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Artificial Damage Induction in the Leaves of Chilli Plants Leads to the Release of Volatiles that Alter the Host Plant Selection Behaviour of *Bemisia tabaci* (Hemiptera: Aleyrodidae)

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ABSTRACT

Chilli plants release volatile organic compounds following insect or mechanical damage. In laboratory experiments, the behavioural responses of *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae) were investigated by using artificially damaged and undamaged chilli plants. The headspace volatiles released by the plants were determined using gas chromatography-mass spectrometry. The results showed that the preference of adult whiteflies varied with the time after damage induction. No significant difference in distribution of whiteflies was noted between artificially damaged and undamaged chilli plants within 1 and 3 h of damage induction. However, whitefly adults preferably aggregated on undamaged chilli plants than on artificially damaged plants after 6 and 24 h of damage induction. Further, the artificially damaged plants were less preferred for subsequent oviposition than undamaged chilli plants. There was a significant quantitative difference ($p < 0.05$) in the levels of volatile monoterpenes, i.e., α -pinene, p-cymene and β -phellandrene, in artificially damaged plants after 24 h of damage induction compared with that in undamaged plants and artificially damaged plants after 1 h of damage induction. The increased emission of these volatile compounds might have altered the preference of whiteflies, leading them to avoid artificially damaged chilli plants.

Key words: *Bemisia tabaci*, artificially damaged, volatiles, terpenes

INTRODUCTION

Economically important crops worldwide are affected by whiteflies *Bemisia tabaci* (Hemiptera: Aleyrodidae). This insect feed on plant phloem, disseminate plant pathogens and produce honeydew that is conducive to the growth of Sooty mould, thus leading to serious annual crop losses (Zalom *et al.*, 1995; Liang *et al.*, 2007; Jiu *et al.*, 2007). In recent times, in Malaysia, many host plants, including brinjal (*Solanum melongena* L.), tomato (*Solanum lycopersicum*) and chilli (*Capsicum annum*), have been affected by whitefly (WF) infestation. In Malaysia, insecticides have been used extensively to eliminate this pest (Syed *et al.*, 2000). However, not only

has this practice increased the resistance of the pest to the active pesticide ingredients (Horowitz *et al.*, 2005) but also destroyed the natural antagonists that control WF infestation.

For instance, the insecticide avermectin efficiently controls the proliferation of larval WF on brinjal and tomato; however, it is also toxic to *Macrolophus caliginosus* which feeds on WFs (Mohd Rasdi *et al.*, 2012). As an alternative to insecticides, the efficiency of plant Volatile Organic Compounds (VOCs) has been increasingly investigated in regulating insect behaviour (Bruce *et al.*, 2005).

As reported by Carroll *et al.* (2006), VOCs are actively involved in the interaction between pest and host. The type of VOCs released might vary depending on the pest species infesting the plants, extent of plant damage and environmental conditions (Gouinguene and Turlings, 2002; Kigathi *et al.*, 2009; Schuman *et al.*, 2009). Plant damage caused by biotic or abiotic stresses induced physiological changes. These changes might induce defence mechanisms which affect the behaviour and infestation performance of insect's pest (Gonzales *et al.*, 2002). The VOCs generally play important roles in mediating insect behaviours such as host plant searching to repel the attacking pest, as well as have a secondary function to attract both parasitic and predatory insects of WFs. Examples of such VOCs are Green Leaf Volatiles (GLVs), terpenes, alkenes, carboxylic acids and alcohols (Holopainen, 2004; Law and Regnier, 1971). However, determining the factors that markedly affect the relationships between insects and their host plants is important (Schoonhoven *et al.*, 2005). In this study, the chemical VOCs produced by undamaged and artificially damaged chilli plants (*Capsicum annum* var. Kulai), were investigated and determined the effects of these VOCs on the behavioural responses of adult WF females.

MATERIALS AND METHODS

WF rearing: A colony of *B. tabaci* was established and maintained in a greenhouse top, at 30-36°C, 80% relative humidity. The colony was reared on chilli plants (*C. annum* var. Kulai) raised in pots. Before the experiment started, newly emerged WF adults were collected from the chilli plants and were sexed under a stereomicroscope (50X). Females could be distinguished from males by their relatively large size and abdomen (Campos *et al.*, 2003). Insects were confined in Erlenmeyer flasks and starved for 2 h before the start of each experiment.

