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DETECTION OF THE EFFECT OF HuLK ON THE 28S GENE OF *BEMISIA TABACI* USING DNA SEQUENCING

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ABSTRACT

This study aims to identify the effect of some types of insecticides on the genetic characteristics of the whitefly, while determining the resulting mortality rate. The experimental environment was then secured by covering the filters with a layer of air- and water-permeable tulle, and then spraying the whitefly and plants with the American insecticide "Hulk" produced by Ancestex at the doses indicated on the cover. Three treatments were carried out with a time interval of 20 days between each treatment. After each treatment, fly samples were collected to extract DNA from them, and to study genetic changes by comparing them with the control sample using polymerase chain reaction (PCR) and genome sequencing techniques to detect genetic mutations.The results of the current research showed the emergence of several deletion and substitution mutations in the 28S gene in strains resistant to whitefly pesticides. Where the results showed DNA-Sequencing through the samples that were studied, which indicated that the herbicide has a mutagenic and not a lethal effect, as the pesticide with its three concentrations and its frequency showed negative effects and the occurrence of genetic mutations on the genome of the whitefly, that is, the greater the concentration of the pesticide and the frequency of its spraying on If the mutagenic effect of this pesticide decreases, that is, the percentage of concentration is inversely proportional to the distinct mutant bands, as the percentage of variation in the samples whose genetic sequence was read decreases as the concentration and frequency of the pesticide increases, and thus these treatments produced strains of whitefly that had high resistance to this pesticide.

Keywords; Bemisa tabaci , Hulk, Deltathor.

Introduction

The white fly, Bemisia tabaci, is one of the most dangerous pests on most agricultural crops, including the tomato crop. This insect belongs to the family of white flies (Aleyrodidiae), belonging to the order Hemiptera, according to the latest taxonomic update, as it was diagnosed and classified for the first time in the world. Gennadius in 1889 when he studied it as a dangerous pest on the tobacco plant and therefore gave it the name of the tobacco white fly, Bemisia tabaci.

In 1897, that is, after eight years, its presence was recorded for the first time in the United States of America as a dangerous pest on the sweet potato plant by the scientist Quaintance and it was called Alerodes inconspicua, and in 1928 its presence was recorded in Brazil by the scientist Bondor on the Euphorbia hirtella plant and it was given the name Bemisia costalmai and also recorded in 1933 in Taiwan

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and called Bemisia hisci (Mound and Halsey, 1978). It is also a serious pest of agricultural crops in Africa, Asia, Central America, South America and the West Indies, where it is also known as the cotton white-Fly. This insect is considered a serious pest on many agricultural crops of economic importance such as tomatoes, cucumbers, eggplants and ornamental plants in greenhouses (Cock, 1986; Heinz and Parrella, 1994).

Chemical pesticides are an effective means of pest control as they rely on chemical compounds capable of disrupting or *inhibiting* the biological processes of target organisms. These compounds are known as insecticides when used against insects. However, these toxic compounds pose an environmental and health risk if not used scientifically (Zuh, et al. 2020).

Most chemical pesticides are toxic compounds that may cause harmful effects on humans and target organisms. Misuse leads to the development of resistance in pests, making control more difficult (Sahar, 2010).

This study aims to analyze molecular changes in the insect Bemisia tabaci after exposure to the pesticide Hulk and to identify the 28s rDNA gene using DNA sequencing technology.

Methodology

1- The method of the experiment

The experiments were conducted in the Laylan district of Kirkuk at a temperature of 30°C, in three stages. These included infecting the plants with whiteflies, followed by treating them with Hulk at concentrations of 250, 500, and 1000 ppm, with 20-day intervals between each treatment. The first treatment was conducted on September 7, 2022.

2- Sample Collection

Whitefly samples were collected using sterile tubes free of chemical contaminants and stored in a 70% ethanol solution for subsequent DNA extraction.

