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Genetic variability of the Mite *Varroa destructor* Isolated from Honey Bees in Iraq and Some Middle Eastern Countries

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Abstract

This study was conducted to isolate and diagnose different isolates of *Varroa* parasite collected from different regions of Iraq, Iran, Turkey, Syria, Egypt, and Jordan. The different *Varroa* isolates were identified using the polymerase chain reaction (PCR) technique to amplify the mitochondrial cytochrome oxidase gene I (mtCOI) to determine the nucleotide sequences generated from these isolates. These nucleotide sequences were analyzed to identify the similarities and differences in the amplified gene regions. The results obtained from the nucleotide base-sequence analysis indicated that all isolates of *Varroa* parasite collected in this study were *Varroa destructor*. Furthermore, the results showed differences in the nucleotide sequences resulting from the amplification of the target mtCOI by PCR. The isolates from Egypt (No.12) and from Syria (No.14) were the most genetically different among the other isolates in this study (17 samples) . By the phylogenetic tree analysis, isolates of parasites numbered with 12 and 14 appeared in separate clades from the other clades of isolates under study. Besides, these isolates (No.12 and No.14) showed differences to other isolates of the same parasite formerly recorded at NCBI. The *V. destructor* isolate of Egypt (12) and Syria (No.14) showed a genetic similarity of 99%.

Key words: *Honey bees, Varroa destructor, Apiculture in Iraq, Polymerase chain reaction.*

Introduction

Bees have an important role in the environment by pollinating both wildflowers and many agricultural crops as they forage for nectar and pollen, in addition to producing beeswax andhoney 19. Like all other living organisms, honey bees are subject to many pests and pathogens that harmfully affect their behavior and production. Among the most important of these pests is Varroa (Varroa destructor), which invaded Iraq for the first time in 1985 and was officially registered as an epidemic pest to honey bees (Apis mellifera L.) in 1987, known then as Varroa jacobsoni (M.H.E.S.R, 1987, 12). The registry was modified afterwards to Varroa destructor according to 6 and based on the classification of the British Natural History Museum for the Varroa sample from Erbil 18 . Since the appearance of Varroa through the year of 1990, the spread of this parasite and the degree of impact was devastating for beehives in Iraq. More than 90% (FAO, 1997) collapsed despite the use of many chemical and natural treatments that limited the effects of Varroa but remained the greatest threat to beekeeping causing direct damages or by diseases that help its spread particularly viral diseases 9. Varroa parasite is affected by geographical location and natural climatic conditions, especially temperature, humidity, and others. This may lead to genetic mutations that contribute to causing morphological, physiological and behavioral changes allowing for their continual reproduction (1,7,10,13,17). According to observations by beekeepers, there are new species of Varroa as confirmed by the Iraqi Ministry of Agriculture could be because of imported bees infected with Varroa parasite 16. Materials and methods:

Sample Collection

Seventeen samples of *Varroa* parasites were collected directly from the infected bee colonies of ten living parasites from adult *Varroa* females for each sample. They were placed in a separate tube of ethyl alcohol (95%), brought to the Department of Plant Protection/ Faculty of Agriculture/ University of Kerbala, and kept at 4°C until the DNA was extracted.

The samples were collected from different regions of south, central and northern Iraq, as well as samples of bees imported into Iraq. Other samples were obtained from some countries of the Middle East, including neighboring countries (Iran, Turkey, and Syria), where bees can enter Iraq naturally by swarming or male travelling (Table 1and Fig. 1). The other method of transmission is the beehive trade, often from three countries (Iran, Turkey, and Egypt), while a few bees enter from Jordan, often bee queens, often in informal ways [5].

Table (1) Sites and coordinates *Varroa* sampling for molecular diagnosis.

