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RESPONSES OF COTTON APHID *APHIS GOSSYPII* AND ITS PREDATORS TO VOLATILE ORGANIC COMPOUNDS EMITTED FROM INFESTED AND UNINFESTED CUCUMBER PLANTS

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ABSTRACT

The response of cotton aphid *Aphis gossypii* adults and their predators *Coccinella septempunctata* and *Chrysoperla carnea* to infested or uninfested cucumber leaves was tested using a Y-shape olfactometer. Aphids were significantly more attracted to uninfested than infested cucumber leaves. *C. septempunctata* was significantly more attracted to the infested leaves with aphids than uninfested cucumber leaves. At the same time, *C. carnea* responded to VOCs from leaves infested with aphids compared to healthy leaves. Moreover, the feeding activity of aphids rustles to release Kairomonal compounds, which can be positively exploited by C. carnea to identify the location of aphids and infested plants. Our results indicated that predators could distinguish between infested and uninfested leaves, and their responses were significant to the differentially released VOCs from infested plants. VOCs were extracted using HS-SPME from *A. gossypii*-uninfested and infested cucumber leaves and analyzed by gas chromatography-mass spectrometry. 35 compounds were identified in uninfested and infested leaves; 7 VOCs were detected in infested cucumber leaves and were not detected in uninfested leaves. In conclusion, the findings from the current study on how cotton aphids and predators respond to infested leaves compared with uninfested leaves and clean air are due to the emission of volatile organic compounds that reflect the traits of the plant host.

Key words: **aphids, beneficial insects, *Chrysoperla carnea*, semiochemicals, VOCs, SPME, olfactometer.**

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INTRODUCTION

Cucumis sativus L. (Cucurbitaceae), cucumber, is an important vegetable crop in Iraq, grown during spring and autumn, and also in greenhouses all year round (Doijode, 2001). Cucumber production in Iraq was approximately 240,000 tonnes in an area of 24,000 hectares in 2020 (CSOI, 2022). Like other vegetables, cucumber suffers from several arthropod pests, including mites and the insect's red pumpkin beetle *Raphidopalpa foveicollis*, melon ladybird *Epilachna chrysomelina*, two-spotted spider mites *Tetranychus urticae*, tobacco whitefly *Bemisia tabaci*, green peach aphid *Myzus persicae*, cotton aphid *Aphis gossypii*, and onion thrips *Thrips tabaci* (Redha et al., 2016; Hooper et al., 2022). In addition, *A. gossypii* (Hemiptera: Aphididae) is one of the most important, causing serious losses in cucumber crops and other cucurbitaceous and non-cucurbitaceous plants (Ali, 2017; Bachmann et al., 2014; Luo et al., 2016). In this context, Aphids are polyphagous insects and are among the main pests that attack around 90% of economically important plants, causing direct and indirect damage to crops (Ali, 2017; Lee et al., 2022). Nymphs and adults of cotton aphids attack

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different plant parts as they feed on the lower side of the leaf (Ahmed, 2022). *A. gossypii* is present in most parts of the world. Direct damage by aphids results from feeding on plant sap, which can cause yellowish spots on the leaves that lead to deformation, curling of the leaves, and dwarfing of the plant, and sometimes death, in addition to losses in both quantity and quality (and value) of the fruit (Ahmed et al., 2022). Honeydew secretions encourage the growth of fungi and impede photosynthesis, respiration, and transpiration (Ahmed, 2022). Arthropods, including aphids, are primary vectors for many plant viruses, which themselves cause damage and loss of value to plants (Ahmed et al., 2021). Aphids can transmit more than one viral disease and cause yield losses of 20-100%, depending on the type of plant, its preference for the aphid, the means of defense it possesses, and environmental conditions (Luo et al., 2016). Volatile organic compounds (VOCs) released by plants play a significant role in plant defense against insects, acting directly as repellents or toxins to pests, or indirectly by recruiting natural enemies, such as predators of pests (Ahmed et al., 2022; Ahmed & Hermize, 2023). Direct defense methods, such as the production or increased expression of toxins, unpalatable compounds, and insect repellents, negatively affect the pest and its behavior (Allmann & Baldwin, 2010). These compounds also have a systemic signaling function, attracting natural enemies and alerting neighboring plants to defend themselves from attack (Rodriguez-Saona & Frost, 2010). Based on Olfactometer experiments and Gas Chromatography Analysis (GC-MS), several studies have shown that M. percicae aphids and their parasitoids and predators are more attracted to terpenoid VOCs, such as limonene, terpinolene, methyl salicylate, and hexanol-3, which are overexpressed in infested plants (Song et al., 2017). Takemoto and Takabayashi (2015) reported that parasitoids are attracted to aphids using the glass Y-shape olfactometer choice experiment based on the VOCs released from infested plants. Earlier experiments (Rodriguez-Saona & Frost, 2010; Ahmed et al., 2021) showed that the infested plant released a variety of VOCs. Other research (Schettino et al., 2017) suggests that injured plants may release different volatile compounds depending on the aphid feeder, which could change the behavior of predators and the attraction of aphids to infested plants. Because aphids feed on plants, VOCs are produced, which significantly attract predators. Contrary to this, a study found (Ahmed et al., 2022) that the VOCs released in these circumstances may serve as useful indicators for predators and parasitoids. This can lead to the reliance on helpful insects to direct them by emitting VOCs from infested plants that attract them. Finding other ways to control pests is a key factor in Integrated Pest Management. This study aimed to identify alternative methods for controlling *A. gossypii* on cucumber in greenhouses using plant VOCs as an integrated pest management (IPM) program in an organic farm.

MATERIALS AND METHODS

Experimental plants: For the Experiment plants, seeds of the cucumber cultivar Super Faris F1 (Vanguard Seeds Company, California, USA) were planted in 1 L pots filled with bark/sand potting mix and maintained in an insect-proof greenhouse. When plants were at the 4-5 leaf stage (n=12 cucumber seedlings, 3 replicates for each uninfested and infested cucumber seedling), they were transferred to rearing cages (40 cm x 40 cm x 40 cm) covered with white mesh to exclude external arthropods. Apterous *A. gossypii* adults (n=50) were introduced in three cages separately when the cucumber plant reached 5 to 6 leaves. Cages containing cucumber seedlings were transferred to the laboratory for the study.