Host plants: Chilli (*C. annum* var. Kulai) seeds were obtained from the Malaysian Agriculture Research and Development Institute (MARDI) Station, Jalan Kebun, Klang. Seeds were soaked in pure distilled water for 8 days to initiate germination. Germinated seeds were transferred to plant cups containing hydroponic growth medium and maintained on a raft made of a punched cylindrical piece of polystyrene. Plants with 3-4 leaves were used for these experiments.

Free-choice bioassays: WFs were allowed to freely feed and oviposit on the artificially damaged and undamaged chilli plants and their position was monitored at 1, 3, 6 and 24 h after release. This study was conducted at the Entomology Laboratory at MARDI in Serdang, Selangor, Malaysia, at a mean temperature of 24±1°C and 60-65% relative humidity. Artificially damaged plants were created by cutting quarter of 3 leaves of plants from the upper, middle and lower strata by using surgical scissors. The plants were placed in cages (60×60×60 cm) at the same level in a circle, 30 cm from the release point, with 5 replicates. Each replicate consisted of 10 plants (5 undamaged and 5 artificially damaged plants). A total of 300 adult WF females were introduced into a cage. The distribution of WFs over the plants was recorded during the light period at 1, 3, 6 and 24 h after

release. After 24 h, the number of eggs per square centimeter on the apaxial surfaces of leaves of the upper, middle and lower plant strata was counted using a stereoscopic microscope at 40X magnification (Naranjo and Flint, 1995).

Collection of headspace volatiles: Headspace volatiles were collected using a Static Headspace (HS) collection system equipped with Solid-phase microextraction, (SPME) fibre using a 65 µm (PDMS/DVB) fiber similar to that described by Tholl *et al.* (2006). The plants with intact root balls that were wrapped in aluminium foil and placed directly in a 3-L glass chamber and capped; the VOCs were then allowed to collect, typically for 60 min. The SPME fibre was then inserted into the HS above the sample and exposed for a fixed time, typically 30 min in the glass container which is sufficient to collect any VOC (Musteata and Pawliszyn, 2007). The VOCs were collected 3 times each time by using a different plant for each treatment. For artificially damaged plants, the first sampling period was after 1 h after the artificial damage was induced, followed by sampling at 24 h. Headspace volatiles emitted from undamaged plants were collected in the same manner under the same condition.

Volatile analysis and identification: A gas chromatography-mass spectrometry GC-MS (QP2010) instrument equipped with a DB-5 msec, column (30.0×0.25×0.25 µm) was used for the identification of the volatiles. Splitless liquid injection of 1 µL of samples was performed. After the sampling was completed, the SPME was retracted into the needle and removed from the glass jar. It was then inserted directly into the GC-MS equipment at 250°C for 2 min for obtaining the thermal desorption spectra to separate and identify the contents of volatile samples. Helium gas was used as the carrier gas. The column temperature was maintained at 40°C for 5 min and then increased by 3°C min⁻¹ to 250°C for 80 min. Compounds were identified by comparing the mass spectra and retention times with those available in the GC-MS system of the National Institute of Standards and Technology (NIST) library. The quantity of VOCs was detected on the basis of the areas of the peaks detected by MS.

Statistical analysis: Comparison of data for feeding preference and oviposition between the artificially damaged and undamaged plants was analysed using t-test ($p < 0.05$). The amounts of chemical volatiles released by the artificially damaged plants after 1 and 24 h and those by undamaged plants were analysed using one-way analysis of variance (ANOVA). The mean amounts of volatiles separated using the GC-MS method were analysed using Tukey's test at $\alpha = 0.05$ level. All data was analysed using the Minitab Statistical Package (v. 16).

RESULTS

There was no significant difference in the preference of *B. tabaci* for artificially damaged and undamaged leaves at both the observation periods (1 and 3 h). The WF's distributed equally between the leaves of the artificially damaged and undamaged chilli plants. In contrast, at 6 and 24 h, the WF's showed greater preference for undamaged plants than for the artificially damaged chilli plants ($p < 0.05$) (Fig. 1). A significant ($F = 82.52$, $df = 1$, $p = 0.001$) difference was also noted in the number of *B. tabaci* eggs per plant among the treatments. As for the adults, the number of WF eggs was significantly higher on undamaged plant leaves than on the artificially damaged plant leaves (Fig. 2).

