



Molecular Characterization and Geographical Distribution of Whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae) in Jordan

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ABSTRACT

The whitefly *Bemisia tabaci* (Gennadius) is a large complex of cryptic species and is considered an invasive pest transmitting many plant viruses. It is a highly polyphagous insect that causes damage and economic losses to many plant species including vegetables, cotton, ornamentals, and weeds either in the open field or the greenhouses. A two-year survey (2016 to 2018) was conducted to biotype *B. tabaci* for B (MEAN1) and Q (MED) biotypes putative species. 18 host plants were screened from 14 different locations with a total of 101 samples that were collected and tested. Microsatellite markers and mitochondrial cytochrome oxidase I (mt COI) gene sequencing were used. The results showed that biotype B is dominant and prevailed in all surveyed locations.

Keywords: *Bemisia tabaci*, microsatellite markers, mitochondrial cytochrome oxidase I, PCR sequencing, biotyping, distribution.

INTRODUCTION

Bemisia tabaci (Gennadius) (Hemiptera: Aleyrodidae) is an invasive pest species of the first 100 invasive cosmopolitan species (Global invasive species database, <http://www.issg.org/database>). It has a wide host range; where more than 900 host plants are infested (Simmons et al., 2008). This pest has widespread and

dispersal nearly all over the world. As a virus vector of more than 111 viruses (Jones, 2003) it causes losses to agriculture production (Mckenzie et al., 2009).

Whitefly adults appeared to differ only slightly in their morphological characteristics which were of little value in distinguishing species (Raymond, 1990). Whitefly *B. tabaci* forms a species complex (De Barro et al., 2011) with at least thirty-six biotypes or host races (Mckenzie et

al., 2012). The genetic groups are morphologically indistinguishable but have differences in geographical distribution, host range, the ability to be a plant virus vector, and other biological traits (Brown et al. 1995). These biotypes were designated primarily into biotypes B, Q, and non-B/Q biotypes. The non-B/Q biotypes include more than 20 biotypes such as A, K, D, E, G, H, L, M, N, etc. *B. tabaci* biotypes identification is only based on different molecular techniques (Perring, 2001). Biotypes B and Q are polyphagous, most invasive, and widely distributed biotypes and considered global invaders by international trade in ornamentals (De Barro and Ahmed, 2011). Biotype B is the Middle East-Asia Minor 1 (MEAM1) and biotype Q is the Mediterranean (MED) putative species respectively (Skaljac et al., 2013), they rarely interbreed although having narrow geographical distance origin. Biotypes A, B, and AB were reported in Jordan using opr-4 primer RAPD markers (Sharaf and Hasan, 2003). However, *B. tabaci* biotype Q had been reported in the surrounding countries of Jordan, such as Syria, Egypt, and Palestine/Israel (Ahmed et al., 2011, Fujie et al., 2009 Horowitz et al., 2003). The biotype Q of *B. tabaci* had the innate ability to rapidly develop a high level of insecticidal resistance; which persists without the selection of insecticides exposure (Mckenzie et al., 2012), it also, causes economic damage. Ecology, biology, and population dynamics of the Aleyrodidae family consider the coexistence of several biotypes (Bel-Kadhi et al., 2008) also monitoring the

biotypes of the *B. tabaci* and its resistance to insecticides is a vital part of its control strategy (Farghaly, 2010).

The previous studies that were conducted regarding the distribution of *B. tabaci* biotypes in Jordan were based on using the RAPD marker (Hasan, 2006). Since biotype Q is not reported in Jordan, more appropriate DNA-based techniques are required to identify *B. tabaci* biotypes such as the mitochondrial cytochrome oxidase I (mtCOI) gene sequence (Ghabeish et al. 2021) and also microsatellite markers, as they are more effective in differentiating *B. tabaci* populations. The objective of this study was to biotyping and determine the geographic distribution of *B. tabaci* biotypes in Jordan using microsatellite markers for the first time and mtCOI gene sequence.

Experimental Methods

Whitefly Collection

Adult whiteflies were collected from different geographical regions where most of the crops were produced, such as the Jordan Valley, uplands, and areas in between various host plants as shown in Table 1, either in the open field or the plastic house using a hand-held aspirator. The pupal stages were collected to confirm the whitefly taxonomy. From 10-30 whiteflies were taken depending on the host plant for each sample, and specimens were kept in 75% ethanol, then stored at -20 °C.

Table1. *Bemisia tabaci* host plants and their locations in Jordan based on the 2016-2018 survey.

