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The Effect of The Insecticide Hulk on Some Genetic Characteristics of The Whitefly and The Cucumber Plant That Parasitizes It

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ABSTRACT

Background: Whitefly, Bemisia tabaci is one of the major injurious sucking pests in tropical and subtropical regions. The effect of the insecticide Hulk, which is used to kill the whitefly, was revealed, and its effect on some of its genes and the genes of the cucumber plant that parasitizes it, was revealed.

Materials and Methods: The current study was conducted in Kirkuk Governorate. DNA was extracted from whiteflies treated with Hulk pesticides and not treated with pesticides as a control, and DNA was also extracted from cucumber leaves treated with the Hulk pesticide and not treated with the pesticide as a control in order to compare them. detecting the specific primer of gene *28s* in the whitefly *Bemisia tabaci* and *Matk* gene in the plant *Cucumis sativus*. Three treatments of each pesticide at three concentrations (25% a quarter lethal, 50% half lethal, 100% lethal).

Results: The research found that 25% of the pesticide Halk killed 20% of the insects, 50% killed 40%, and 100% killed 70% within 24 hours. Pesticide Hulk found that cucumber plant mutations in the *Matk* gene and gene *28s* had the best insecticidal effects against whitefly Bemisa tabaci.

Conclusions: Overall, results suggest that ethanolic DNA extracts pesticide Hulk is the occurrence of mutations in the Matk gene in the plant *Cucumis sativus* and gene *28s* in the whitefly *Bemisia tabaci* showed the highest insecticidal effects on whitefly *Bemisa tabaci*.

Keywords- *Cucumis sativus*, Whitefly, Matk gene, gene *28s*, pesticide Hulk.

I. INTRODUCTION

The whitefly *Bemisa tabaci* belongs to the family of whiteflies (Aleyrodidae) within the order Hemiptera (Liu et al., 2022). It is considered one of the most important pests that cause economic losses to vegetable and ornamental crops globally in tropical and subtropical regions that provide suitable environments for its growth and reproduction, where it parasitizes the whitefly feeds on most crops of the Solanaceae family, such as hot peppers, tomatoes, and potatoes, and it also feeds on some crops of the cucurbit family, such as cucumbers (Novaes et al., 2020). Excessive and repeated exposure of the whitefly to chemical pesticides leads to mutations and changes in the genetic material, leading to the emergence of resistant strains of the fly with high resistance to these pesticides. These mutations are linked to a specific gene that leads to the removal of toxicity resulting from exposure to pesticides (Pym et al., 2023). Plants exposed to these pesticides suffer from phenotypic changes or deformities, such as burns, wilting of leaves, and tissue damage. Pesticide components may accumulate in plant tissues, and this may affect food safety and expose people to health risks when consuming them, as well as physiological changes, represented by a decrease in the rate of photosynthesis and increased stress conditions. Which together lead to a decrease in yield (Shahid et al., 2021). One of the most important molecular techniques used in studying insects and plants is the polymerase chain reaction (PCR), which allows the specific DNA sequence of the gene in question to be amplified, by amplifying the target genes. It is a DNA sequence reading

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technique - sequencing, which includes studying the activity of genes in tissues. Different types or in selected conditions, as this technology made it possible to determine genetic activity and its role in the problems studied by determining the extent of gene expression in insects and plants (Zhu et al., 2020).

II. MATERIALS AND METHODS

2.1 Experiment design

The current study was conducted in Kirkuk Governorate at a temperature of 30°C, and the soil was of a mixed type. The land was prepared and plowed well, then the cucumbers were planted in cork boxes, transported to the land, and planted in 7 divided replicates. Each replicate contained 12 seedlings, with a distance of 40 cm between each seedling. Then the plant was infected with the white fly and left for several days until the infestation was complete. Each plant was individually wrapped in transparent tulle fabric to prevent the white fly from escaping from it. This cover only allows air and the chemical pesticide to enter. After growth and infection, Halk pesticide was used according to proven instructions. Three treatments of each pesticide at three concentrations (25% a quarter lethal, 50% half lethal, 100% lethal). The pesticide was diluted with distilled water and for every 1 liter of water, 1 ml of chemical pesticide was added. The period was 25 days between each transaction.

2.2 Methods of collecting whitefly samples and cucumber leaves

Special, sterile tubes with a size of 50 ml were used to place the fly. Then control samples, were collected from an agricultural replicate not treated with the pesticide for comparison. The remaining fly was collected after each treatment with the pesticide and was preserved in 70% ethanol for the time of DNA extraction from it. Growing leaves of cucumber plants were taken from an agricultural replicate that was not treated with the pesticide as control samples to make a comparison, and samples were collected from the growing leaves of the plant under study after each treatment with the pesticide.