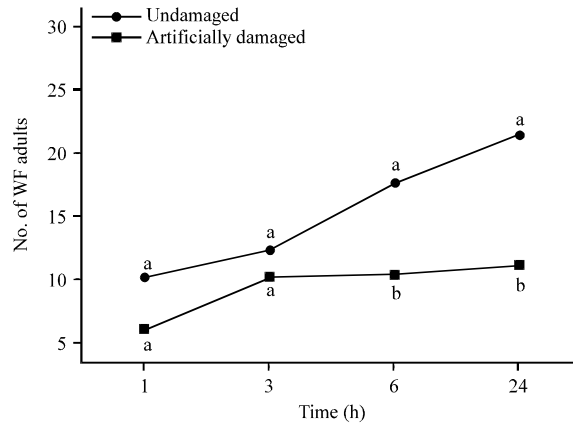


Fig. 1: Total mean No. of adult WF in undamaged and artificially damaged chili plant at different sampling hours. Means with same letters are not significantly different at $p < 0.05$ (Tukey test)

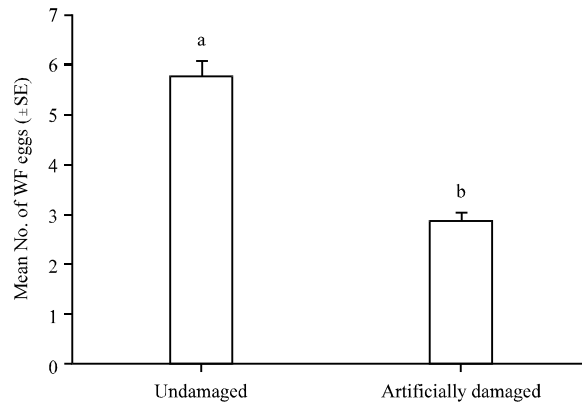


Fig. 2: Oviposition preference of WF among undamaged and artificially damaged chili plant. Mean with different letters are significant at a level of $\alpha = 0.05$ (Turkey's test)

Volatile organic compounds emanating from the plants: A total of 33 VOCs were identified from the chilli plants. The volatiles included terpenoids (monoterpenes C10, sesquiterpenes C15 and triterpenes C30), GLVs, aldehydes, hydrocarbons, ketones and fatty acids (Table 1). The chemical compound profiles did not show qualitative differences among them, with the exception of 2 compounds of GLVs: The production of *Z*-2-hexen-1-ol ($p = 0.034$) and (*E*)-2-hexen-1-yl acetate ($p = 0.000$) was significantly higher in the artificially damaged than the undamaged chilli plants. Similarly, 3 compounds of monoterpenes β -phellandrene ($p = 0.022$), α -pinene ($p = 0.004$) and *p*-cymene ($p = 0.031$) were also emitted in significantly higher quantities by artificially damaged plants after 24 h than by the undamaged plants and artificially damaged plants after 1 h treatment. However, there were no significant differences between treatments in the amount of sesquiterpenes, aldehydes, hydrocarbons, fatty acids, ketones and triterpenes (Table 1).

The total amounts of monoterpenes and aldehyde released from the artificially damaged plants after 24 h were significantly different from those released by the undamaged and artificially damaged plants after 1 h ($F = 3.64$, $df = 2$, $p = 0.031$ and $F = 4.83$, $df = 2$, $p = 0.014$, respectively; Fig. 3). In contrast, there were no differences in the quantity of volatile hydrocarbons, fatty acids, sesquiterpenes, ketones and triterpenes between the undamaged and artificially damaged chilli

Table 1: Volatiles organic compound released from artificially damaged chili plants collected at 1 and 24 h after treatment