Location	Common name	Scientific name	Coordination
Ghawr Al-Sammar	Melon	<i>Cucumis melo L.</i>	N31° 03.432' E35° 29.490'
Ghawr Al-Mazraah	Eggplant	<i>Solanum melongena L.</i>	N31° 12.981' E35° 31.370'
Ghawr Al-Mazraah	Squash	<i>Cucurbita maxima</i>	N31° 15.447' E35° 30.842'
Ghawr Essal	Melon	<i>Cucumis melo L.</i>	N31° 10.927' E35° 32.105'
Wadi Bin Hammad	Squash	<i>Cucurbita pepo L.</i>	N31° 16.629' E35° 40.874'
Wadi Bin Hammad	Cucumber	<i>Cucumis sativus L.</i>	N31° 17.050' E35° 40.739'
Wadi Shueib	Sweet pepper	<i>Capsicum frutescens L.</i>	N31° 55.223' E35° 39.284'
Ghawr Al-Safi	Cauliflower	<i>Brassica oleraceae L.</i>	N31° 03.450' E35° 28.849'
Karameh	Tomato	<i>Solanum lycopersicum L.</i>	N31° 56.537' E35° 34.880'

Sail Al Karak	Lettuce	<i>Lactuca sativa L.</i>	N31° 12.100' E35° 41.434'
Gwair	Broccoli	<i>Brassica oleracea L.</i> var. <i>italica</i> Plenck	N31° 09.714' E35° 44.888'
Wadi Al Mujib	Tobacco	<i>Nicotiana tabacum L.</i>	N31° 25.933' E35° 49.433'
Dair Alla	Potato	<i>Solanum tuberosum L.</i>	N32°03.295' E35°35.222'
Al Tuneib, Amman	Cucumber	<i>Cucumis sativus L.</i>	N31° 47.073' E35° 55.693'
Mubes Al Baqa'a	Louisiana cut flowers	-	N32° 06.149' E35° 51.441'
Mubes Al Baqa'a	Gerbera cut flowers	<i>Gerbera spp L.</i>	N32° 06.162' E35° 51.468'
Dair Alla	Black nightshade	<i>L. Solanum nigrum</i>	N32° 05.698' E35° 35.130'
Ghawr kabit	Beans	<i>Phaseolus vulgaris L.</i>	N32° 04.088' E35° 34.022'
Wadi Al Mujib	Hot pepper	<i>Capsicum annum L.</i>	N31° 25.234' E35° 49.691'
Ghawr Al-Mazraah	Watermelon	<i>Citrullus lanatus</i> (Thunb) Matsum and Nakai	N31° 15.781' E35° 30.777'
Ein Sarah	Common amaranthus	<i>Amaranthus retroflexus L.</i>	N31° 12.000' E35° 40.617'

Sampling was started in October 2016 and extended for two years until October 2018. Samples were collected randomly from each agricultural season from a particular area (Jordan Valley, uplands, and the areas in between). Host plants ranged from vegetables, and ornamentals, to weeds, and 101 specimens were collected to be tested for biotypes B and Q.

DNA Extraction

Identification of *B. tabaci* samples was confirmed by comparing the collected pupae (red-eyed late fourth nymphal stage) with a reference and were observed and sexed by taking the larger whiteflies (the females) under a binocular stereomicroscope (Motic) (Qiu et al., 2009). Six females were selected for the DNA extraction and to be stored to be used later when needed. Single whitefly was analyzed for DNA extraction. Insects were dried well from the ethanol and a single whitefly was macerated by a sterile micro pestle in 10 µl 2% CTAB solution (Bio basic inc.) in a 1.5 ml tube, at 65 °C on thermoshaker for one hour, then 1 µl proteinase K (Qiagen) and 1 µl RNase (GeneDireX) were added in dry bath for 10 min with

shaking and then 10 µl chloroform: isoamyl alcohol (24:1) were added, then the mixture was vortexed for 30 sec, after the appearance of two layers, it was centrifuged at the maximum speed 14000 rpm for 10 min then the upper layer (the supernatant) was transferred into a new 1.5 ml tube and 25 µl of absolute ethanol were added to precipitate DNA to form a pellet which was stored at -20 °C overnight or for one hour, then it was centrifuged for 10 min at the maximum speed of 14000 rpm, after that 25 µl of 75% ethanol were added to wash the DNA, then it was centrifuged for 5 min, then completely dried the pellet from alcohol at room temperature, afterwards pellet was dissolved in 20 µl of TE buffer or nuclease water free and kept at -20 °C for further use (Chen et al., 2010).