2.3 How to extract DNA from whiteflies and cucumber leaves:

DNA extraction is done through the following steps, according to Chamoun and Oliveira-Costa (2019).

After collecting the remaining whiteflies and the growing tips of the plant leaves, they were ground with liquid nitrogen using a ceramic mortar to obtain a fine powder. 0.5 grams of it was placed in a 2-ml Ependorf tube and 700 microliters of Lysis buffer were added to it. Then we placed the samples in a water bath for an hour. Stirring it every 10 minutes, after removing the samples from the water bath, they were left at room temperature to cool for 5 minutes, adding 200 microliters of protease, the sample was mixed well and kept in the refrigerator for 5 minutes, then the samples were placed in the centrifuge and centrifuged at 14,000 rpm. d for 5 minutes, then keep the tube with the filter, add 600 microliters of the sample into a new 2 ml Ependorf tube, add 750 microliters of Binding buffer (DNA binding solution). Leave the samples for 5 minutes at room temperature, mixing the sample well. Then transfer 600 microliters of the sample to a colloidal tube, pouring the solution at an angle of 545 degrees to the wall of the filter tube to avoid puncturing the filter. The samples were centrifuged at 10,000 revolutions for only one minute in the centrifuge with the sediment removed. Then we added 500 microliters of washing buffer to the sample and centrifuged the sample at 10,000 revolutions/min for a minute. The sediment was removed and 500 microliters of Wishing buffer. The sample was centrifuged in a centrifuge at 10,000 rpm for one minute. Then we dried the sample in a centrifuge at 13,000 rpm for 3 minutes. Then the sediment filter was removed and placed in a 2 ml Ependorf bottle, and 150 microliters of DNA Elushtion were added. Leave The samples are placed vertically for 5 minutes, after which they are centrifuged in a 10,000 rpm centrifuge for 2 minutes, after which a DNA sample is obtained. The sample is then left at room temperature for 24 hours, then stored in the refrigerator until use and completion of the experiment.

2.4 Electrophoresis of DNA on (Agarose gel) from Incect and plant

Electrophoresis is used to identify DNA fragments following DNA extraction or to detect the PCR reaction when standard DNA is present to discriminate band size from the Agarose gel result.

2.5 Preparation of agarose gel

According to Sambrook et al. (1989) produced the agarose gel by dissolving 1.5 g agarose in 100 ml TBE buffer solution and condensing it at 1.5%. We heated the agarose to boiling and let it cool to 45-50°C. After installing the comb to produce sample holes, we poured the gel onto the dish with the agarose support plate. Pour the gel carefully to avoid air bubbles and let it cool for 30 minutes. The comb is gently removed from solid agarose. Electrophoresis tanks are filled with TBE insulating material to cover the gel once the plate is put on the platform.

2.6 Preparation samples:

According to Maniatis et al. (2004), mix 3 µl of processor loading buffer (Intron/Korea) with 5 µl of DNA material for electrophoresis. Transfer the mixed mixture to gel holes. For 1-2 hours, a 7-volt per centimeter squared electric current was delivered to migrate the tincture to the opposite side of the gel. UV testing at 336 nm was performed on the gel. For staining, the gel was submerged in a pool with 3 µl of Red Safe Nucleic Acid and 500 ml of distilled water.

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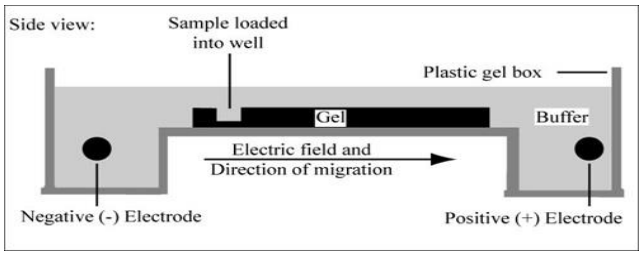

Figure 1: electrophoresis Working system

2.7 Safe red solution for DNA staining

This novel DNA stain is both safe and serves as a substitute for the conventional ethidium bromide (EtBr) stain in the context of DNA detection in agarose gel. When the dye is attached to DNA, it produces a green fluorescence. When attached to DNA, the new spot exhibits two distinct peaks of fluorescence excitation. The first shifts at a wavelength of 309 nm and the second at 419 nm. It also contains a single excitation visible at 514 nm. The Red Safe fluorescence emission associated with DNA is centered at 537 nm. The RedSafe 20,000x DNA staining solution is also as sensitive as EtBr and should be chosen instead of EtBr to detect DNA in agarose gels.