Insect rearing: Cotton aphid *A. gossypii*, seven-spot ladybird *C. septempunctata*, and green lacewing *C. carnea* were reared in a laboratory at the Department of Diagnosing Natural Enemies, Directorate of Plant Protection, Ministry of Agriculture, Iraq. Cotton aphid *A. gossypii* adults were collected from the cucumber field located in Abu Ghraib near Baghdad city (within 15 km). Cotton aphids were reared on cucumber seedlings (as above) after confirmation of identity via using a dissecting microscope by an entomologist at

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the Department of Plant Protection, University of Baghdad. Seedlings were transferred to insect-proof cages (as above) and provided with a zip door on one side to facilitate the entry and exit of the pot into the cage. Aphids were reared under laboratory conditions at 25±2 °C and 60-70% RH using a Hygrometer and Thermometer (ThermoPro TP50, CA, USA) and an 8:16 day/night photoperiod. Adults of seven-spot ladybirds *C. septempunctata* were collected from the wild-infested plants sweep-net. A pair of seven-spot ladybird adults was transferred to the aphid-infested cucumber seedlings. A piece of cotton moistened with water was placed in each cage to maintain humidity. Cucumber plants were continuously checked for ladybird eggs; when found, they were removed with a soft brush and micro dissecting needles (BioQuip, CA, USA). Eggs were transferred to Petri dishes. Upon hatching, larvae were transferred to cages containing infested cucumber plants with aphids, where the larvae of the seven-spot ladybirds continued to feed on cotton aphids. Then, they were transferred to Petri dishes when the larvae reached the pupal stage and incubated at 24 °C in an incubator until adults formed (Albittar et al., 2016). Moreover, the adults of the green lacewing were collected from the infested weeds by cotton aphids. Green lacewings were reared under laboratory conditions at 25±2 °C, 60-70% RH, and 16 h light/day. Adults were fed a sugar solution consisting of equal proportions of honey, water, palm pollen, and yeast (19). After eggs were laid and hatched, larvae were isolated and transferred to 50 ml glass tubes to prevent self-predation; each larva was placed in one glass tube. Larvae were fed on the eggs of the Mediterranean flour moth *Ephestia kuehniella*, after exposure to 2°C for 20 days in incubators. Pupae were also isolated individually in glass tubes until they reached the adult stage (Jalloud et al., 2013).

Extraction of VOCs using HS-SPME

Cucumbers uninfested and infested by *A. gossypii* were divided into two groups and kept in fabric cages covered by fine mesh to prevent further infestation of cucumber seedlings by aphids and other insects. The first group of cucumber seedlings was infested with *A. gossypii*, and the other group was free of infestation. Each cage contained three replicates of potted seedlings. When the cucumber seedlings reached the 5-6 leaf stage, one group of plants was infested with cotton aphids and left for five days before VOCs collection. Uninfested plants were kept for the same period under the same conditions, minus aphids. Headspace solid-phase microextraction (HS-SPME) was used to extract VOCs from uninfested and infested cucumber plants. The fiber SPME type was 50/30 µm divinylbenzene/carboxen/polydimethylsiloxane (PDMS/Car/DVB) (Sigma-Aldrich, Australia, Catalogue Number 57347-U). Four uninfested and four infested cucumber leaves were placed into separate sterile 1 L glass jars, which were sealed with aluminium foil and left for 6 h (sealing time) to concentrate the VOCs inside. Then, a fiber of SPME was inserted into the headspace of the glass jar over the cucumber leaves to extract the VOCs. The SPME fiber was left for 2 h (extraction time) at 25± 2 °C, 60-70% RH. The SPME fiber was then withdrawn into the fiber needle, removed from the sample's headspace, and injected directly into the GC-MS device for VOC analysis as described (Ahmed et al., 2022).

GC-MS analysis of extracted VOCs

The VOCs extracted by HS-SPME were analyzed using an Agilent Technologies 7820A Gas Chromatograph (GC) instrument connected to an Agilent Mass Spectrometer (MS) E5977 (Santa Clara, CA 95051, USA). The separation column used was fused-silica capillary tubing DB-35ms (30m × 250µm × 0.25µm) (Agilent Technologies J and W GC Column, USA). The analysis method was set up in the GC-MS before injecting the SPME fiber into the GC injection port. Injector and detector temperatures were 250 and 280 °C, respectively. The carrier gas was Helium (He) at a flow rate of 1 mL/min. The GC oven temperature was held at 50 °C for 2 min and then increased by 5 °C/min to 250 °C. Electron-impact ionization spectra were obtained at 70 eV, with mass spectra recorded from 40 to 550 amu. The VOCs were identified using the chemical library of the

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National Institute of Standards and Technology (NIST, 2008) MS database.

Y-shape Olfactometer set up and assessment: A Y-shape Olfactometer made of glass was used to conduct the behavioral response of *A. gossypii* and their natural enemies to uninfested and infested cucumber plants. The glass Y-shaped Olfactometer consisted of two arms, 7 cm long and 2 cm in diameter, linked by plastic tubes, with two 2 L glass desiccators. Each desiccator was connected to a pure air source filtered through activated charcoal, with an airflow of 100 ml/min (Ahmed et al., 2021). The mixtures of VOCs released from infested and uninfested leaves were stored inside sealed desiccators, and the aphids and predators were released into the Y-tube base individually (N=30) for each insect species (**Figure 1**). The response of aphids and their predators (*C. septempunctata* and *C. carnea*) was tested using the following three treatments: (a) cucumber leaves infested with aphids compared to filtered air; (b) uninfested cucumber leaves compared to filtered air, and (c) cucumber leaves infested with aphids compared to uninfested leaves. One individual aphid or one species of predator insect (N=30) was released in the central arm of the Olfactometer for a distance of 2 cm. Responses were recorded after 5 min; as soon as the individual moved to one of the side arms, the direction of response was recorded, and the insect was removed and replaced with a new individual. Three replicates were used for each experiment. After each test using 10 individuals, all instrument parts were treated with 70% ethanol, washed with water, and dried in an oven at 200 °C for 1 h. Three concentrations of methyl salicylate (10, 50, and 100 µl/mL) were used to test the behavior of two predators, *C. septempunctata* and *C. carnea* (N=30 for each predator), using a Y-tube olfactometer (Figure 1). A concentration of 50 µl/mL was used in the greenhouse, where cucumber plants were kept free of chemical pesticides and allowed to grow naturally, with infestation by cotton aphids and the natural enemies growing under greenhouse conditions.

