen bonding is a function of the amino groups, so it is expected that any modification in these groups will increase the probability of mutations occurring, and where there are a large number of Simple and common chemicals are able to modify the DNA chain and increase the frequency of self-mutation from tenfold up to a thousandfold or more depending on the factor itself and the conditions of treatment, and although mutagenic factors affect purines or pyrimidines, they also affect all genetic genes (Fatima , 1994).

The chemical compounds have been divided regarding their ability to cause damage to the DNA (Deoxyribonucleic acid) and that many compounds possess this ability. Mutagens are carcinogenic substances Many researchers have mentioned that various chemicals and physical factors may have an inhibitory effect on the repair systems of the DNA chain (Deoxyribonucleic acid) and thus will lead to the recurrence of the malignant mutation through environmental pollution called for by the chemicals. The center of this process is that many environmental pollutants are carcinogenic and mutagenic, depending on their ability to cause DNA damage (Al-Baku` and Muhammad, 2022).

The objectives of the study is to detect the phenotypic and molecular changes of cucumber plants treated with insecticides, and to detect the effect of repeated spraying of deltathor insecticides on the

plant genome, as well as to diagnose the plant using the genetic sequencing technique of DNA (Deoxyribonucleic acid).

Methodology

1- The method of the experiment

The current study was conducted in Laylan sub-district of Kirkuk Governorate, the temperature was 30C and the soil was mixed, and it was carried out in three stages, which are: Then, cucumbers are planted in the form of 7 repetitions, divided into 12 seedlings, each seedling 40 cm apart. The second stage after growth was using chemical pesticides (Deltathor) and according to the concentrations written on the box, the pesticide was treated with three treatments and three concentrations: a lethal dose, a half-lethal dose, and a quarter-lethal dose (250ppm, 500ppm.1000pmm) and between each treatment 20 days. The first transaction was on 7/9/2022.

2- collecting leaf samples of cucumber

Special and pure tubes that do not contain a chemical substance were used for the purpose of collecting samples of plant leaves after each chemical pesticide treatment and keeping them in 70% ethanol alcohol until DNA extraction is performed. Control samples were collected from the growing leaves of cucumber plant from an agricultural plant that was not treated with chemical pesticides for the purpose of comparison.

3- Molecular study

a- DNA extraction

Take a complete sample of the leaves of the cucumber plant, wash it and sterilize it using 70% ethanol. This sample is placed in a ceramic mortar and liquid nitrogen is added to it. The sample is crushed well until it turns into powder, then the powder is transferred, then placed in sterile glass tubes, and added Directly 3 ml of the CTAB extraction solution prepared in the first paragraph, which was taken directly from a water bath at 65 °C. We start by stirring the tubes so that the mixture becomes homogeneous, then they are incubated in a water bath at a temperature of 65 °C for 60-90 minutes, with continuous stirring every 15 minutes.

The tubes are then removed from the water bath and left for 15 minutes to reach room temperature. Add to it 1 ml of the previously prepared SDS solution kept in the water bath at 65 °C, and start stirring the tubes for 1 minute until the mixture is homogeneous.

The tubes are removed from the centrifuge, and we notice that two layers have formed. The upper layer is withdrawn with a fine pipette and transferred to new, sterile tubes. The same volume of chloroform solution: isoamyl alcohol 24:1 is added to it. Then the tubes are stirred quietly for 15 minutes and placed in a centrifuge and centrifuged at a speed of 10,000. revolution/minute for 10 minutes (Al-Sakmani, 2010). The same amount of phenol-chloroform-isoamyl solution prepared and preserved in a dark tube is added, and the tubes are placed after stirring in a centrifuge and centrifuged at a speed of 10,000 revolutions/minute for 10 minutes.

The tubes are lifted, the upper layer is removed, and transferred to new, sterile tubes. The same volume of cooled isopropanol solution is added to it, while the tubes are stirred gently so that the alcohol mixes with the sample well. During this time, white threads will be formed that can be observed, which are DNA threads. The tubes are transferred to the centrifuge again under the same conditions above. After the completion of the centrifugation, the tubes

are lifted and we notice that the DNA strands have been deposited at the bottom of the tube in the form of a white mass

After that, we pour the solution quietly, then add the washing solution to the tubes, transfer them to the centrifuge, and also centrifuge at a speed of 10,000 revolutions for 10 minutes. After completion, the tubes are removed from the device, then we pour the solution also quietly, and the tubes are left for a period until they dry completely, then we add 100-200 TE buffer and leave the threads until they dissolve, then the samples are kept in a stock sample at a temperature of 20 °C until used.