Methods used to determine biotypes: two molecular techniques were used to distinguish whiteflies' biotypes including microsatellite fragment analysis (De Barro et al., 2003) and analysis of mtCOI gene sequence (Frohlich et al., 1999).

Microsatellite Markers

The primers set Bem 23-F 5'-CGGAGCTTGCGCCTTAGTC-3' and Bem 23-R 5'-

CGGCTTTATCATAGCTCTCGT-3' (BGI Co. China) were used (De Barro et al., 2003). Polymerase chain reactions (PCR) for the microsatellite primers were conducted in a 25 µl total reaction composed of 12.5 µl of 2X master mix with standard buffers (One Taq Quick-Load), 8.5 µl sterile H₂O (Nuclease-Water Free), 0.5 µl of the forward primer 10 µM, 0.5 µl of the reverse primer 10 µM, and 2 µl of the DNA. The reactions were run under a thermal regime of 95 °C for 7 minutes followed by 40 cycles of 30 seconds at 95 °C (denaturation), 45 seconds at 55 °C (annealing), 40 seconds at 72 °C (extension), and a final step of 72 °C for 5 minutes, then stop at 4 °C overnight (Bio-Rad DNA Engine). PCR products were separated on 2.5% agarose gels, and bands were visualized by Red Safe Nucleic Acid Staining Solution (20,000X), viewed with a UV light source, and photographed by the gel documentation system (Alpha Innotech doc.).

MtCOI PCR Amplification and Sequencing

A fragment of the mtCOI gene (866 bp) was amplified by PCR using the universal primers C1-J-2195 (5'-TTGATTTTTTGGTCATCCAGAAGT-3') and TL2-N-3014 (5'-TCCAATGCACTAATCTGCCATATTA-3') (Frohlich et al., 1999). DNA was amplified in a final volume of 25 µl, containing 1x of Taq-buffer, 1.0 mM MgCl₂, 200 µM dNTPs, 200 nM of each primer, 1 U Crimson Taq DNA polymerase (New England Biolabs, MA, USA), and 2 µl of DNA. PCR conditions were an initial 1 min at 94 °C (denaturation), followed by 30 sec. at 94 °C (denaturation), 1 min at 45 °C (primer annealing), and 1 min at 72 °C (amplification) for 35 cycles, and 10 min at 72 °C. PCR products were separated on 1% agarose gels, and bands were visualized by ethidium bromide staining and viewed with a UV light source. Eight selected PCR amplicons (866 bp) were recovered and purified using the Wizard DNA Clean-up System kit (Promega, U.S.A.) according to the manufacturer's instructions.

Purified PCR products were ligated into the plasmid pGEM T-Easy vector (Promega). Plasmids were transformed into *Escherichia coli* JM109 according to the manufacturer's instructions. Recombinant colonies were

screened and their plasmids were isolated using the Pure Yield Plasmid Miniprep System kit (Promega, USA), digested with EcoRI (New England Biolabs) to determine insert size, and analyzed using agarose gel electrophoresis. Nucleotide sequences were obtained by Macrogen (Korea) from two cDNA clones and were analyzed using sequence analysis and data management software from Invitrogen, Vector NTI Advance™ 10 (InforMax, USA). Sequences were deposited in the Genbank under accession numbers (MW514031 and MW514038). The nucleotide sequences were compared with sequences available from the NCBI database using the BLASTn program in GenBank (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>).

Results

All of the specimens surveyed were of B biotype or MEAN1 putative species except a few samples that showed no reactions or did not show specific PCR amplified bands or fragments for Q or B biotypes indicating either the presence of other biotypes or the failure of the PCR reaction. Figures (1-3) respectively give clear pictures of the *B. tabaci* B biotype prevalence in all the surveyed regions in Jordan despite the different environmental conditions and factors affecting them, these regions are extending from the uplands to the lowest areas in the Jordan Valley and including the areas in between.

The *B. tabaci* biotype B or MEAN1 putative species has a 220 bp band size as shown in Figures 1-3 compared with the control. The specific amplified product (410 bp) of the Q biotype or the MED putative species was not seen in any of the tested samples except in the positive control. The distribution of the B biotype in Jordan is shown in Figure (4). Furthermore, the 866-bp fragments of the mtCOI sequences were obtained from 8 samples of *B. tabaci* collected in this study from different hosts and regions followed by sequencing. BLASTn analysis showed that the nucleotide sequences shared 99 to 100% identity to mtCOI sequences of *B. tabaci* B biotype or MEAN1 putative species available in the NCBI database.