Volatile compound	RT	Undamaged	Damaged (h)		p-value
			1	24	
Monoterpenes					
α -pinene	7.73	0.060±0.048 ^b	0.006±0.006 ^b	1.627±0.623 ^a	0.031
β -pinene	9.23	0.023±0.023 ^a	Nd	0.213±0.095 ^a	0.071
Limonene	10.85	0.410±0.225 ^a	0.070±0.051 ^a	0.123±0.061 ^a	0.251
p-cymene	10.60	0.016±0.008 ^b	Nd	0.043±0.003 ^a	0.004
Geranylacetone	23.23	0.323±0.117 ^a	0.330±0.092 ^a	0.440±0.210 ^a	0.829
(E)- β -ocimene	11.15	Nd	0.033±0.033 ^a	0.046±0.024 ^a	0.414
β -phellandrene	22.46	Nd	Nd	0.320±0.115 ^a	0.022
β -2-carene	11.65	0.076±0.076 ^a	0.250±0.250 ^a	Nd	0.525
Triterpenes					
Squalene	56.18	1.690±0.882 ^a	6.33±5.84 ^a	1.57±1.14 ^a	0.574
Sesquiterpenes					
α -humulene	23.55	0.723±0.078 ^a	0.480±0.060 ^{ab}	0.556±0.012 ^b	0.060
(E)-caryophyllene	22.73	0.950±0.737 ^a	0.006±0.006 ^a	0.013±0.008 ^a	0.272
Copaene	21.26	0.157±0.123 ^a	0.010±0.005 ^a	0.016±0.008 ^a	0.330
Aldehydes					
Heptanal	6.93	Nd	0.006±0.006 ^a	0.043±0.017 ^a	0.062
Nonanal	13.16	0.243±0.158 ^a	0.463±0.200 ^a	1.193±0.434 ^a	0.130
Decanal	11.96	0.433±0.068 ^a	0.870±0.335 ^a	4.00±1.55 ^a	0.065
Octanal	10.01	Nd	Nd	0.953±0.496 ^a	0.090
Ketones					
5-hepten-2-one, 6-methyl	9.41	0.070±0.032 ^a	0.160±0.037 ^a	0.477±0.191 ^a	0.098
Green-leaf volatiles					
Hexanal	5.91	Nd	0.076±0.076 ^a	0.110±0.058 ^a	0.414
Z-2-hexen-1-ol	5.85	Nd	0.170±0.055 ^a	0.033±0.033 ^{ab}	0.039
(E)-2-hexen-1-yl acetate	6.03	Nd	0.026±0.003 ^a	Nd	0.000
3Z)-hex-3-enyl	9.96	Nd	0.810±0.810 ^a	Nd	0.422
Hex-3-enal <cis	4.68	Nd	0.407±0.332 ^a	0.076±0.076 ^a	0.363
(E)-2-hexenal	5.56	Nd	0.587±0.414 ^a	0.076±0.076 ^a	0.257
Fatty acids					
Hexadecanoic acid	35.16	0.237±0.109 ^a	0.480±0.460 ^a	0.103±0.088 ^a	0.645
Hydrocarbons					
Eicosane	37.71	0.513±0.062 ^a	0.510±0.036 ^a	0.840±0.204 ^a	0.182
Tetradecane	21.70	0.196±0.067 ^a	0.103±0.014 ^a	0.840±0.204 ^a	0.291
Hexacosane	48.45	4.99±2.01 ^a	5.21±2.06 ^a	5.430±0.818 ^a	0.984
Tridecane	21.56	0.286±0.072 ^a	0.163±0.006 ^{ab}	0.120±0.023 ^a	0.083
Undecane	12.76	0.296±0.061 ^a	0.183±0.059 ^{ab}	0.043±0.043 ^b	0.047
Dodecane	15.85	0.296±0.073 ^a	0.180±0.050 ^a	0.243±0.008 ^a	0.342
Heptacosane	45.58	3.04±1.03 ^a	1.153±0.142 ^a	1.637±0.272 ^a	0.162
Pentadecane	24.35	0.086±0.052 ^a	Nd	0.110±0.086 ^a	0.424
Decane	9.53	0.250±0.055 ^a	0.146±0.036 ^{ab}	0.103±0.003 ^a	0.085

Values are Mean±SE (three replicates), Nd: Not detected, Means followed by the same letters within each row are not significantly different (p>0.05), RT: Retention time

plants after 1 and 24 h ($F = 0.13$, $df = 2$, $p = 0.881$; $F = 0.47$, $df = 2$, $p = 0.645$; $F = 2.45$, $df = 2$, $p = 0.108$; $F = 3.51$, $df = 2$, $p = 0.098$ and $F = 0.61$, $df = 2$, $p = 0.574$, respectively) (Fig. 3). On the other hand, the GLVs were released in greater amounts by artificially damaged plants after 1 and 24 h of treatment than the undamaged plants ($F = 4.62$, $df = 2$, $p = 0.014$) (Fig. 3).