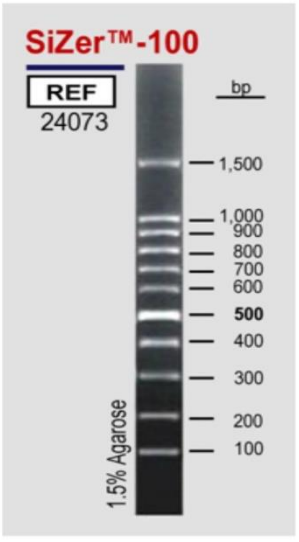

Figure 2: SiZer DNA Markers (intron)

2.8 The primers used to the interaction

Dilute primers using free ddH2O to 100 mol/µl with stock solution after freeze-drying and store at -20. Test 10 µl of stock solution with 90 µl of free water to create 10 mol/µl suspension starter. Reduce ddH2O to 100 µl via IDT (Integrated DNA Technologies, Canada) (Williams et al., 1990).

The primer used to the interaction

1) The specific primer of gene 28s

Primer	Sequence	Tm (°C)	GC (%)	Product size
Forward	5'-GAG AGT TMA ASA GTACGT GAA AC- 3'	**52.3**	44	**860-600 base pair**
Reverse	5'-TCG GAR GGAACC AGC TAC TA - 3'	56	42	

The primer used to the interaction

2) The specific primer of gene *Matk*

Primer	Sequence	Tm (°C)	GC (%)	Product size
Forward	′5ACTGTATCGCACTA TGTATCA -3′	506.	38 %	-400 650 base pair
Reverse	5′ - GCATCTTTTACCCART AGCGAAG-3′	55	45 %	

2.9 Maxime PCR PreMix kit (i-Taq) 20µlrxn (Cat. No. 25025)

The Maxime PCR PreMix Kit from iNtRON includes many possibilities for specialized experiments. A Master's competence is doubled in this solution. Maxime PCR Pre Mix Kit (i-Taq) has many components. One PCR reaction requires i-Taq DNA Polymerase, a dNTP combination, and a reaction buffer in one tube. This gadget provides excellent results in an easy way. One benefit is that it includes all the PCR components, so we just need template DNA, a primer set, and distilled water. The second step includes adding gel loading solution to assist electrophoresis and gel loading. Untreated. The method is simple and efficient for several sample studies.

2.10 Diagnosis of the Gene

Table1: Mixture of specific interaction for diagnosis gene

Components	Concentration
Taq PCR PreMix	5 µl
Reverse primer	10picomols/µl (1 µl)
Forward primer	10picomols/µl (1 µl)
DNA	2 µl
Distill water	16 µl
Final volume	25 µl

To guarantee response component mixing, gloves were worn and the components were centrifuged for 17 seconds. The tubes in the thermopolymer, device, and initiator applied his own program:

Initial Denaturation	95°C	5 min	1 cycle
Denaturation -2	95°C	45 sec	35 cycle
Annealing	58°C	45 sec	
Extension-1	72°C	45 sec	
Extension -2	72°C	45 sec	1 cycle

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Bemisia tabaci						
No.	Type of substitution	Location	Nucleotide	Sequence ID with compare	Source	Identities
1	Transition	129	T/C	ID: MH757981.1	*Bemisia tabaci* isolate 137 28S ribosomal RNA gene,	86%
	Transition	131	A/G			
	Transversion	132	A/C			
	Transition	133	T/C			
	Transition	137	G/A			
	Transition	138	T/C			
	Transition	142	G/A			
	Transition	147	A/G			
	Transition	154	T/C			
	Transition	159	T/C			
	Transition	165	C/T			
	Transition	166	T/C			
	Transition	169	T/C			
	Transversion	198	A/C			
	Transition	199	A/G			
	Transversion	201	T/G			
	Transition	202	T/C			
2	Transition	129	T/C	ID: MH757981.1	*Bemisia tabaci* isolate 137 28S ribosomal RNA gene,	86%
	Transition	131	A/G			
	Transversion	132	A/C			
	Transition	133	T/C			
	Transition	137	G/A			
	Transition	138	T/C			
	Transition	142	G/A			
	Transition	147	A/G			
	Transition	154	T/C			
	Transition	159	T/C			
	Transition	165	C/T			
	Transition	166	T/C			
	Transition	169	T/C			
	Transversion	198	A/C			
	Transition	199	A/G			
	Transversion	201	T/G			
	Transition	202	T/C			
3	Transversion	115	A/T	ID: MH757981.1	*Bemisia tabaci* isolate 137 28S ribosomal RNA gene,	80%
	Transition	128	T/C			
	Transition	129	T/C			
	Transition	131	A/G			
	Transversion	132	G/C			
	Transition	134	G/A			
	Transition	137	G/A			
	Transversion	142	T/A			
	Transition	147	A/G			
	Transversion	148	T/G			
	Transition	154	T/C			
	Transition	159	T/C			