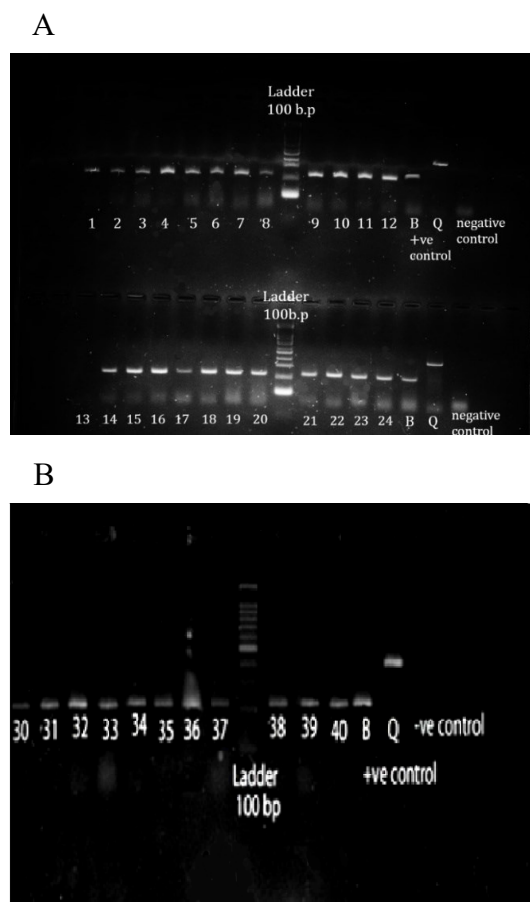


Figure (1): Microsatellite marker results, A specimens from 1-24 in two combs and the control B 220 bp, Q 410 bp, negative control, and the DNA ladder 100 bp (in the middle), B DNA bands for whitefly specimens from 30-40.

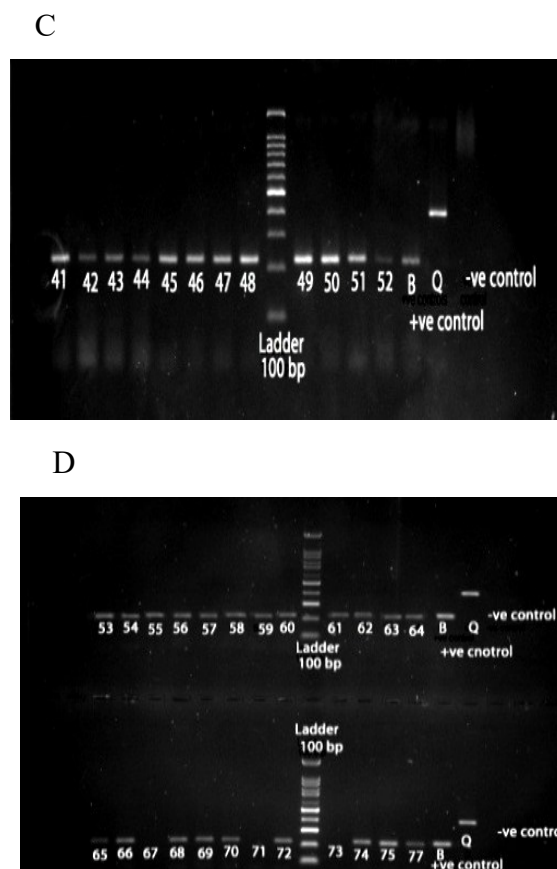


Figure (2): Microsatellite marker results, C DNA bands for whitefly specimens from 41-52, D DNA bands for whitefly specimens in the two combs from 53-77, and the control B 220 bp, Q 410 bp, negative control, and the DNA ladder 100 bp (in the middle).