Sample No	Country	Place	Latitude	Longitude	Sample date
1	Iraq	Dohuk	37°07'54.0"N	42°42'13.5"E	14/04/2018
2	Iraq	Najaf	32°05'07.0"N	44°34'39.8"E	19/02/2018
3	Iraq	Sulaimanya (Egyptian product)	35°34'55.1"N	45°16'09.4"E	28/03/2018
4	Turkey	Sharnaq	37°15'08.8"N	42°23'36.3"E	03/04/2018
5	Iraq	Karbala	32°35'09.7"N	44°09'25.3"E	25/02/2018
6	Iraq	Muthanna	31°19'05.6"N	45°18'05.3"E	28/02/2018
7	Iraq	Basra	30°44'38.2"N	47°42'47.7"E	01/03/2018
8	Iraq	Diwaniya	32°03'21.7"N	45°08'50.7"E	06/01/2018
9	Iran	Ahwaz	30°43'30.3"N	48°27'00.4"E	20/01/2018
10	Iraq	Erbil	36°06'58.0"N	\44°00'55.0"E	15/03/2018
11	Jordan	The Jordan Valley	31°59'42.3"N	35°35'09.2"E	13/12/2017
12	Egypt	Kafr El Sheikh	31°13'03.6"N	30°33'17.8"E	13/12/2017
13	Iraq	Najaf Iran product	32°03'52.0"N	44°28'13.1"E	20/01/2018
14	Syria	Rif Dimashq	33°28'11.5"N	36°21'44.9"E	13/12/2017
15	Iraq	Dhi Qar	31°02'01.2"N	46°17'33.7"E	23/01/2018
16	Iraq	Wasit	33°03'12.3"N	44°35'52.5"E	02/02/2018
17	Iraq	Kirkuk	35°34'33.6"N	44°21'52.6"E	15/03/2018

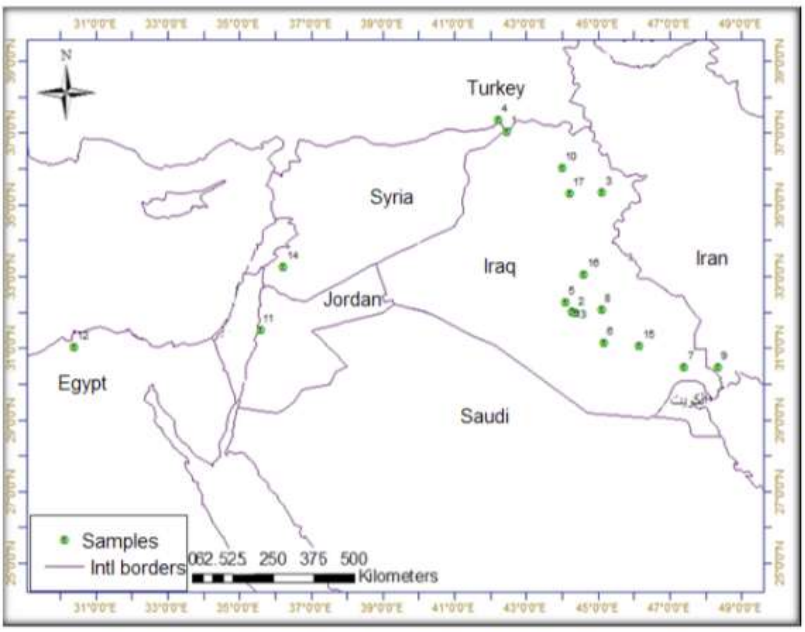

Figure (1) Map shows sites of *Varroa* Sampling, Source: The researcher by GIS software, ArcMap 10.5

Genomic DNA Extraction and PCR Amplification of mitochondrial cytochrome oxidase I (mtCOI) in *Varroa* genome

To extract DNA from the *Varroa* parasite, a DNA extraction kit (Favorgen, Cat No. (FAPGK 001) was used following the steps recommended by the manufacturer. PCR was performed using the Maxime PCR PreMix (i-Taq) kit (Cat. No. 25026) with the primer pair (CO1-F: TTGATTTTTGGTCATCCAGAAGT and CO1-R: TCCAATGCACTAATCTGCCATATTA) to amplify the mitochondrial cytochrome oxidase I (mtCOI) gene [15].

The target region of *Varroa* genome was amplified for all samples to be diagnosed using the following PCR steps and conditions: Initial DNA denaturation for 5 minutes at 94°C, followed by 35 cycles each consisting of final denaturation for 30 seconds at 94°C, primer annealing for 30 seconds at 58°C and then an initial extension of the amplified PCR product for 1 min at 72°C with the completion of PCR steps by one cycle of the final extension at 72 ° C for five minutes [15]. PCR-amplified products were electrophoretically separated on a 1% agarose gel for 140 min at 80 V, 400 mA and visualized with ethidium bromide staining under UV illumination and images were captured using Vilber Lourmat, Taiwan gel documentation system.

Nucleotide sequence analysis of DNA of *Varroa* Samples

To identify the nucleotide sequences of the PCR-amplified products of the various *Varroa* samples, the PCR products were sent to the Korean Macrogen company with both forward and reverse primers (CO1-F / R) used in DNA amplification to determine the species of *Varroa* parasite in the sample collection sites, and the degree of similarity and difference in the sequences of the nucleotide sequences of the amplified DNA of *Varroa*. The nucleotide sequences of the DNA product was processed into the database available at the National Biotechnology Information Center (NCBI) using the Basic Local Alignment Search Tool (BLAST) [24]. The phylogenetic tree was constructed using the MEGA6 program [20], using the Neighbor-joining method.