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4	Transition	109	A/G	ID: MH757981.1	*Bemisia tabaci* isolate 137 28S ribosomal RNA gene,	81%
	Transversion	117	C/G			
	Transition	129	T/C			
	Transition	131	A/G			
	Transversion	132	A/C			
	Transition	133	T/C			
	Gap	135	G			
	Transversion	138	G/C			
	Gap	143-144	T			
	Transition	154	T/C			
	Transversion	158	T/G			
	Transversion	159	A/C			
	Transversion	164	T/G			
	Transition	167	T/C			
	Transversion	169	A/C			
	Transversion	170	C/A			
	Transversion	174	C/G			
	Transversion	191	T/G			
5	contamination					
6	Transition	129	T/C	ID: MH757981.1	*Bemisia tabaci* isolate 137 28S ribosomal RNA gene,	82%
	Transition	131	A/G			
	Transversion	132	A/C			
	Transition	133	T/C			
	Transition	137	G/A			
	Transition	138	T/C			
	Transition	142	G/A			
	Transition	147	A/G			
	Transition	154	T/C			
	Transition	159	T/C			
	Transversion	160	T/G			
	Transition	165	C/T			
	Transition	166	T/C			
	Transition	169	T/C			
	Transversion	181	A/C			
	Transversion	198	A/C			
	Transition	199	A/G			
	Transversion	201	T/G			
	Transition	202	T/C			
	Transition	208	g/A			
	Transversion	209	g/T			
	Transition	210	g/A			

2.11 Revealing the sequence of nitrogenous bases of the 28s gene and the Matk gene:

Sequencing DNA for the genes included in the study is done by taking the PCR product included in the study in a volume of 07 microliters with a pair of primers and sending it to Macrogen Biotechnology Company in South Korea. After the results arrived from the company, the results were read according to the sequences and according to Basic Local Alignment. Search Tool (BLAST) and the National Center for Biotechnology Information (NCBI) global website to determine the sequence of the studied genes and compare the current results with it.

III. RESULTS AND DISCUSSION

Results of the effect of the chemical pesticide Hulk in controlling the insect 24 hours after treatment. The results of the research showed that treatment with a

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concentration of 25% of the pesticide Halk gave the lowest percentage of killing the insect, 20%, while a concentration of 50% gave a percentage of killing of 40%, while a concentration of 100% gave the highest percentage of killing, reaching 70% within 24 hours, as in Table (2).

Table 2: The effect of the pesticide Hulk on adult whiteflies

Concentration	*100%*	*50%*	*25%*
Kill percentage Duration 1 day	*70%*	*40%*	*20%*
Control treatment	*0.0*	*0.0*	*0.0*
The average concentration in killing is	*58*	*58*	*37*

The death that occurs in the first period of exposure is due to its effect on contact with these pesticides, and as time progresses, the cumulative death rate increases, and the systemic infectious action of these pesticides is added by contact (Senn *et al*., 2000). The Hulk pesticide showed greater killing effectiveness on whiteflies as it contains two active substances (al acetamiprid and abamectin). Thus, it gave high killing rates when used in high concentrations and controlled the whitefly because this insect is difficult to control because it has genes that have the ability to develop And the formation of high resistance against pesticides. It is necessary to find an insecticide that contains more than one effective substance to reduce and combat whitefly resistance and at the same time be harmless to the environment. These results were consistent with the results of Payton *et al*. (2020) in his study conducted on aphids, which showed that the killing rate achieved by the pesticide reached 70% at high concentration, while at low concentration it reached 30%. These results were similar to the results of El-Ashry and others (2021) that the halk pesticide has a very high effectiveness of up to 70% in killing whiteflies treated with the pesticide, as it was shown that the killing effect comes from its toxic effects on adults. The halk pesticide has high effectiveness in controlling the insect. It is one of the chemical control methods that can help reduce the economic losses of agricultural fields infected with whiteflies. These results agreed with the results of Bansal *et al*. (2023) in his study that the halk pesticide works to reduce the whitefly colony treated with the same pesticide, and this effect is due to the high toxicity of the pesticide on adults, which are highly sensitive to the toxicity of the pesticide. These results were consistent with the results of Berrouk *et al*. (2023) that high concentrations of the pesticide lead to tissue changes in insects treated with the pesticide. These changes are represented by cell death, decreased pigmentation rates, and changes in muscle fibers, which supports the high research results on the toxicity of the pesticide used. Genomic DNA extraction results from insects and plants