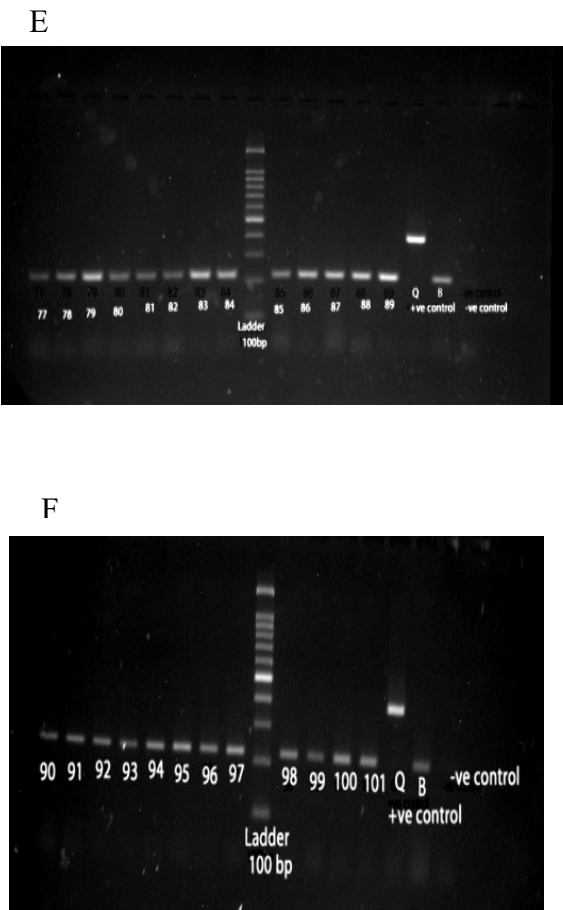


Figure (3): Microsatellite marker results, E DNA bands from 78-89, and F DNA bands from 90-101, and the control Q 410 bp, B 220 bp, negative control, and the DNA ladder 100 bp (in the middle).

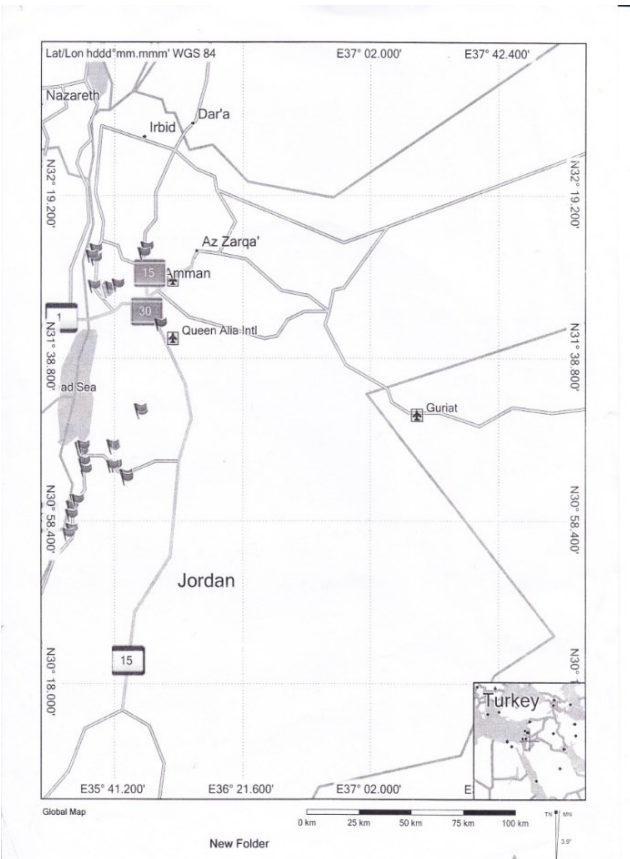


Figure (4): Map of the distribution of the Bemisia tabaci biotype B in Jordan marked by flags.

Discussion

Sharaf and Hasan (2003) distinguished two biotypes using three RAPD primers and primer OPR-4 detected two biotypes clearly, biotype A and biotype B with different bands and the ratio between them was 25%:75% respectively suggesting that biotype B may reach the completion of a new species stage in the future replacing A, and this may confirm what has been found in this study, only B biotype is dominant. Hasan (2006) later continued investigations using RAPD PCR to survey the *B. tabaci* biotypes from 9 sampling sites in Jordan Valley (Ghawr region) and the uplands, from the cultivated plants and the wild plants, he found that there were at least three biotypes A, B, and AB distributed in percentage as 12.5%, 75%, and 12.5%, respectively. Recently Ghabeish et al. (2021) conducted a study for three years in which 111 whiteflies were collected from different hosts and locations in Jordan and were identified by molecular techniques as (mtCOI) gene and morphologically. They found that there were nine whitefly species, in addition to the *B. tabaci* biotype B or the (MEAN1) putative species. Thus, it is obvious from these studies that biotype B had a trend to be dominant in Jordan and compete with the other biotypes A, AB, to be the only biotype, as has been revealed in our study. Therefore, both biotype A and AB may be replaced by biotype B in Jordan as reported by Costa et al., (1994) where biotype B replaced biotype A in the USA in the 1980s.