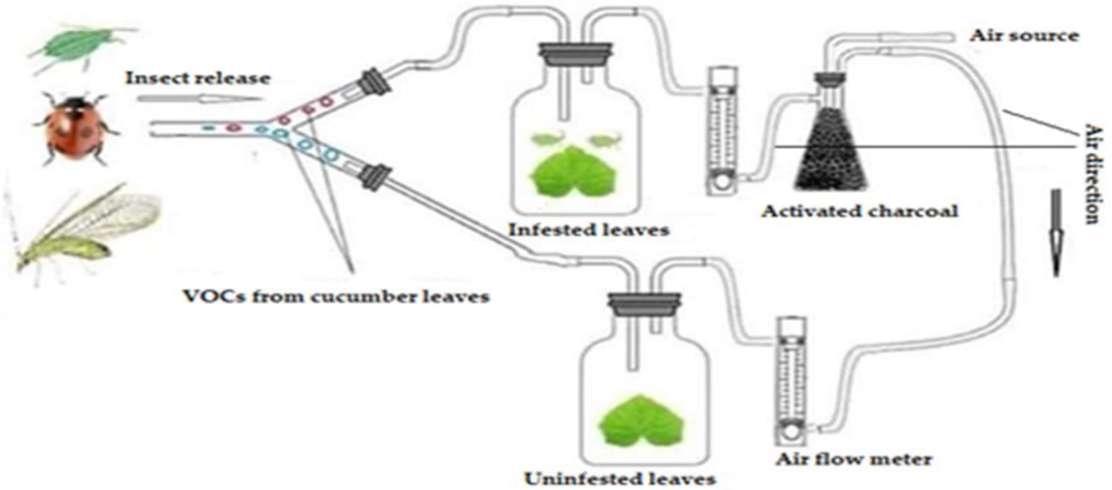

Figure 1. Components of the Y-shape olfactometer used with three insect species: *Aphis gossypii*, *Coccinella septempunctata*, and *Chrysoperla carnea*. (Ahmed et al., 2022)

Statistical analysis

Analysis of differences in VOC emissions between infested and uninfested cucumber leaves was performed using MetaboAnalyst version 5.0 to generate P-values and hierarchical heat maps (Chong et al., 2018). Differences in the means were compared by using the least significant difference test (P≤0.05). The peak area was divided by 100,000 for each compound to reduce the numerical values. Y-shape Olfactometer choice experiments were analyzed using the Chi-Square test at the probability (P-value) level of 0.05 using the Statistical Package of SPSS Version 9.1 (IBM Company, CA, USA).

RESULTS AND DISCUSSION

Identification of the insects: Cotton aphid *A. gossypii*, seven-spot ladybird *C.*

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septempunctata, and green lacewing *C. carnea* were collected from cucumber plants and accompanying weeds located in the field of cucumber in Abu Ghraib near Baghdad city, Iraq. The species of *A. gossypii*, *C. septempunctata*, and *C. carnea* were identified by entomologists at the Iraqi Natural History Research Center and Museum, University of Baghdad, Iraq.

1. *Aphis gossypii* (Glover, 1877) (Hemiptera, Aphididae).
2. *Coccinella septempunctata* (Linnaeus, 1758) (Coleoptera, Coccinelidae).
3. *Chrysoperla carnea* (Stephens, 1836) (Neuroptera, Chrysopidae).

GC-MS analysis of VOCs: The qualification and quantification of volatile chemicals released from cucumber leaves infested with or uninfested with cotton aphids were evaluated. The data obtained from chemical analysis of VOCs from uninfested and infested cucumber leaves by *A. gossypii* are reported in **Table 1**. About 35 compounds were identified from both treatments. Significant differences were found between the infested and uninfested cucumber plants. Aphid infestation altered the chemical profile of the cucumber plants. The chemical profile of VOCs emitted from the infested leaves was higher than that from the uninfested plant. Especially, d-limonene, methyl salicylate, undecane, octadecanoic acid, hexadecane, corticosterone, bis-trimethyl, 2-methyl butyl benzoate, t-cadinol, and oleic acid make up the majority of the changes in the composition of the VOCs coming from these treated plants. However, some compounds were detected by GC-MS analysis in infested cucumber leaves but not in uninfested leaves, including phenol, guaifenesin di-tms, 6-methyl-5-hepten-2-one, carbonic acid, 3,3-dimethyl-2-pentanol, z,e-farnesene, and acetic acid.

Table 1. Volatile organic compounds released from uninfested and infested cucumber cultivar Super Faris leaves by *Aphis gossypii* using HS-SPME technique

