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Behavioural Response of Whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae) Towards Volatile Organic Compounds Emitted from Eggplant Leaves

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ABSTRACT

The whitefly *Bemisia tabaci* is considered one of the most destructive insect pests that can attack several vegetable crops worldwide. The behavior of choosing a host plant by whitefly can be significantly influenced by volatile organic compounds. The aim of this investigation is to identify and bioassay the volatile organic compounds emitted from uninfested and infested eggplant leaves with *B. tabaci*. The current study focuses on the interaction between whitefly and eggplant with the identification of volatile organic compounds emitted from leaves that were collected by headspace solid phase microextraction fiber and gas chromatography mass spectrum analyzed. Both uninfested and infested eggplant leaves released a total of 11 compounds. The amount of volatile organic compounds emitted from affected eggplant leaves by whitefly was higher than from uninfested eggplant leaves. Using a Y-shaped olfactometer bioassay, whitefly preferred to attract toward volatile emitted by eggplant leaves in both uninfested and infested eggplant leaves. Our results suggest these chemical compounds can play a key mediator in host finding behavior. The exploitation of one or more of these compounds can apply in an integrated management program of this pest as a lure in the sticky trap or manipulating insect behavior.

KEYWORDS: Semiochemicals; *Solanum melongena*; VOCs; Y-shape olfactometer; IPM.

الخلاصة

تعتبر حشرة الذبابة البيضاء *Bemisia tabaci* واحدة من أكثر الآفات الحشرية التي تصيب مختلف محاصيل الخضروات واشجار الفاكهة ونباتات الزينة في جميع أنحاء العالم. يتأثر سلوك الذبابة البيضاء في اختيار النبات العائل عن طريق تأثيرها بالمركبات العضوية المتطايرة. هدفت الدراسة الى تشخيص واختبار المركبات العضوية المتطايرة المنبعثة من أوراق الباذنجان غير المصابة والمصابة بحشرة الذبابة البيضاء *B. tabaci* فضلا عن التفاعل بين الذبابة البيضاء ونباتات الباذنجان. جمعت المركبات العضوية المتطايرة المنبعثة من الأوراق باستخدام تقنية الفايبرات SPME وتحليها باستخدام الكروماتوغرافيا الغازية. شخصت 11 مركب عضوي متطاير من أوراق الباذنجان الغير المصابة والمصابة بحشرة الذبابة البيضاء. كانت تراكيز المركبات العضوية المتطايرة المنبعثة من أوراق الباذنجان المصابة أعلى منها من أوراق الباذنجان غير المصابة. اوضحت نتائج مقياس الشم Olfactometer الزجاجي ان حشرات الذبابة البيضاء تنجذب نحو أوراق الباذنجان غير المصابة والمصابة عند مقارنة انجذابها مع معاملة الهواء. تشير النتائج إلى أن هذه المركبات الكيميائية يمكن أن تلعب دور رئيسي في سلوك الحشرة في إيجاد النبات العائل ويمكن استخدام المركبات العضوية المتطايرة في برنامج إدارة متكامل لهذه الآفة في المصائد اللاصقة.

INTRODUCTION

Around the world, eggplant *Solanum melongena* L. belonging to the Solanaceae family, is a common vegetable. Attacks by numerous insect pests, especially the whitefly *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae), significantly negatively impact the production of eggplant [1]. The polyphagous insect known as the greenhouse whitefly significantly negatively impacts the world's agriculture industry [2]. The nymphs and adults of whitefly can directly damage plants by sucking the plant sap, which has a negative impact on plant growth and development, and indirectly by excretion of honeydew on the leaves [3]. In order to plants defend themselves from insect herbivores, plants produce morphologically and chemically complex structures on the surface of their leaves, such as changes in trichomes (outgrowth from the plant epidermis), and produce toxic substances that have a detrimental impact on the behavior or growth of the pests [4]. Additionally, plants may defend themselves through indirect means, such as by producing and releasing volatile organic compounds (VOCs) in response to insect feeding [5]. VOCs are released in massive quantities after the infestation of herbivores [6]. Various studies have demonstrated that even without the plants' visual stimuli by insect pests, interactions between VOCs and their host plants play a significant role in how insects behave [7]. Whiteflies are known to respond to subtle changes in their olfactory environment and use volatiles to select the most suitable host [8]. Some experiments on plant VOCs have shown that there are chemical compounds produced by infested plants that can be used as signals for herbivores after insect-feeding activities [6]. We predicted that volatile emissions from the uninfested and infested plants would be the main factor influencing whitefly attraction. Hence, the purpose of this investigation is to identify, and bioassay the volatile organic compounds (VOCs) emitted from uninfested, and infested eggplant leaves with *B. tabaci*.