-1 28S gene in the whitefly *Bemisia tabaci*

2-Matk gene in the plant *Cucumis sativus*

Cucumis sativus						
No.	Type of substitution	Location	Nucleotide	Sequence ID with compare	Source	Identities
1	Transition	3140	A/G	ID:KX231329.2	Cucumis sativus hybrid chloroplast of Chinese long green (CFND) x Korean solid green (MGLCC),	99%
2	Transition	3080	A/G	ID:KX231329.2	Cucumis sativus hybrid chloroplast of Chinese long green (CFND) x Korean solid green (MGLCC),	99%
3				ID:KX231329.2	Cucumis sativus hybrid chloroplast of Chinese long green (CFND) x Korean solid green (MGLCC),	100%
4				ID:KX231329.2	Cucumis sativus hybrid chloroplast of Chinese long green (CFND) x Korean solid green (MGLCC),	100%
5	Transition	3140	A/G	ID:KX231329.2	Cucumis sativus hybrid chloroplast of Chinese long green (CFND) x Korean solid green (MGLCC),	99%

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6				ID:KX231329.2	Cucumis sativus hybrid chloroplast of Chinese long green (CFND) x Korean solid green (MGLCC),	100%
7	Transversion	3206	C/A	ID:KX231329.2	Cucumis sativus hybrid chloroplast of Chinese long green (CFND) x Korean solid green (MGLCC),	99%
8				ID:KX231329.2	Cucumis sativus hybrid chloroplast of Chinese long green (CFND) x Korean solid green (MGLCC),	100%
9				ID:KX231329.2	Cucumis sativus hybrid chloroplast of Chinese long green (CFND) x Korean solid green (MGLCC),	100%
10	Transition	3393	A/G	ID:KX231329.2	Cucumis sativus hybrid chloroplast of Chinese long green (CFND) x Korean solid green (MGLCC),	99%

The results showed that using the pesticide Hulk and increasing the concentration with the process of repeatedly spraying the pesticide with the same concentrations has negative effects on the genome of the cucumber plant, as the more we doubled the concentration and repeated the pesticide, the lower the appearance of mutations, and thus the percentage of killing the plant increases the higher the concentration, as this pesticide is considered lethal and not mutagenic when used in concentrations. These results agree with Kim *et al*., (2006) in that the Hulk pesticide has toxic effects on the overall cellular functions of the plant, which affects them phenotypically and physiologically. Continuous exposure to this pesticide in plants leads to the decomposition of the chlorophyll pigment, thus reducing plant productivity as a result of a decrease in the rate of photosynthesis. Also, acetamiprid causes a severe lack of nutrients in plants grown in soil exposed to spraying with this pesticide, as the pesticide interferes with the absorption of other elements that the plant needs in addition. Therefore, it can lead to a reduction in the rate of flowering in plants through its effect on flowering hormones and causes an imbalance in the hormonal regulation of many other hormones, especially which in turn leads to stunting and poor flower formation. It also agreed with Brigante Costa *et al*. (2021) on the effect of Hulk pesticide on pollinators, such as bees, that feed on the nectar of plants exposed to this pesticide, as it reduces their efficiency in pollination and thus reduces the fertility rate of the plant.

It also agreed with Qiu Xu *et al*. (2022). Accumulation of Hulk pesticide in the soil leads to negative effects on plants through its effect on soil microbial communities, which play a major role in the nutrient cycle in the soil and which plants depend on for nutrition. It was agreed with Zhange Yin *et al.* (2022) that repeated exposure to the pesticide increases plant susceptibility. To bacterial and viral diseases and many other pests as a result of an imbalance in the enzymatic defense mechanism.

IV. CONCLUSION

The ethanolic extracts tested in this study, particularly those of pesticide Hulk showed is the occurrence of mutations on cucumber plants in the Matk gene in the plant *Cucumis sativus* and gene *28s* in the whitefly *Bemisia tabaci* showed the highest insecticidal effects on whitefly *Bemisa tabaci* the potential to be developed into compounds for the management of whitefly *Bemisa tabaci*. Further study will evaluate these extracts using bioassays to identify B. tabaci's insecticidal metabolites. We want safer B. tabaci treatments in the long run. These natural items may be essential to integrated pest control.

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