Chiel et al. (2007) reported the dominance of the biotype B of *B. tabaci* field populations on vegetables like sweet pepper, cucumber, squash, eggplant, and tomato, as has been found in this study and they reported Q biotype on cotton and sage, which was not detected in Jordan yet; the B biotype was dominant in field populations sampled from sweet pepper, cucumber, squash and eggplant (75–100%), on the other hand, the Q biotype dominated in cotton and sage plots (80–100%). B biotype was distributed and dispersed in all the surveyed areas in Jordan on different vegetables, ornamentals, and weeds as shown in Figure 4 and Table 1. This could be attributed

to the lack of both good control measures and local quarantine regulations. Bel-Kadhi et al. (2008) had made molecular characterization of the *B. tabaci* that were collected from nine sites in southern Tunisia using Bem 23 microsatellites markers and found that biotype B was found in seven of the surveyed areas yielding 220 bp bands, however, in two other sites of Dous and Bazma, showed both B and Q populations the last which showed 410 bp bands indicating the presence of Q biotype in Dous region. These results showed that biotype Q was found only in one region out of nine surveyed sites indicating the dominance of biotype B in all the surveyed regions. Alhudaib et al. (2014) found that the B biotype is dominant in the Al-Ahsa region of Saudi Arabia where the specimens were collected from nine different locations. Al-Shehi and Khan (2013) surveyed different geographical regions in Oman during 2011-2012 for the *B. tabaci* biotypes using microsatellite markers Bem 23F and Bem 23 R also, identified its secondary symbionts associated with whitefly populations collected and found that only biotype B was detected from the whitefly populations which was collected from different vegetable crops and were infected with the secondary symbiont *Hamiltonella* sp.

Horowitz and Ishaaya (2014) found that since 2009 there was a significant shift in the ratios of the *B. tabaci* biotypes B and Q in the cotton fields, biotype B is becoming predominant over the Q biotype and they demonstrated that the B biotype is more competitive than Q biotype under untreated conditions. Crowder et al. (2010) supposed that the high competitiveness trait of the B biotype was due to its ability to copulate more effectively than the Q biotype thus resulting in faster population growth and therefore, the exclusion of the Q biotype. The predominance of the B biotype in the surveyed areas in Jordan may be explained due to the fast-growing B biotype populations of *B. tabaci* as a more competitive biotype than the Q biotype, and to its adaptation to different crops grown in various geographical regions in Jordan, in addition to this, the quarantine regulations on the longboarders as well as the long distance between the infested areas in the

surrounding countries where the Q biotype was reported and the Jordanian areas that were surveyed may also explain the prevalence of the B. tabaci biotype B and exclusion of biotype Q in Jordan. Our results are in line with the results reported by Ghabeish et al. (2021) which indicate the prevalence of the biotype B or the (MEAN1) in Jordan.

Conclusion

From this study, it is concluded that the Q biotype or the MED putative species is absent until now, and the biotype B or (MEAN1) putative species is the dominant biotype in all the screened hosts and locations. For future studies, it is recommended to conduct investigations on the B. tabaci resistance against certain insecticides in correlation to the biotypes and the secondary symbionts of B. tabaci.

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التوصيف الجزيئي والتوزيع الجغرافي للذبابة البيضاء *Bemisia tabaci* (Hemiptera: Aleyrodidae) في الأردن

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ملخص

الذبابة البيضاء *Bemisia tabaci* (Gennadius) هي مجموعة كبيرة ومعقدة من الأنواع الخفية وتعتبر آفة غازية ناقلة للعديد من الفيروسات النباتية. هي حشرة متعددة العوائل تسبب ضرراً وخسائراً اقتصادية للعديد من أنواع النباتات متضمنة الخضروات والقطن ونباتات الزينة والأعشاب إما في الحقل المكشوف أو البيوت المحمية. لقد تم عمل حصر لمدة سنتين من عام 2016 ولغاية 2018 لتوصيف *B. tabaci* للنوعين الحيويين المفترضين B (MEAN1) و Q (MED). تم مسح 18 عائلاً نباتياً من 14 موقعاً مختلفاً بما مجموعه 101 عينة تم جمعها واختبارها. تم استخدام معلمات *microsatellites* وسلاسل جين *mtCOI*. وبينت النتائج أن النوع الحيوي B هو السائد وينتشر في جميع المواقع التي تم حصرها.

الكلمات الدالة: *Bemisia tabaci*، علامات الأقمار الصناعية الدقيقة، سيتوكروم أوكسيداز الميتوكوندريا I، تسلسل PCR، التمييز الحيوي، التوزيع