MATERIALS AND METHODOLOGIES

Eggplant plants and whitefly rearing

Eggplant *S. melongena* L. variety Zomorrod F1 (Enza Zaden Company, Nether-lands) seeds were obtained from a local market and grown in the plastic of 1 Kg pots containing potting soil mixture under greenhouse conditions; when eggplant seedlings reached the 5-6 leaves stage, they were used in the experiment.

The greenhouse whitefly, *B. tabaci* was collected from greenhouses of eggplant located at the University of Baghdad. The whitefly insects were reared on potted eggplant seedlings using cages size 40 × 40 × 40 cm made from aluminum tubes and covered by mesh to prevent other pest infestation. Cages that contained eggplant seedlings were placed in a greenhouse. *B. tabaci* were introduced into the cage for the eggplant infestation and kept for at least two successive generations before being used in the experiment.

Headspace collection of plants volatiles

The headspace of volatile organic compounds collection was extracted from both infested and uninfested eggplant using SPME fiber type PDMS/ CAR/ DVB (carboxen/ polydimethyl siloxane/ divinylbenzene) 2 cm long 50/30 µm of the thickness (Manufacture of Sigma- Aldrich). To extract VOCs, eggplant leaves were carefully placed in a 2-L glass jar and tightly covered the surface of the jar with aluminium foil, and left for 24 h for acclimatization prior to volatile col-lection (sealing time). SPME fiber was inserted through the aluminium foil into the headspace of the sample for 30 min to collect the VOCs and then analyzed by GC-MS.

Chemical analysis by GC-MS

Headspace samples were analyzed with Agilent Gas Chromatography GC mod-el 7820A (Manufacture of Agilent Technology, USA) connected to Agilent Mass Spectrometer (MS) E5977. The SPME fiber was injected into the injection port of GC at the temperature of 270 ˚C for 1 min. Helium was the gas carrier with a gas flow of 20 ml min−1. The VOCs were shifted in spitless mode to the GC column type DB-35ms (30m× 250µm× 0.25 µm), located in the GC oven. The total run time of GC-MS was 45 min. Based on the retention time (RT) of each compound, the VOCs were determined by using the Alkane C7-C40 standard and MS database of the National Institute of Standards and Technology library (NIST 2008) [9].

Y-shape Olfactometer Experiments

Using a glass Y-shape olfactometer, the behavior of whiteflies toward infested and uninfested eggplant leaves was examined. The bioassays of whitefly adult’s attraction were conducted using a two-armed Y-tube olfactometer in a closed system. Each arm of olfactometer length was 7 cm with a diameter of 2 cm. The olfactometer was set up as described by [9][9]. Clean air was used by filtering air with distilled water after it had been passed through a flask containing activated charcoal. Prior to the experiment, the Y-shape was linked to an air pump using flow meters set to 10 mL/min airflow and maintained for 15 min for the stability of the air system inside of the olfactometer. In this set-up, air steam was passed through a sealed desiccator with a capacity of 2 liters that contains different experimental treatments such as (1) comparison between infested eggplant leaves and filtered air; (2) comparison between uninfested eggplant leaves and filtered air; and (3) comparison between infested and uninfested eggplants. Three replicates were used for each treatment, and 20 adults of *B. tabaci* were individually released at the base of the Y-shape. After every 10 adults with *B. tabaci* was

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tested in the olfactometer, all glassware was washed and cleaned [10].

Statistical analysis

MetaboAnalyst online software version 5.0 was used to statistically compare the emission of VOCs from infested and uninfested eggplant leaves [11]. In order to minimize the peak area numerical value, the peak area value was reduced by dividing by 100,000 using Microsoft Excel 2016. Y-shaped Olfactometer choice experiments were analyzed using the Chi-Square likelihood test (P-value) level of 0.05 using SPSS software Version 26 (IBM Company, New York, USA)