No.	Compound	RT [1]	Infested mean area ±SD [2]	Uninfested mean area ±SD	P-value
1	carbon dioxide	0.51	255.57±23.29	329.66±434.23	0.731
2	1,2-propanediamine	1.30	174.44±55.71	226.34±296.33	0.081
4	benzoic acid, ethyl	5.32	203.57±163.29	177.41±47.46	0.341
5	benzaldehyde,ethyl	7.61	186.76±86.51	478.43±346.41	0.024*
6	d-limonene	8.12	228.33±9.90	48.96±39.78	0.016*
7	nonane, 2-methyl	9.31	309.55±204.24	281.18±211.29	0.359
8	2-propanol	9.84	217.21±129.35	363.83±231.98	0.283
9	decane	11.15	243.18±104.42	212.58±175.08	0.070
10	phenol	11.94	240.17±199.31	ND [3]	0.250
11	methyl salicylate	12.17	214.98±72.183	23.79±17.42	0.011*
12	undecane	14.11	163.24±65.68	59.46±13.35	0.267
13	guaifenesin di-tms	15.65	38.25±7.36	ND	0.055
14	octadecanoic acid	15.87	256.28±214.80	40.10±26.79	0.074
15	hexadecane	16.09	285.17±266.48	113.58±110.80	0.015*
16	heptane,2,6-dimethyl	16.22	328.61±222.80	276.36±273.71	0.487
17	nonanal	17.02	260.48±82.04	453.84±248.54	0.001*
18	1-heptadecyl ester	18.35	295.92±279.65	396.09±320.99	0.031
19	2-ethoxypropane	19.06	111.98±86.85	348.61±230.12	0.281
20	n-heptadecan-1	19.38	129.24±104.83	104.61±86.96	0.168
21	fumaric acid	19.68	243.45±238.01	11.40±6.25	0.268
23	1,3-dioxolane-5-ol	22.38	56.22±0.25	6.79±5.47	0.059
24	corticosterone,bis-trimethyl	23.15	371.98±278.03	6.67±5.59	0.027*
25	6-methyl-5-hepten-2-one	23.51	331.29±226.26	ND	0.026
26	carbonic acid	24.68	248.55±190.93	ND	0.024*
27	3,3-dimethyl-2-pentanol	24.71	534.94±341.78	ND	0.029*
28	2-methylbutyl benzoate	25.42	158.13±135.57	2.59±1.23	0.068
29	z,e-farnesene	26.83	71.86±5.91	ND	0.052*
30	tridecane, 2-methyl-	27.06	57.71±7.19	19.61±17.88	0.472
31	acetic acid	27.18	166.09±132.35	ND	0.024*
32	t-cadinol	28.86	106.87±93.32	18.21±16.08	0.274
33	oleic acid	29.37	151.06±128.76	24.97±21.14	0.349
34	oxalic acid	31.37	150.92±122.35	107.06±72.76	0.274
35	malonic acid	40.75	369.84±178.40	467.83±328.71	0.580

[1] RT is the retention time of compounds
[2] SD is the standard deviation of the peak area
[3] ND is not detected by GC-MS.
* Significant differences at the level of 0.05

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A heat map (**Figure 2**) illustrates the hierarchical clustering of VOCs from infested and uninfested plants, indicating correlations and differences among the components detected by GC-MS. Grouping uninfested and infested cucumber leaves into two different groups emphasized the differences between treatments; the heat map displays the chemical profile for VOCs with the value of compound characterized by dark red, orange, and dark blue for the maximum (2) as represented by a dark red, average (0) as represented by white, and minimum (–2) as represented by dark blue. The first cluster contained the VOCs released by uninfested leaves, while the second cluster contained those VOCs released by infested leaves.

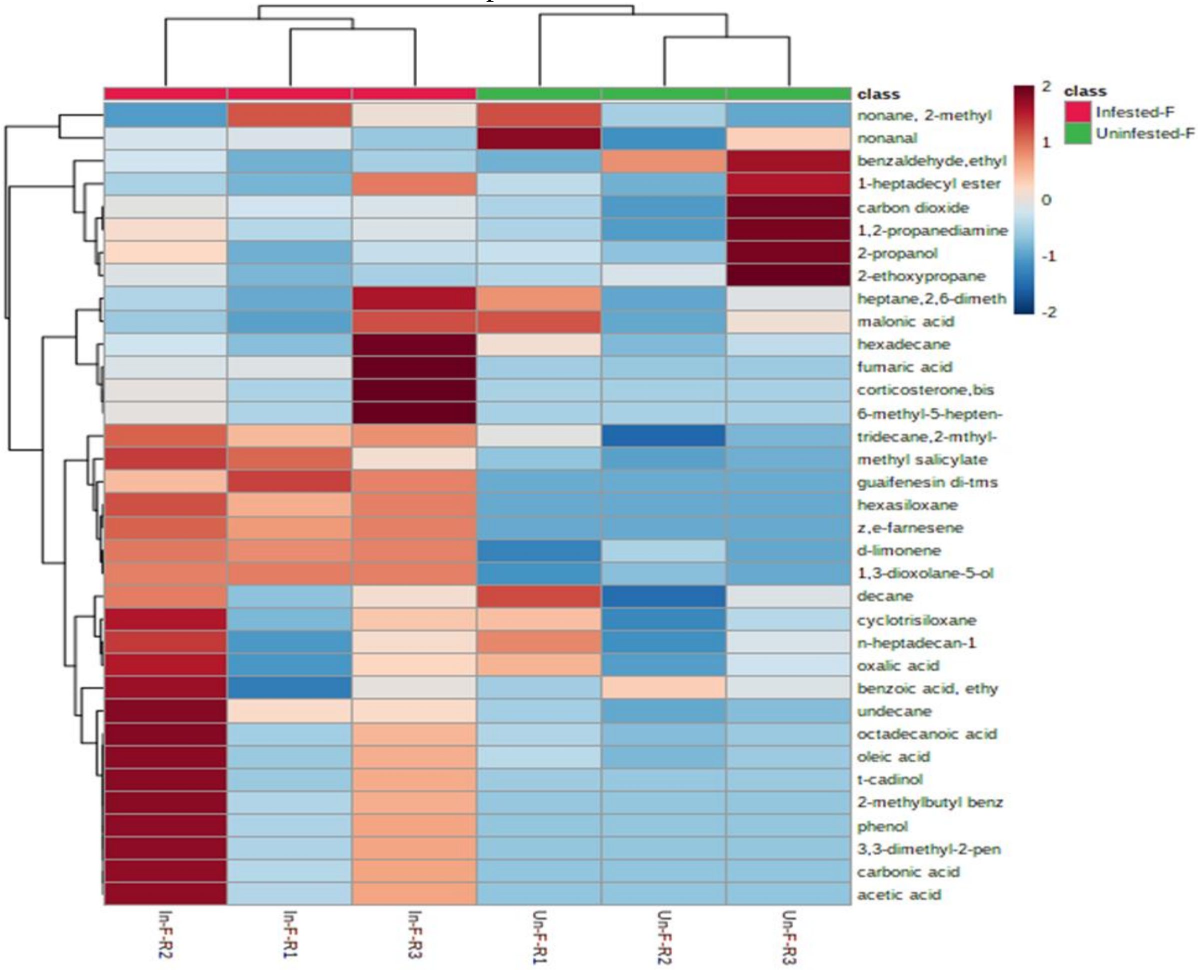

Figure 2. Heat map of VOCs released from *Aphis gossypii*-uninfested and infested cucumber leaves. Clustering is measured by the distance between samples using correlation and clustering algorithms. The abundance of metabolites recorded in each sample is illustrated as high (deep red) to low (deep blue; see legend, top right).