The acronym	
D3M3	Deltathor pesticide, 100% concentration, 3rd treatment
D3M2	Deltathor pesticide, 100% concentration, 2nd treatment
D3M1	Deltathor pesticide, 100% concentration, 1st treatment
D2M3	Deltathor pesticide, 50% concentration, 3rd treatment
D2M2	Deltathor pesticide, 50% concentration, 2nd treatment
D2M1	Deltathor pesticide, 50% concentration, 1st treatment
D1M3	Deltathor pesticide, 25% concentration, 3rd treatment
D1M2	Deltathor pesticide, 25% concentration, 2nd treatment
D1M1	Deltathor pesticide, 25% concentration, 1st treatment
C	Control

b- Determination of concentration and purity for DNA

The concentration and purity of the DNA was measured using a Nano drop device. If I take one drop of the previously extracted and preserved genomic DNA and put it in the place designated for the sample, then a command is given to the device to measure after the device is calibrated on the same dissolving solution, then the device will give a measurement of concentration in ng/ml and purity accurately, then the sample is diluted until The concentration becomes 50 ng/ml and is kept by freezing until use.

c- **Electrophorsis of Genomic DNA**

The acarose gel is prepared at a concentration of 1%, which is used for genomic DNA migration, by dissolving 1 g of acarose powder in 100 ml of 1X SP using a microwave heat source, then the solution is left to cool to 55 °C, then the gel is poured into the transmigration tray after installing the special comb By forming wells and pouring quietly to avoid the formation of bubbles, then the gel is left to solidify, the comb is lifted after making sure that the gel has solidified, then the tray is transferred to the migration basin that contains an appropriate amount of Sp 1X solution.The

migration process takes place by mixing 5 microliters of DNA sample with 3 microliters of loading buffer using a micropipette, then the sample is placed in the designated hole. The electrical relay device is operated by passing an electric current with a voltage difference of 3 volts / cm from the size of the migration basin. And after adjusting the electrodes, the direction of the samples must be towards the positive electrode to the extent that the samples reach before the end, and the migration process takes 1-2 hours

d- **RAPD- PCR**

In this type of reaction, migration is carried out using an acarose gel at a concentration of 5.1%, which is prepared by dissolving 5.1 g of acarose powder in 35 ml of a 1X SP solution using a Microwave heat source, then the solution is left to cool slightly and poured in the same way as above. Upon migration, 5 microliters of the product of the PCR-RAPD reactions are taken, and each sample is accurately loaded into the gel pits. The volumetric marker, section bp300-100, is placed in a dedicated hole on one side of the gel, then the relay is turned on by passing an electric current with a voltage difference of 3 volts / cm. After completing the adjustment of the electrodes, the direction of the migration of the samples should be towards the positive electrode to the extent that the samples reach before the end of the gel. This process takes 2-5.1 hours. RAPD reactions were carried out according to Williams et al. (1990) on cucumber plant samples, starting with 10, and they are as follows.

Table 1: Primers of RAPD PCR

no	Primer	Sequence 5´→→→ 3´	No	Primer	Sequence 5´→→→ 3´
1.	OP A-01	CAGGCCCTTC	6	OP C-08	**TGGACCGGTG**
2.	OP A-06	GGTCCCTGAC	7	OP H-16	**TCTCAGCTGG**
3.	OP B-04	GGACTGGAGT	8	OP J-04	**CGGAACACGG**
4.	OP B-12	CCTTGACGCA	9	OP B-20	**GGACCCTTAC**
5.	**OPB-14**	**TCCGCTCTGG**	**10**	**OP C-16**	**CACACTCCAG**

After the completion of the reaction, the tubes are removed from the thermopolymer device and the samples are preserved by freezing. Upon deportation, 5 microliters are withdrawn from the tubes and loaded onto the agarose gel prepared in advance at a concentration of 5.1% with the volume index as mentioned in paragraph 3-1-5 second-2, then the samples are transferred to the gel and after At the end of the migration time, the gel is removed from the relay and placed in a UV transilluminator, and the gel is photographed.

Table 2: Steps of PCR

Steps	Temp (°C)	Time	Cycle no.
Initial Denaturation	94	**5 min**	**1**
Denaturation -2	93	**45 sec.**	**40**
Annealing	36	**1 min.**	
Extension-1	72	**1.5 min**	

Extension -2	72	**7 min**	**1**

e- Primers preparation

Primers were dissolved in free ddHO to a final concentration of 100 µL/µL as a stock solution, and the stock was kept at -20. To prepare a concentration of 10 µL/µL as a suspension primer, 10 µL of the stock solution was added to 90 µL of free water ddH2O to reach a final volume of 100 µL.