RESULTS AND DISCUSSION

Identify VOCs by GC-MS

The volatiles produced by both infested and uninfested eggplant was significantly different; around 11 volatile compounds were detected in eggplants. *B. tabaci* can change the quality and quantity of VOCs in the infested plant. However, as shown by GC-MS analysis, some volatile chemicals from the infested leaves with whitefly were emitted in a greater quantity when compared to the uninfested leaves. These compounds were 6-decenal, decane, (e)-2-hexenal, and the compound of (z)-3-hexenol were also greater in infested eggplant leaves compared to the uninfested ones (Table 1). Moreover, the mean compound peak area that was released from infested eggplant leaves was higher than the mean peak area in the uninfested eggplant leaves in ethyl acetate, 3-octenone, nonanone, 2,4-octadienal, tridecane, methyl salicylate and pentadecane (Figure 1).
The volatile compounds analysis by HS-SPME demonstrated quantifiable variations in the volatile produced from infested eggplant leaves with whitefly compared with uninfested leaves of the Zummord cultivar of eggplant.

Table 1. Mean+STD of Volatile compounds detected in of infested and uninfested eggplant with *Bemisia tabaci*.

RT[1]	KI[2]	compounds	Uninfested leaves	Infested leaves
3.42	720	ethyl acetate	234.52	0
5.93	927	(E)-2-hexenal	0	11.52
6.58	982	(Z)-3-hexenol	0	10.25
6.83	1004	decane	0	44.40
8.19	1131	3-octenone	54.41	8.79
8.70	1182	nonanone	348.89	52.32
9.35	1249	2,4-octadienal	33.43	12.67
9.77	1295	6-decenal	0	19.29
9.81	1299	tridecane	49.77	10.48
11.29	1472	methyl salicylate	38.49	0
11.53	1500	pentadecane	191.84	0

[1] RT= retention time; [2] KI= Kovats retention index.

The released quantities of ethyl acetate, 3-octenone, nonanone, 2,4-octadienal, tridecane, methyl salicylate and pentadecane decreased in the number of infested plants in parallel with uninfested plants. As a result of the whitefly infestation, however, 6-decenal, decane, (e)-2-hexenal, and (z)-3-hexenol emissions increased. This result is in line with the findings of [8], who claimed that the Trialeurodes vaporariorum infestation was a major factor in the variations in plant volatile emissions from infested plants compared to uninfested plants. In addition, Darshanee et al. [8] also reported that (Z)-3-hexenol was one of the VOCs that raised after the infestation with T. vaporariorum and this is consistent with the increase of the emission of the compound (Z)-3-hexenol from our results. According to a study by Wei and Kang [12], the chemical (Z)-3-hexen-1-ol directly affects how various herbivores behave. Curiously, (Z)-3-hexen-1-ol was not present in uninfested eggplant. The outcomes for (e)-2-hexenal and 6-decenal were the same. However, compared to uninfested eggplant, infested eggplant showed a surprising ability to create some VOCs that attracted *B. tabaci*. However, we might presume that these compounds contribute significantly to the plants' allure.

Olfactometer bioassay

Results of using the Y-tube olfactometer for testing the attractiveness of white-fly *B. tabaci* (n = 20 for each replicate) towards infested and uninfested eggplant are reported in figure 2. Results showed significant differences between the treatments. In specific, 81.77 % of whitefly adults were attracted to the VOCs released from infested eggplant, while 18.33% made a choice toward uninfested eggplant (Chi-square (χ^2) =40.96, Degree of freedom (df)=1, (*P*-value) $P < 0.001$).