Aphids and their predators utilize volatile organic compounds (VOCs) released by cucumber plants to find and identify their host plants. A glass Y-shape olfactometer is used to assess the attraction of *A. gossypii* to both uninfested and infested cucumber leaves caused by aphid-feeding activity. In the same context, the aphid predators *C. septempunctata* and C. carnea could distinguish between *A. gossypii*-infested and uninfested cucumber leaves, and their favored response to VOCs released from the infested plants was significantly higher than that of the uninfested plants. Additionally, because aphid feeding leads plants to emit VOCs, treating infested leaves had a greater impact on the plant's response than treating uninfested leaves. Predictably, predators preferred VOCs emitted by leaves infested with their prey, *A. gossypii*. The current study showed qualitative and

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quantitative differences among VOCs emitted by *A. gossypii* in uninfested and infested cucumber leaves. In contrast to earlier investigations (Rodriguez-Saona & Frost, 2010; Ahmed et al., 2022), our study revealed various VOCs emitted by cucumber leaves upon infestation by the cotton aphid. According to another study (Schettino et al., 2017), we assumed that damaged plants might release volatile chemicals that attract aphids, which might alter aphid predators and their attraction to infested plants. The VOCs released by cucumber plants during aphid feeding attract predators. The VOCs produced in these situations may serve as helpful cues for predators and parasitoids, in contrast to the findings of Ahmed et al. (2022). The dependence of beneficial insects on the information provided by released VOCs from infested plants that attract them (Schettino et al., 2017). Comparing the headspace composition of plants with and without aphid infestations, there were apparent variations, as reported in **Table 1**. The observed qualitative differences, particularly for d-limonene, methyl salicylate, un-decane, octadecanoic acid, hexadecane, corticosterone, bis-trimethyl, 2-methyl butyl benzoate, t-cadinol, and oleic acid. Some of the mentioned VOCs, such as d-limonene and methyl salicylate, already serve as aphid alarm VOCs and are known to recruit natural enemies (Hatano et al., 2008). It seems that *C. septempunctata* and *C. carnea* are susceptible to d-limonene and methyl salicylate as attractants. One of the key distinguishing factors between the two sample groups was methyl salicylate, a well-known volatile produced by aphids via the salicylic acid pathway (Ponzio et al., 2016). Numerous studies have assessed how it affects predators and parasitoids by attracting them (Taveira et al., 2009; Mathur et al., 2013). The VOCs of Phenol, n-heptadecan-1, Me-thyl-5-hepten-2-one, and Carbonic acid were not found in the headspace of uninfested cucumber plants with aphids, probably because cucumber plant tissues do not damage as described in (Silva et al., 2017), who reported that some alcohol, monoterpene, and ester chemical classes were not found in the uninfest

Olfactometer bioassay: The Y-shape olfactometer bioassay responses of *A. gossypii* and three of its predators are reported in (**Figure 3**). *A. gossypii* was attracted to infested cucumber leaves by 6000% compared to the clean air treatment (30.00%) (χ^2=10.57, df =1, P-value=0.0007, n=30). *A. gossypii* was more attracted to uninfested leaves than to clean air, at 46.67 and 16.67% (χ^2=10.98, df=1, P-value=0.0007, n=30), respectively. Furthermore, the comparison of *A. gossypii* attraction to uninfested and infested leaves revealed they were more attracted to uninfested leaves, 60.00 and 16.67% (χ^2=12.74, df=1, P-value=0.0001, n=30), respectively. On the other hand, in (**Figure 4**), the behavioral results of *C. carnea* adults showed a greater attraction to infested leaves (63.33%) than to clean air (36.66%) (χ^2=9.326, df=1, P-value=0.0028, n=30). *C. carnea* showed no significant preference for uninfested leaves and clean air at 43.33 and 56.66% (χ^2 =5.37, df=1, P-value=0.0371, n=30), respectively. However, *C. carnea* was significantly more attracted to infested leaves than to uninfested leaves, at 70.00 and 30.00% (χ2=12.25, df=1, P-value=0.0001, n=30), respectively. The olfactometer assay showed the behavioral response of predator *C. septempunctata* (**Figure 5**). Attraction to infested leaves by cotton aphids and seven-spot ladybirds was significantly greater than to clean air, and the percentage of attraction towards treatments was 60.00 and 30.00% (χ^2=10.57, df=1, P-value=0.0007, n=30), respectively. However, the attraction of *C. septempunctata* to infested leaves was significantly greater than to the clean air treatment, at 63.33 and 36.66% (χ^2=9.326, df=1, P-value=0.0028, n=30), respectively. In the attraction towards the uninfested leaves compared with the clean air, *C. septempunctata* selected uninfested leaves by 56.67% and 43.33% (χ^2=5.376, df=1, P-value=0.0371, n=30), respectively. Conversely, the attraction of *C. septempunctata* towards infested leaves was significantly greater than the attraction to uninfested leaves, at 63.33 and 36.66% (χ^2=9.326, df=1, P-value=0.0028, n=30), respectively