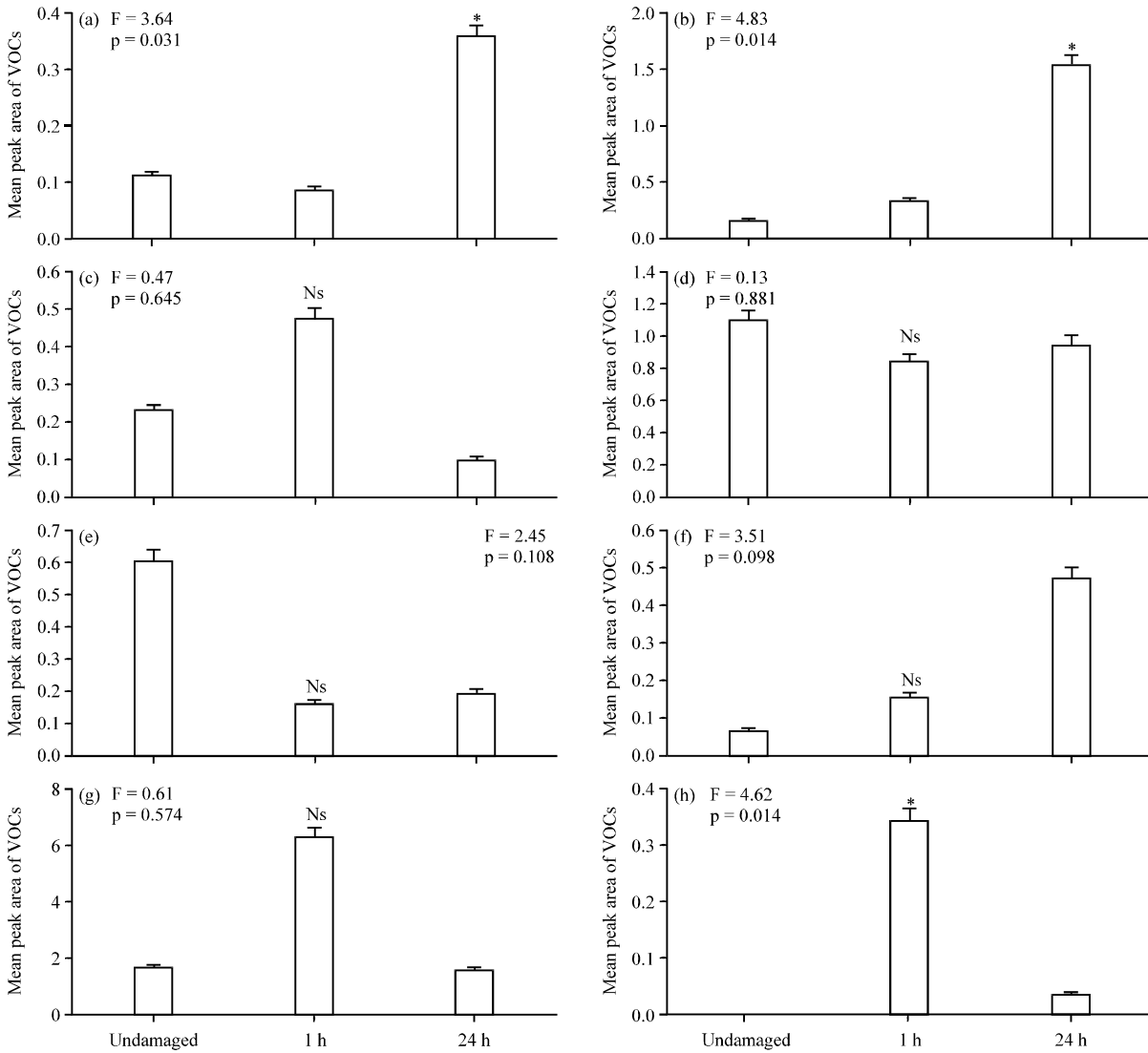


Fig. 3(a-h): Comparison of the total peak areas of Volatile Organic Compounds (VOCs) (a) Monoterpenes, (b) Aldehydes, (c) Fatty acids, (d) Hydrocarbons, (e) Sesquiterpenes, (f) Ketones, (g) Triterpenes and (h) GLV emitted from chili plants. The data is mean of three replications \pm SE. Ns = Not significant, * Asterik indicate significant differences between artificially damaged (1, 24 h) and undamaged chili plant