3- Molecular Study

a- DNA Extraction: DNA extraction was performed according to the company's instructions.

b- Electrophoresis: The samples were then separated using electrophoresis in a 1% agarose gel for approximately two hours.

c- Primers: A set of specific primers was used to study genetic changes (Table.1)

Table 1: primer for 28S gene

Primer	Seq.	Tm	GC (%)	size
Forward	5'- GAG AGT TMA ASA GTACGT GAA AC- 3'	52.3	44	**600-860 bp**
Reverse	5'- TCG GAR GGAACC AGC TAC TA - 3’	56	42	

a- The mechanism of action of the polymerase chain reaction: The tubes were placed in the thermopolymer device and the initiator applied its own program as follows (Table 2):

Table 2: Steps of PCR

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

b- Sequencing of 28S gene

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DNA sequencing was performed using a 0.7 µl PCR product using a specific set of primers. Samples were sent to Macrogen Biotech in South Korea for sequencing. After receiving the results, they were analyzed using the BLAST tool available on the NCBI website to determine the gene sequence and match it to internationally registered reference sequences.

Results and discussion

Results of the 28S DNA polymerase chain test in whiteflies:

Gel electrophoresis revealed bundles from 28S DNA extracted from whiteflies (Fig. 1).

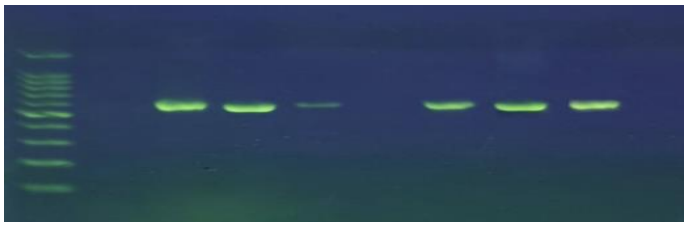


Fig. 1: Electrophoresis of 28S gene

The sequencing technique, after being first The Sanger method for DNA sequencing, developed in 1977 by Frederick Sanger and his team, has since become the most widely used method for gene sequencing for nearly four decades. First commercialized by Applied Biosystems in 1986, it has become the gold standard in sequencing. Although it has been superseded by next-generation technologies in large genomic projects, the Sanger method is still used in small-scale studies and for validating deep sequencing results, due to its long reads exceeding 500 nucleotides and high accuracy of nearly 99.99%. It is currently used in public health applications and epidemic surveillance (Crossley et al., 2020).

According to the current study's findings, whitefly pesticide-resistant strains have developed a number of deletion and substitution mutations in the 28S gene. Whereas the pesticide's three concentrations and frequency revealed negative effects and the occurrence of genetic mutations on the whitefly's genome, the results of DNA-Sequencing through the samples that were studied indicated that the herbicide has a mutagenic and not a lethal effect. that is, the higher the pesticide's concentration and the more frequently it is sprayed on The amount of variation in the samples whose genetic sequence was read decreased as the concentration and frequency of the pesticide increased, and if the mutagenic effect of this pesticide decreases, that is, the percentage of concentration is inversely proportional to the distinct mutant bands, then these treatments produced strains of whiteflies that had high resistance to this pesticide. Two types of DNA substitution mutations exist. Transitions involve bases of similar structure because they are interchanges of two-ring purines (A G) or one-ring pyrimidines (C T). Transversions entail the exchange of one-ring and two-ring structures because they involve the substitution of pyrimidine bases for purine bases (Gupta, et al, 2020).

When a purine base (consisting of two rings, such as A or G) is replaced by a pyrimidine base (consisting of one ring, such as T or C), or vice versa, this is known as a genetic mutation (Ali et al., 2021). These mutations can be triggered by ionizing radiation or alkylating agents, or they can occur spontaneously and can only be corrected by spontaneous reversal.In genetics and molecular biology, the term "transition" refers to a point mutation that converts one nucleotide from one purine to another (A to G) or one nucleotide from one pyrimidine to another (C to T). Transitions make up about two out of every three single nucleotide polymorphisms (SNPs) (Aziz, et al, 2022).

Sequencing results of the 28S gene of the whitefly

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Ribosome inactivation proteins (RIPs) are enzymes that possess RNA N-glycosidase activity. They remove a base at the conserved α-sarcin/ricin site of the 28S rRNA molecule, leading to a permanent halt in intracellular protein synthesis. RIP genes were first discovered in the genomes of some mosquito and whitefly species, and analyses indicate that they originated from a single horizontal gene transfer from a donor bacterium. RIP gene expression levels have also been shown to change across different developmental stages. Recent findings also indicate the presence of these genes in the genomes of whiteflies of the family Aleyrodidae (Lapadula et al., 2020).