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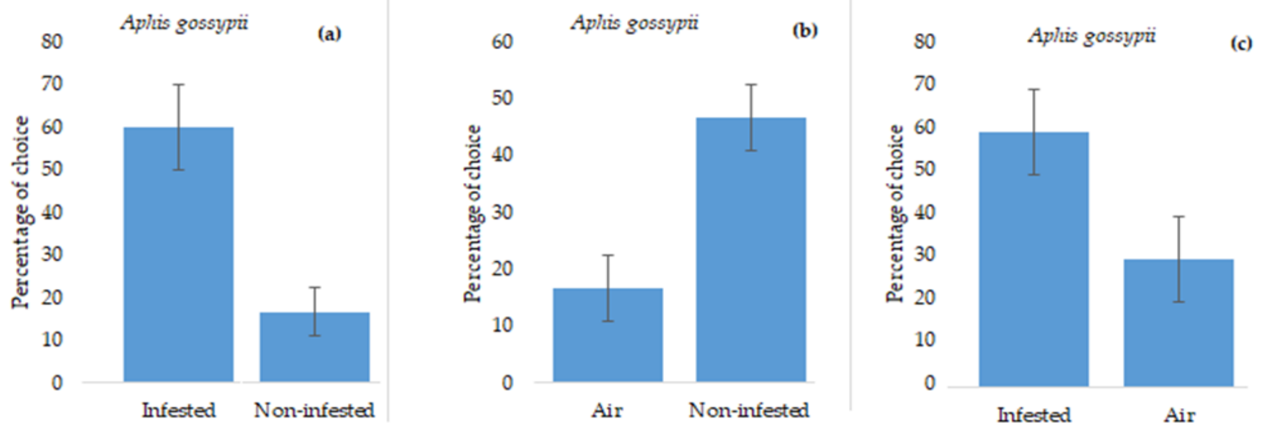

Figure 3. Behavioral response of Aphis gossypii to VOCs released from A. gossypii-infested and uninfested cucumber leaves, (a) Comparison between infested and uninfested leaves, (b) comparison between uninfested leaves and clean air, (c) comparison between infested and clean air. All results are presented with standard deviation (SD).

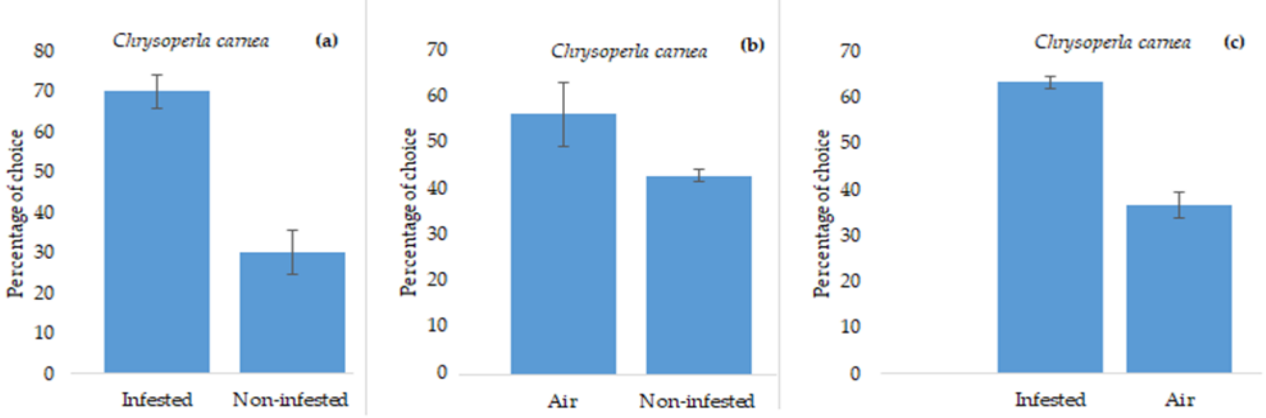

Figure 4. Behavioral response of Chrysoperla carnea to VOCs released from A. gossypii-infested and uninfested cucumber leaves, (a) Comparison between infested and uninfested leaves, (b) comparison between uninfested leaves and clean air, (c) comparison between infested and clean air. All results are presented with standard deviation (SD).

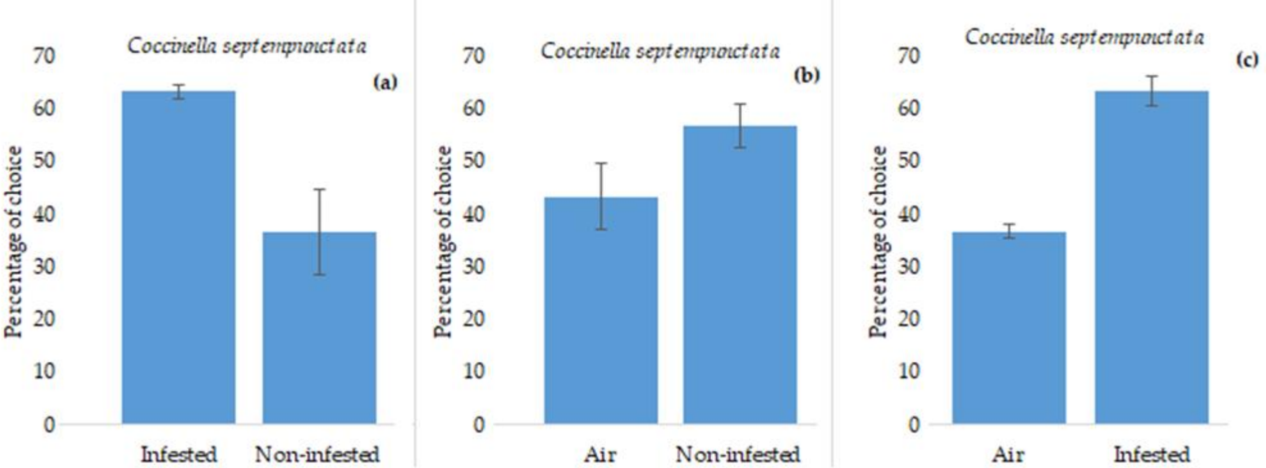

Figure 5. Behavioral responses of *Coccinella septempunctata* to VOCs released from *A. gossypii*-infested and uninfested cucumber leaves, (a) Comparison between infested and uninfested leaves, (b) comparison between uninfested leaves and clean air, (c) comparison between infested and clean air. All results are presented with standard deviation (SD).