DISCUSSION

In the free-choice study, WF females showed preference in feeding and oviposition for undamaged chilli plants over artificially damaged plants after 6 and 24 h of treatment (Fig. 1). The volatile concentrations of artificially damaged chilli plants were different from those of the undamaged plants: The GLVs were absent in the undamaged chilli plants or in the artificially damaged plants before the damage was induced (Table 1). This suggests that artificially damaged plants either lack important cues that promote host acceptance or produce deterrents that actively repel WFs. Generally, the GLVs are known to be released immediately after mechanical damage or insect herbivore infestation, eliciting the characteristic smell of freshly cut grass

(Hatanaka, 1993; Buttery *et al.*, 1987; Allmann and Baldwin, 2010). Therefore, the artificially damaged chilli plants emitted GLVs, especially Z-2-hexen-1-ol and (E)-2-hexen-1-yl acetate, in significantly higher amounts 1 h after the damage than 24 h later. The GLVs released by plants are often attractive to insects that have similar feeding habits. For example, tea aphids show positive attraction to GLVs from tea shoots (Han *et al.*, 2012). However, little is known about the differential effects of plant volatiles on the behaviour response of *B. tabaci*. In our study, however, WF females were not attracted to chilli plants at 1 h after the induction of artificial damage. This was probably because of the limited amount of GLVs released. This is in agreement with the findings of Sharkey (1996), who suggested that the total amount and duration of GLVs released by plants is likely to be small relative to other biogenic hydrocarbons. The levels of monoterpenes such as α -pinene, p-cymene and β -phellandrene emitted by artificially damaged chilli plants after 24 h were significantly higher than those produced by undamaged and artificially damaged chilli plants after 1 h of damage induction (Table 1). Previous studies on resistance have shown that monoterpenes, including p-cymene and α -pinene, play a role in host choice of insects since they are toxic to herbivores. Interestingly, they can indirectly protect plants by attracting predators of the herbivores (Brown *et al.*, 1995; Keeling and Bohlmann, 2006). For example, p-cymene has been reported to be toxic to the western flower thrips *Frankliniella occidentalis* (Janmaat *et al.*, 2002) and used as a repellent against mosquitoes (Park *et al.*, 2003) and α -pinene found in rosemary oil was repelled the onion aphid *Neotoxoptera formosana* (Hori and Komatsu, 1997). Further, both α -pinene and p-myrcene were also reported to act as a repellent against *Tribolium castaneum* adults (Kim *et al.*, 2012).

Generally, WFs are known to possess olfactory receptors and are attracted to host plant odours (Visser, 1986). These characteristics indicate that female WFs might have the ability to select their host plants on the basis of not only host quality but possibly also predator-related cues (Nomikou *et al.*, 2003). In general, plant volatiles are known to attract natural enemies and herbivores are found to prefer odours from healthy plants (Turlings *et al.*, 1998). For example, the oviposition rate of *B. tabaci* was reduced on the leaves exposed to lacewing larvae (Legaspi *et al.*, 1996). Moreover, adult WFs avoided cucumber plants with predatory phytoseiid mites (Nomikou *et al.*, 2003). Similarly, in our study, the change in the quantities of monoterpenes, especially α -pinene, p-cymene and β -phellandrene, in the artificially damaged chilli plants at 24 h of treatment was used as a cue by female WFs to avoid feeding and egg deposition on them. It is hypothesized that artificial damage induction might have led to the emission of volatiles that attracted predators or parasitoids of the WFs which might have been the reason for the significant oviposition preference shown by adult WFs on undamaged plants (Fig. 2). This preference for undamaged plants might be attributed to the minor amounts of monoterpenes released by these plants.

CONCLUSION

In conclusion, results indicate that artificial damage induction in chilli plants altered the preference of WFs which showed higher preferences for undamaged plants than for the artificially damaged plants after 6 and 24 h of treatment. Monoterpenes were found to be emitted significantly by the chilli plants after 24 h of damage induction, these seem to have affected the host plant selection of WFs. This finding is in agreement with those of previous studies that indicated that monoterpenes serve as functional signals to repel WFs in tomato plants (Bleeker *et al.*, 2009). However, further studies are warranted to determine the effect of the individual, pure VOCs on the

behaviour and physiology of *B. tabaci*. This might improve the understanding of the changes in the metabolism of plants, induced for repelling or attracting WFs and might reveal new methods to improve crop protection.

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