Table 3: primer for matk gene for plant

Primer	**Seq.**	**Tm (°C)**	**GC (%)**	**Size**
Forward	5′- ACTGTATCGCACTATGTATCA -3′	50.6	%38	**650-400 bp**
Reverse	5′ - GCATCTTTTACCCARTAGCGAAG-3′	55	%45	

f- The mechanism of action of the polymerase chain reaction

The components were mixed well and centrifuged by a Microfuge device for 17 seconds to ensure mixing of the reaction components, taking into account wearing gloves. The tubes were placed in the thermopolymer device and the initiator applied its own program as follows:

Initial Denaturation	**95°C**	**5 min.**	**1 cycle**
Denaturation -2	95°C	45 sec	35 cycle
Annealing	85°C	45 sec	
Extension-1	72°C	45sec	
Extension -2	72°C	7 min.	1 cycle

Results and discussion

Molecular study results using RAPD indicators:

Genomic DNA extraction results

The DNA was isolated by the modified method. It was deduced from the method mentioned by (Al-Sakmani, 2017) on the authority of (2013 Haung) that the method used in the purification described by Al-Sakmani (2017) is the same steps as the extraction method except for the step of adding liquid nitrogen and grinding. This method helped to obtain The DNA is of perfect purity, as shown in picture (1), and it is suitable for PCR reactions, and it takes place in a standard time that does not exceed one and a half hours. - 1.8) The purity of the DNA was measured using the Nano drop device, and the dilution was set at 50 nanograms per microliter.

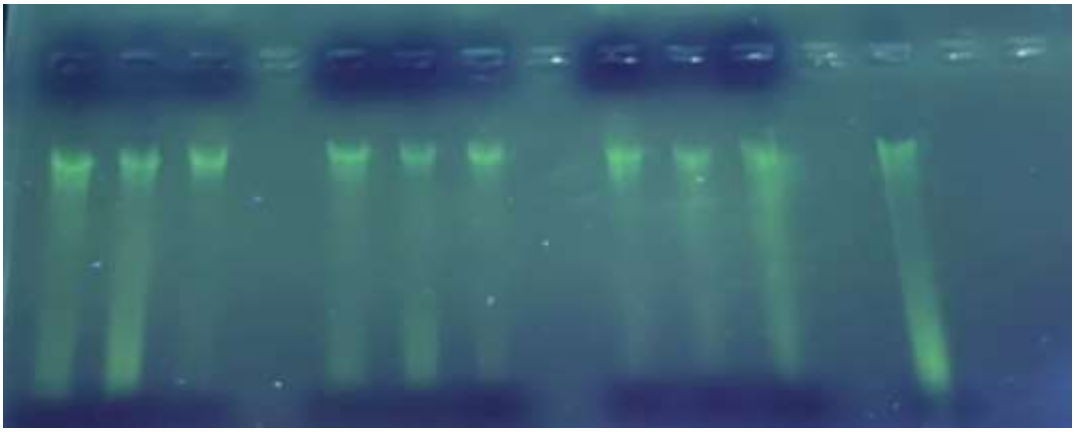

Figure 1: electrophoresis of matk gene

Table 4: Mutations produced in cucumber plants treated with Deltathor

Results of RAPD-PCR indicators on cucumber plants:

The primers of the pesticide at three concentrations used on the cucumber plant produced different bands distributed between the monomorphic and polymorphic bands, which were detected on the agarose gel in the presence of the DNA marker (DNA ladder100bp). The primers on the genome of the samples were 244 sites, of which 171 were divergent sites. The primer 6P was characterized by the highest number of bands produced, reaching 47 bands, while the two primers P3 produced the lowest number of bands, which amounted to 16 bands. The results of the primers showed a clear variation, as it ranged between 400-1700 bp, as if the lowest molecular size was 400 bp in the primer 2P, and the highest molecular size was in each of the primers 4P and 6p, as the molecular size was 1700 bp for each.