The genomic sequences of 28S were analyzed in five samples. In this study, a single genetic locus, consisting of 600 nitrogenous bases, was amplified. Sequence analysis was performed by Mega 6 software and showed changes in the nucleotide sequence of the 28S gene compared to other globally known genes. These mutations included Transition and Transversion.

The results of the analysis of 1st sample showed that this sample obtained 17 changes, between 3 exchange changes and 14 transmission changes, compared to the ncbi ID: MH757981.1 sample. And these changes were in several locations, at site 129, thymine was transferred to the cytosine site, and at site 131, adenine was transferred to the guanine site, and at site 132 adenine was exchanged at the cytosine site, and at site 133 thymine was transferred to the cytosine site, and at site 137 the guanines were transferred adenine site, At position 138, thymine was transferred to the cytosine site, at position 142, guanine was transferred to the adenine site, at position 147, adenine was transferred to the guanine site, at position 154, thymine was transferred to the cytosine site, at position 159, thymine was transferred to the cytosine site, and at position 165, thymine was transferred to the site of cytosine. Cytosine is transferred to the thymine site, and at site 166 thymine is transferred to the site of cytosine, and at site 169 thymine is transferred to the site of cytosine, and at site 198 adenine is exchanged at the site of cytosine, and at site 199 adenine is transferred to the site of guanines, and at site 201 thymine is transferred to the site of guanine, and at Site 202 Thymine has been transported to the cytosine site.

The results of the analysis of 2nd sample showed that this sample obtained 17 changes, between 3 exchange changes and 14 transmission changes, compared to the ncbi ID: MH757981.1 sample. And these changes were in several locations, at site 129, thymine was transferred to the cytosine site, and at site 131, adenine was transferred to the guanine site, and at site 132 adenine was exchanged at the cytosine site, and at site 133 thymine was transferred to the cytosine site, and at site 137 the guanines were transferred At site 138, thymine moved to the site of cytosine, and at site 142, guanine moved to the site of adenine, and at site 147, adenine moved to the guanine site. At position 154, thymine was transferred to the cytosine site, at site 159, thymine was transferred to the cytosine site, at site 165, cytosine was transferred to the thymine site, at site 166, thymine was transferred to the cytosine site, at site 169, thymine was transferred to the cytosine site, and at site 198, exchange took place. Adenine moved to the cytosine site, and at site 199 adenine moved to the guanine site, and at site 201 thymine moved to the guanine site, and at site 202 thymine moved to the cytosine site.

At 3rd sample, The results of the analysis showed that this sample obtained 12 changes, between 4 exchange changes and 8 transmission changes compared to the ncbi ID: MH757981.1 sample. And these changes were in several locations, at site 115, adenine was exchanged at the site of thymine, and at site 128, thymine was transferred to the site of cytosine, and at site 129, thymine was transferred to the site of cytosine, and at site 131, adenine was transferred to the site of the guanines, and at site 132, the guanines were exchanged at the cytosine site, and at site 134 the guanines were transferred to the adenine site, and at site 137 the guanines were transferred to the adenine site, At site 142, thymine was exchanged at the adenine site, at site 147, adenine was transferred to the guanine site, at site 148, thymine was exchanged at

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the guanine site, at site 154, thymine was transferred to the cytosine site, and at site 159, thymine was transferred to the cytosine site.

4th sample, The results of the analysis showed that this sample obtained 18 changes, between 10 exchange changes, 6 transmission changes, and 2 gap changes compared to the ncbi ID: MH757981.1 sample. And these changes were in several locations, at site 109 adenine was transferred to the guanine site, and at site 117 cytosine was exchanged at the guanine site, and at site 129 thymine was exchanged at the cytosine site, and at site 131 adenine was transferred to the guanine site, and at site 132 adenines were exchanged at the cytosine site, and at site 133 thymine was transferred to the cytosine site, and at site 135 the presence of the guanine gap, and at site 138 the guanine exchange at the cytosine site, and at site 143-144 the presence of a thymine gap, At site 154, thymine was transferred to the cytosine site, at site 158, thymine was exchanged at the guanine site, at site 159, adenine was exchanged at the cytosine site, at site 164, thymine was exchanged at the guanine site, at site 167, thymine was exchanged at the cytosine site, and at site 169, exchange took place. Adenine is at the site of cytosine, and at site 170, cytosine is exchanged at the site of adenine, and at site 174, cytosine is exchanged at the site of guanine, and at site 191, thymine is exchanged at the site of guanine.