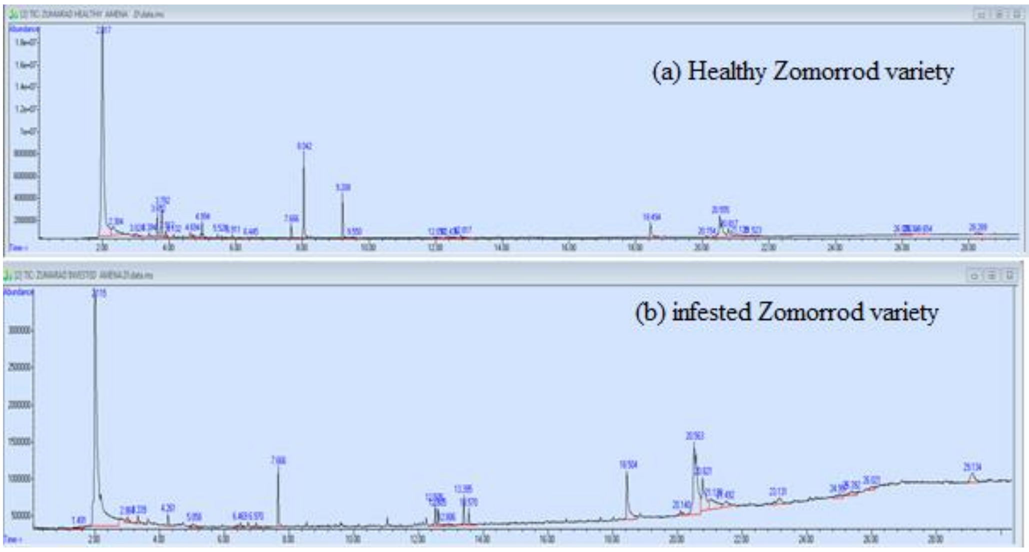

Figure 1. GC-MS analysis for eggplant variety zomorrod. (a) Represented the GC-MS analysis for healthy eggplant, and (b) Represented infested eggplant with *Bemisia tabaci*.

In addition, VOCs released from infested eggplant were more attractive to *B. tabaci* adults were 88.33%, compared with clean air, which was 11.67% (χ^2 =57.76, df=1, P < 0.002). While the findings also indicated that *B. tabaci* were noticeably more attractive to the uninfested eggplant leaves by 88.33% versus. 11.67% in clean air, (χ^2 =57.76, df=1 and P < 0.002).

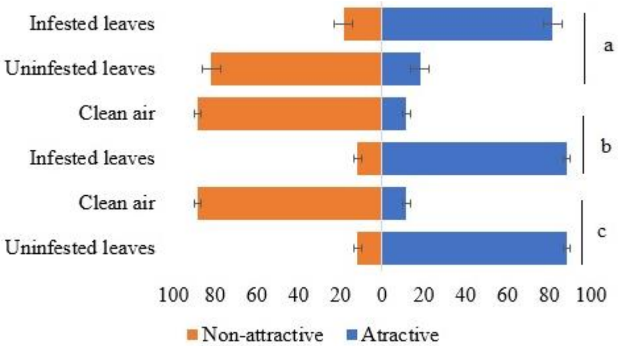

Figure 1. Preference of *Bemisia tabaci* to three different treatments (a) infested eggplant versus uninfested eggplant (b) infested eggplant versus clear air (c) uninfested eggplant versus clear air.

Our findings showed that constitutive volatiles from eggplant could be a significant factor in the choice of *B. tabaci* host plants. That was corroborated by the outcomes of Y-tube olfactometer bioassays. In this investigation, we discovered that *B. tabaci* significantly preferred both uninfested and infested plants over clean air. Whereas whiteflies were attracted to infested eggplant leaves in large numbers compared with the attraction to intact eggplant leaves. Once initially targeting the host plant, *B. tabaci* mainly considers plant olfactory cues to locate the host plant [7]. The quantity of volatile emissions from infested eggplant is strong enough to make them more attractive than uninfested eggplants, which is a plausible explanation for our findings. *B. tabaci* may possibly use olfaction as the principal trigger during the host-finding behavior. Insects that feed on plant sap can activate the salicylic acid (SA) signaling pathway to make it easier for them to provide, reducing the effectiveness of jasmonic acid defenses [13]. It is conceivable that *B. tabaci* has the capacity to alter volatile emissions from uninfested plants. This is consistent with Birkett et al. [14], who reported that T. vaporariorum-infested plants emit volatile blends that are quantitatively different from those produced by uninfested plants. Therefore, it can be said that *B. tabaci*'s reaction to the VOCs released by the infested plants is an inherent trait. Add the conclusion of the results.

CONCLUSIONS

In summary, our experiments received remarkable results, showing that infested eggplant could attract a significantly larger quantity of whiteflies than could in-tact eggplants. Our study demonstrated that decane, (e)-2-hexenal, (z)-3-hexenol, and 6-decenal as important cues for behavioral changes of *B. tabaci*. Even though we have confirmed that vast numbers of whiteflies were attracted to infested eggplants based on the four volatile compounds, the volatile blends accountable for attracting such a massive number of whiteflies in odour-mixed natural conditions are still nevertheless tough to determine. Hence, to the utilization of selected volatiles for developing sustainable semiochemical management, moreover, experiments should be performed to get assure the volatile com-pound composition that successfully attracts whiteflies under greenhouse or field conditions.

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Informed Consent: All patients gave their written informed consents before inclusion.

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