The results of the study showed the presence of distinct bands (absent bands), (unique bands), as shown in Table (4), as if the total distinct bands resulting from prefixes were 143 bands, as if 80 were unique and 63 were absent. The samples treated with the pesticide 25% were characterized by the highest percentage of distinct bundles amounting to 58 bundles, followed by the samples treated with the pesticide 100%, which produced 42 bundles, followed by the samples treated with the pesticide 50%, which produced 41 distinct bundles, as if the total distinguished bundles from the concentration of 25% for the studied treatments is 58 A bundle of which 23 are unique and 35 are absent, as the total bundles for the first treatment of this concentration were 24 distinct bundles, while the third treatment had 19 distinct bundles and the second was 15 distinct bundles

As if the total distinct bundles of 100% concentration for the three treatments of the pesticide are 42 bundles, of which 16 are unique and 26 are absent, as the total bundles for the second treatment of this concentration reached 22 distinct bundles, while the first treatment was 14 distinct bundles and the third was 6 distinct bundles, as if the total distinct bundles were from a concentration of 50% for the treatments The studied is 41 bundles, of which 21 are unique and 22 are absent, where the total bundles for the first and second treatment of this focus were equal to 14 distinct bundles, while the third treatment had 13 distinct bundles

While the primers of deltathor at three concentrations used on cucumber plants produced different bands distributed between the general monomorphic bands and the divergent polymorphic ones, they were detected on the agarose gel in the presence of the DNA marker (DNA ladder100bp), as the results of the primers showed different patterns of bands, as if the total number of bands identified It has primers on the sample genome is 258 bands, of which 157 are distinct bands. The primer 6 P and

p4 were distinguished by the highest number of bands produced, reaching 66 bands, while the two primers P5 produced the lowest number of bands, amounting to 16 bands. The results of the primers showed a clear discrepancy, as it ranged between 1700-200 bp. As if the lowest molecular size is 200 bp in primer 2p and p4 and the highest molecular size is in both primers P3 and 6p, as the molecular size was 1700 bp for each.

The results of the study showed the presence of distinct bands (absent bands), (unique bands), as shown in the table, as if the total distinct bands resulting from the primers of this pesticide were 157 bands, as if 86 were unique and 71 were absent, and they were distinguished Samples treated with pesticide 100% with the highest percentage of distinct bands amounted to 58 bands, followed by samples treated with pesticide 50%, which produced 53 bands, followed by samples treated with pesticide 25%, which produced 46 distinct bands. As if the total distinct bundles produced from a concentration of 100% for the three treatments of the pesticide are 58 bundles, of which 31 are unique and 27 are absent, as the total distinct bundles for the first treatment of this concentration reached 27 distinct bundles, while the third treatment had 16 distinct bundles and the second was 15 distinct bundles.

While the total distinct bundles produced from the concentration of 50% for the studied treatments was 53 bundles, of which 32 were unique and 21 were absent, as the total bundles for the second and third treatment equal to this concentration were 20 distinct bundles, while the first treatment was 13 distinct bundles, while the total distinguished bundles produced from the concentration was 25% For the studied treatments, there are 46 bands, of which 23 are unique and 23 are absent. The total number of distinct bands for the second and third treatment equal to this focus is 17 distinct bands, while the first treatment had 12 distinct bands.

The results of the study showed that there are more distinct bands in the deltathor herbicide than the herbicide perishable, and therefore this indicates that the treatments of the herbicide deltathor are the ones with a greater effect than the herbicide, as these bands are used as a diagnostic and discriminatory characteristic for those species. The appearance of these bands in one type indicates the occurrence of a mutation in a specific site It led to the recognition of the initiator of this site and the emergence of the unique band, as well as the absent bands, as a mutation occurs in the site that recognizes the initiator only in one species without the other species, which leads to the cancellation of that recognition and the band disappears, and this is consistent with the results of most researchers (Glaucia et al., 2016; Bajpai , 2019; Brito, 2008).

As the toxicity of pesticides on plants, despite their effectiveness in the short term, their intensive use leads, in the long term, to plant damage and a clear decline in the productivity of the land, because pesticides and chemical fertilizers kill living organisms and biomolecules that are beneficial to the soil and necessary for its fertilization, and thus lead to (chemicals).) to a significant decline in soil quality (Darwish, et.al. 2022).

An example of the role of the farmer in destroying the soil is his neglect of the plowing process immediately after the end of the agricultural season, with the aim of getting rid of weeds before they produce seeds. the earth. Year after year, the farmer loses the ability to control these weeds and uses chemical herbicides, most of which are considered carcinogens, some of which cause damage to the central nervous system and genetic and reproductive abnormalities in humans, while others lead to hormonal imbalance in the body (Sharma, et.al. 2022).

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