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In the experiments testing the chemical compound methyl salicylate (MS) and its role in attracting aphid predators using the olfactometer. **Table 2** shows the percentage of attraction of the predators *C. septempunctata* and *C. carnea* to the chemical compound MS, uninfested and infested leaves with *A. gossypii*. The statistical analysis showed significant differences between uninfested leaves and MS-treated leaves. The results of the experiment treating uninfested leaves with the chemical compound MS at a concentration of 10 µl showed that the predator C. septempunctata was equally attracted in both treatments, with 05% attraction. While the comparison between uninfested cucumber leaves and MS compound at a concentration of 50 µl, the attraction went towards the compound by 63.33% more than the attraction to uninfested leaves by 33.33%. At 100 µl, the results showed that the predator C. septempunctata was attracted to uninfested leaves by 53.33% and to MS by 30%. On the other hand, in the comparison between three concentrations (10, 50, and 100 µl), the results indicated that *C. septempunctata* was attracted to the MS compound by 53.33, 76.66, and 73.33%, respectively. The attraction of the ladybird toward infested leaves was 33.33, 20, and 26.66%, respectively. The results of **Table 2** showed the response of the predator *C. carnea* when comparing uninfested and infested cucumber leaves treated with MS compound at three concentrations (10, 50, and 100 µl). The results indicated that, when comparing uninfested leaves and the MS compound at a concentration of 10 µl, the percentage of predator attraction to the compound was 60.21%, compared with 36.33% for uninfested leaves. When comparing the two treatments between uninfested leaves and MS compound at a concentration of 50 µl, the *C. carnea* was attracted to MS chemicals more than they were going toward uninfested leaves, with a percentage of 63.33% and 33.33%, respectively. While the comparison results in the attraction of the predator *C. carnea* to uninfested leaves and to the MS compound at a concentration of 100 µl, the percentage of predator attraction to the MS compound was 30% and 66.66% towards uninfested cucumber leaves. Whereas the percentage of attraction of *C. carnea* in the treatment of infested leaves and MS compound at different concentrations of 10, 50, and 100 µl were 43.33, 33.33, and 73.33% toward infested cucumber leaves, respectively. The attraction of *C. carnea* was 56.66, 66.66, and 23.33% toward the MS compound, respectively.

Table 2. Behavioral responses of *Coccinella septempunctata* and *Chrysoperla carnea* to different concentrations of methyl salicylate.

Predator names	Treatments	Response percentage to methyl salicylate 10µl	50µl	100µl
Chrysoperla carnea	Methyl salicylate	60.21	63.33	30
	Uninfested leaves	36.33	33.33	66.66
	Chi-Square	8.62*	9.43*	8.78*
	P-value	0.0078	0.0026	0.0076
	d.f	1	1	1
Chrysoperla carnea	Methyl salicylate	56.66	66.66	23.33
	Infested leaves	43.33	33.33	73.33
	Chi-Square	5.376*	9.37*	11.97*
	P-value	0.0371	0.0007	0.0001
	d.f	1	1	1
Coccinella septempunctata	Methyl salicylate	50	63.33	30
	Uninfested leaves	50	33.33	53.33
	Chi-Square	0.00 NS	9.43*	11.63*
	P-value	1	0.0026	0.0001
	d.f	1	1	1
Coccinella septempunctata	Methyl salicylate	53.33	76.66	26.66
	Infested leaves	33.33	20	73.33
	Chi-Square	7.25*	12.63*	11.72*
	P-value	0.0076	0.0001	0.0001
	d.f	1	1	1

***Indicates a significant difference P<0.05 (Chi-square test)**

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Based on olfactometer experiments, the findings demonstrate that *A. gossypii* preferred uninfested and infested cucumber plants, as the infested plants emitted several VOCs that can affect the tested insects' behavior. In the current investigation, glass Y-shape olfactometer bioassays of predators and parasitoids revealed that they were most strongly attracted to infested cucumber plants infested with cotton aphids, demonstrating that odor was a factor in their selection. Additionally, comparisons of GC-MS results showed that aphids fed on plants released only specific VOCs, indicating the importance of plant-associated chemicals in attracting parasitoids to their herbivore hosts. In addition, it has been reported that VOCs have an important ecological role in herbivorous insects (Taveira et al., 2009; Silva et al., 2017). VOCs produced by aphid-infested cucumber plants were substantially more attractive to aphid predators than volatiles from uninfested plants. The Y-shape olfactometer was used to assess the aphid *A. gossypii*'s reaction to the host plant. The results showed that the aphid was significantly attracted to both uninfested and infested plants compared to clean air choices. Cotton aphids prefer to feed on the young new leaves. Therefore, a prolonged high aphid population could move to new uncolonized leaves to reduce feeding competition and improve food quality for aphid development. Aphids can find their host plant by odor recognition (Jalloud et al., 2013; Chong et al., 2018). While parasitoids and predators were significantly attracted to infested cucumber plants, suggesting that the cucumber plant's volatiles had an impact on the aphid's and natural enemies' behavior. Beneficial insects such as predators and parasitoids may respond to volatile chemical compounds released from plants by aphid infestation; *Aphidius ervi* can detect infested plants by the 6-methyl-5-hepten-2-ol compound released in response to aphid attack (Albittar et al., 2016). Aphids can find their hosts in response to host VOCs, color, and size, and this attraction may serve as an excellent guide for them. Our results agree with previous studies (Schettino et al., 2017), which confirm that aphids find their hosts mainly through plant chemical cues. The study also showed that aphids' response to uninfested and highly infested plants tends to the volatile organic compounds emitted in high concentrations after feeding activity. Ladybirds are a biological control factor that limits the spread of aphids. Adult ladybirds can detect their prey (aphids) through olfactory signals. Plant VOCs may affect both phytophagous insects, such as aphids, and their natural enemies, and several variables will affect the adaptive importance of predator responses to chemical interactions between uninfested and infested plants. Our findings on predators' attraction to infested cucumber plants are consistent with what was reported (Bahlai et al., 2008; Vucetic et al., 2014), which declared that the ladybird *C. septempunctata* could be influenced by aphids *M. persicae* infestation and released VOCs, especially terpenoid compounds like (e)-nerolidol from onion and potato plants. The olfactometer bioassay results showed that ladybirds are more attracted to VOCs released from infested leaves than to those from uninfested leaves, because these blended volatile compounds, such as limonene and methyl salicylate, signal the presence of insect infestations on cucumber plants. Yoon et al. (2010) reported that the predator Harmonia axyridis was attracted to the prey, M. persicae, feeding on Chinese cabbage, and that it located infested plants via VOCs. Accordingly, aphids' attraction to plant volatiles can be explained as a response to feeding on the host plant (Will & van Bel, 2006; Zust & Agrawal, 2016). This indicates that natural enemies use chemical signals to locate their prey, such as volatile compounds emitted by aphid-infested cucumber plants, and this was also shown by Hatano et al. (2008). Similarly, *C. septempunctata* adults were attracted to the volatile organic matter from the infested leaves more than the uninfested leaves due to the *A. gossypii* feeding that stimulates an increase in the emission of volatile compounds in a higher concentration. Olfactometer experiments revealed that *C. septempunctata*'s food-seeking behavior may be significantly influenced by aphid volatiles. The reactions seen in these studies could be the natural ability of