5th sample, The results of the analysis for this sample showed that 22 changes were obtained between 6 exchange changes and 16 transmission changes in sample ncbi ID: MH757981.1. And these changes were in several locations, at site 129, thymine was transferred to the cytosine site, and at site 131, adenine was transferred to the guanine site, and at site 132 adenine was exchanged at the cytosine site, and at site 133 thymine was transferred to the cytosine site, and at site 137 the guanines were transferred At site 138, thymine was transferred to the site of cytosine, and at site 142, guanine was transferred to the site of adenine, and at site 147, guanine was transferred to the site of adenine, and at site 415, thymine was transferred to the site of cytosine, At site 159, thymine was transferred to the cytosine site, at site 160, thymine was exchanged at the guanine site, at site 165, cytosine was transferred to the thymine site, at site 166, thymine was transferred to the cytosine site, at site 169, thymine was transferred to the cytosine site, and at site 181, exchange took place. Adenine at the cytosine site, at site 198 adenine exchanged at the cytosine site, at site 199 adenine transferred at the guanine site, at site 201 thymine moved to the guanine site, at site 202 thymine transferred to the cytosine site, and at site 208 thymine exchanged at the guanine site guanine transferred to the adenine site , and at site 209 the two laws were exchanged at the thymine site, and at site 210 the two laws were transferred at the adenine site.

Where the authors (Rajamohan and colleagues, 2001) report that ribosomal inactivating proteins (RIPs, Ribosome inactivating proteins) are RNA N-glycosidases that remove ribosomes in the conserved alpha-sarcin/ricin loop of 28S rRNA, leading to an irreversible halt of its synthesis. protein. The researchers (2017, Lapadula, Ayub) also indicated that coding gene segments are widespread in plants but rare within bacterial and fungal strains. Previous studies showed the presence of RIP genes in the genomes of mosquitoes belonging to the Culicinae subfamily. Tis was the first description of the existence of RIPs in metazoans.

Our research also indicated that many mutations occurred in these genes, including translocation and reversal in several locations of the gene. These mutations led to a variation in the nitrogenous bases, which is the result of the use of pesticides. Furthermore, researcher (Lapadula et al., 2020) Recent studies have revealed that RIP genes in the mosquito Aedes aegypti are transcribed, and that their expression levels change according to different developmental stages. Other research, including a study by (Ballinger & Perlman 2019), has shown that these genes play a functional role in the defense systems that symbionts develop against arthropod infestations by their natural enemies. In Hemiptera (whiteflies – Aleyrodidae),

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RIP genes have been found to be evolutionarily distinct from those in mosquitoes, being evolutionarily related to plant-derived RIP genes.

Altogether, the results for the 28S gene encoding RIP genes are attributed to different insect strains. Our results indicate that a portion of the 28S genes may fulfill an important functional niche in insects leading to recurrent selection either in terms of horizontal transfer of genetic material or by symbiotic interaction.

Breeds Registration

The obtained strains were registered in the National Information Bank (NCBI), and a serial number was obtained with the registered code QQ815951.1.

conclusion

The study showed that the use of insecticides such as Hulk and Deltathor to control the whitefly on cucumber plants was associated with clear mutagenic effects at the molecular level. DNA analyses and RAPD techniques showed a high compatibility with the biokill ratios. We found that increasing the concentrations of the active ingredients in these insecticides did not lead to an improvement in their control effectiveness, but rather contributed to enhancing the undesired mutagenic effects.

Conflict of Interest

The study recommends the need to limit the excessive or repeated use of these insecticides. It is also important to instruct competent authorities, such as the Ministry of Health and agricultural authorities, to limit their recommendations and discourage farmers from repeating their use. Agricultural authorities should also be directed to search for safer and more effective alternatives. Advanced molecular markers such as SSR, SNR, and PCR-RFLP can be relied upon to evaluate insect resistance, while expanding the application of molecular studies to include other insect pests and enhancing genetic control strategies as a sustainable option for agricultural pest management

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