Results and Discussion

Molecular diagnosis of *Varroa* Isolates

The results of DNA extraction from *Varroa* isolates and their processing (PCR) showed the possibility of replicating the PCR-amplified products and the expected size (approximately 880 nitrogen bases) using the forward and reverse buffer (mtCOI_F/ R) (Fig.2).

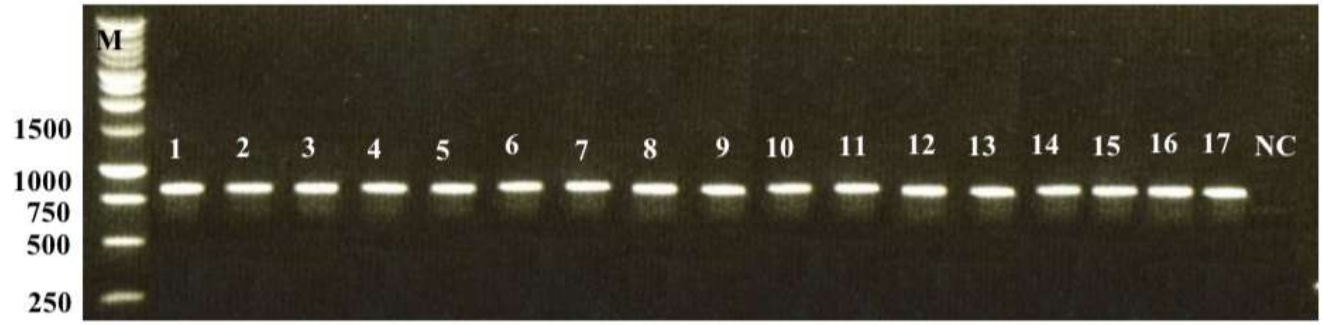

Figure (2) PCR-amplified products, using the mtCOI_F / mtCOI_R (PCR) pair of *Varroa* isolates from Duhok (1), Najaf (2), Sulaymaniyah (imported bees) (3), Turkey (4), Karbala (5), Al Muthanna (6), Basrah (7), Diwaniyah (8), Iran (9), Erbil (10), Jordan (11), Egypt (12), Najaf (imported bees) (13), Syria (14), Thiqar (15), Wassit (16), and Kirkuk (17). M= 1Kbp DNA ladder marker. NC: Control treatment (without adding DNA to the other PCR components). The results of the nucleotide sequence analysis of the amplified DNA products for *Varroa* isolates using BLAST showed that all isolates of the current study belong to *V. destructor*. The isolates from Egypt (No.12) and Syria (No.14) gave the most different in the nucleotide sequences with 99% similar with the other isolates belonging to the same parasite isolated in this study from different regions in some Middle Eastern countries (Table 2 and Fig. 3).

Figure (3) Similarity and difference in nucleotide sequence alignments of PCR-amplified products of isolates from Duhok (No. 1), Egypt (No. 12), and Syria (No. 14

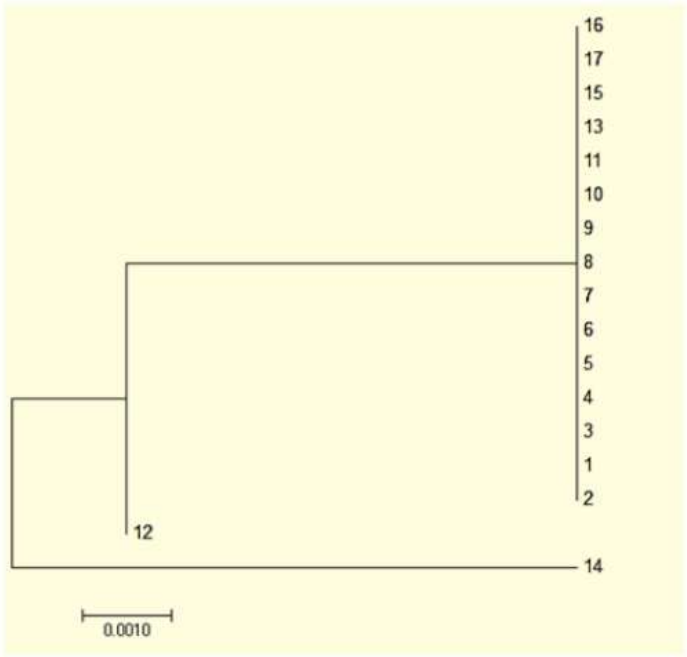

Figure 4: The Neighbor-Joining tree analysis showing the genetic relationship of the *V. destructor* isolates of Iraq and some Middle Eastern countries.