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coccinellid adults to find their prey or the outcome of associative learning (Ninkovic et al., 2001). In addition, green lacewing *C. carnea* is attracted to the infested cucumber leaves due to the volatile compounds emitted due to the infestation of *A. gossypii*. The response of green lacewing to compounds released by aphid infestation also plays a role in attracting predators, as these VOCs are among the factors that can locate the host plant and prey (Schettino et al., 2017). Green lacewing *C. carnea* was more attracted to infested cucumber leaves than to uninfested plants and clean air because predatory insects could distinguish volatiles of plants due to the attack by their preferred prey species. Parasitoids can locate their host by releasing plant volatiles (Alkhafaji & Ahmed, 2023). The infested cucumber with cotton aphids-induced plant volatile methyl salicylate (MS) has been extensively researched for its ability to attract natural enemies for pest control. MS is marketed as a spray or in slow-release lures, and MS application has enhanced the abundance of natural enemies in several crops (Lee et al., 2022). Our results indicated that MS could affect the behavior of *C. septempunctata* and *C. carnea* and attract them to synthetic MS. Only MS significantly attracted adults of *C. septempunctata*, *C. carnea*, and syrphid flies. However, the synthetic methyl salicylate showed excellent attractiveness to adult *C. septempunctata* at doses between 100 and 300 mg. According to a subsequent dose-response test at the highest tested concentration of this chemical, a substantial decrease in trap catch was noted (Zhu & Park, 2005; Alkhafaji & Ahmed, 2023). Under greenhouse conditions, we studied how differently attractive accessions affect aphids and their natural enemies, thereby enabling predators and parasitoids to take advantage of them and achieving sustainable horticulture with less pesticide use.

CONCLUSION

Volatile organic compounds (VOCs) are emitted by insects as an indirect defense, attracting natural enemies such as predators. These volatile organic compounds act as warning signals, alerting natural enemies to the danger posed by insect infestation. The results of evaluating the behavioral response of *A. gossypii* towards infested leaves more than uninfested leaves and clean air, as a result of the emission of volatile organic compounds that indicate the characteristics of the plant host, the response of the *C. carnea* to infested leaves more than uninfested leaves and clean air, because the predator can distinguish the infested plant by volatile compounds. *C. septempunctata* is attracted to the infested leaves compared with the uninfested leaves because of the olfactory signals emitted from the infested plant due to the aphids feeding that stimulate the plants to emit volatile compounds in higher concentrations and attract predators to the infested plant.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHOR/S DECLARATION

We confirm that all Figures and Tables in the manuscript are original to us. Additionally, any Figures and images that do not belong to us have been incorporated with the required permissions for re-publication, which are included with the manuscript.

Author/s signature on Ethical Approval Statement.

Ethical Clearance and Animal Welfare

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AUTHOR'S CONTRIBUTION STATEMENT

Conceptualization – Yonglin, Qasim, and Hind; Data curation – Qasim and Xin; Investigation – Qasim and Hind; Methodology - Qasim and Hind; Project administration – Yonglin, Xin and Qasim; Resources - Qasim and Hind; Supervision – Qasim and Yonglin; Writing original draft - Hind; Writing, reviewing, and editing – Qasim, Yonglin, Xin and Hind. All authors have read and agreed to the published version of the manuscript.

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استجابة حشرة *Aphis gossypii* ومفترساتها للمركبات العضوية المتطايرة من نباتات الخيار المصابة وغير المصابة

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المستخلص

اختبرت استجابة حشرة من القطن البالغة *Aphis gossypii* ونوعين من المفترسات *Coccinella septempunctata* و*Chrysoperla carnea* لأوراق الخيار المصابة أو غير المصابة باستخدام مقياس شمي على شكل Y. كانت حشرات المن أكثر انجذابا إلى اوراق الخيار غير المصابة من المصابة. كان المفترس *C. septempunctata* أكثر انجذابا للأوراق المصابة بحشرات المن أكثر من أوراق الخيار غير المصابة. في نفس الوقت، استجاب المفترس *C. carnea* للمركبات العضوية المتطايرة من الأوراق المصابة بـ *A. gossypii* مقارنة بالأوراق السليمة. إن نشاط تغذية حشرات المن ادت الى إطلاق مركبات الكايرومونات والتي يمكن أن تعمل على جذب *C. carnea*، والتي من خلالها يمكنهم تحديد موقع حشرات المن والنباتات المصابة. أشارت نتائجنا إلى أن المفترسات يمكن أن تميز بين الأوراق المصابة وغير المصابة، وكانت الاستجابة معنوية للمركبات العضوية المتطايرة المنبعثة من النباتات المصابة. استخلصت المركبات العضوية المتطايرة باستخدام تقنية HS–SPME من أوراق الخيار. شخصت 35 مركبا في الأوراق غير المصابة والمصابة بواسطة GCMS، وحددت 7 مركبات عضوية متطايرة في أوراق الخيار المصابة ولم تظهر في الأوراق غير المصابة. إن نتائج الدراسة اشارت الى ان سلوك حشرات المن ومفترساتها يتأثر في رد فعلها تجاه الأوراق المصابة أكثر من الأوراق غير المصابة بسبب إطلاق المركبات العضوية المتطايرة من النبات العائل.

الكلمات المفتاحية: المن، الحشرات النافعة، اسد المن، مركبات عضوية متطايرة، فايبرات SPME .