The results of the nucleotide sequence analysis for *Varroa* isolates from Egypt (No.12) and other isolates recorded at NCBI indicated that there is a difference in the nucleotide sequences, wherein the genetic similarity ranges between 86-99%. The Neighbor-joining tree analysis showed the isolate (No.12) was appeared in a separate clades from other isolates previously recorded at NCBI (Figure. 5).

The results also of the nucleotide sequence analysis of the PCR-amplified mtCOI gene of *Varroa* isolates from Syria (No. 14) showed the presence of a clear genetic variation in the nucleotide sequences with the nucleotide sequences of the other isolates previously recorded at NCBI giving a similarity of 99%. The Neighbor-Joining tree analysis showed that the isolate No.14 was appeared in a separate clade from the other clades of the same parasite previously registered in NCBI (Figure 6).

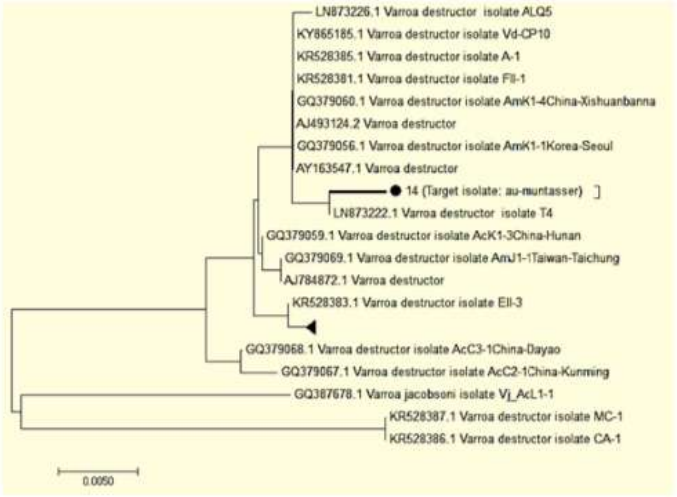

Figure 5: The Neighbor-Joining tree analysis showing the genetic relationship of the *V. destructor* isolates of Syria (No.14) and other isolates registered in NCB(.

In this paper, seventeen isolates of *V. destructor* were isolated from different regions of southern, central, and northern Iraq, as well as the Middle Eastern countries of Iran, Turkey, Syria, Egypt, and Jordan, and identified by PCR amplification and sequencing the PCR-amplified products. By determining and comparing the nucleotide sequences identified in this study with sequences previously registered in NCBI, it was found that two isolates of *V. destructor* (No.12 and No.14) were not previously isolated and identified; therefore, the identified sequences have registered in Genbank under the accession numbers: MK482687 and MK509767, respectively.

In the current study, PCR was used to diagnose 17 different isolates of the *Varroa* parasite collected from different parts of Iraq and some Middle Eastern countries by targeting the mitochondrial cytochrome oxidase I (mtCOI). PCR has been used efficiently in the diagnosis of many organisms; microorganisms (such as fungi) and insects (*Bemisia tabaci*) [2,3] to avoid diagnostic problems based on morphological characters. Although phenotypic diagnosis is useful in categorizing the organisms under study into smaller groups before starting other methods of diagnosis, there are many problems associated with the phenotypic diagnosis of organisms, including the need of the diagnosis conductor to the high experience, especially for similar fungal species as well as the need for long time and substantial efforts ([4,11, 14]. There are also other factors that may affect these morphological characters, including the type and nature of growth, moisture and light that can affect some of the morphological characteristics of the insect. Several researchers have also used the difference

between the mtCOI-based sequence to diagnose *Varroa* isolates from different regions of the world using PCR technology [6,8,22,23]

Conclusion

The results obtained from the nucleotide base-sequence analysis indicated that all isolates of *Varroa* parasite collected in this study were *Varroa destructor*. Furthermore, the results showed differences in the nucleotide sequences resulting from the amplification of the target mtCOI by PCR. The isolates from Egypt (No.12) and from Syria (No.14) were the most genetically different among the other isolates in this study (17 samples) . By the phylogenetic tree analysis, isolates of parasites numbered with 12 and 14 appeared in separate clades from the other clades of isolates under study.

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Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Department of Plant Protection/ Faculty of Agriculture/ University of Kerbaand all experiments were carried out in accordance with approved guideline.

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