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Research Article

Chilli plants (*Capsicum annuum* L. var Dumay) volatiles induced by *Aphis gossypii* (Hemiptera: Aphididae) and *Spodoptera litura* (Lepidoptera: Noctuidae)

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Abstract
Chilli plants respond to injury caused by herbivorous insects by emitting various volatile compounds. Insects that infest have different mouths; the difference in mouth type is thought to influence the volatiles released by plants. This research aimed to identify volatile compounds released by chilli plants (*Capsicum annuum* L. var Dumay) due to infestation by chewing and piercing-sucking insects. Capture of volatile compounds using the Headspace Solid-Phase Micro Extraction (SPME) method. The results showed differences in the composition of volatile compounds released by chilli plants infested by herbivores with different mouth types. Chilli plants not infested by herbivores release as many as 34 compounds. There were 24 volatile compounds released by chilli plants infested by *Aphis gossypii* and 27 compounds infested by *Spodoptera litura*. Infestation by herbivores with different mouth types triggers the synthesis of new compounds. Undecane was the only specific compound produced by chilli plants infested by *A. gossypii* and *S. litura*. In addition, herbivore infests trigger an increase in the proportion of some volatile compounds. The compound with the highest proportion in chilli plants infested by *A. gossypii* was eucalyptol (12.92%), while that infested by *S. litura* was o-xylene (11.77%). Naturally, the volatile substance with the highest proportion was cis-3-hexenyl acetate (21.22%). These findings can be the basis for developing more effective and sustainable pest control strategies and support further understanding plant defence mechanisms against herbivore infestation.

Keywords: *Aphis gossypii*; eucalyptol; Solid Phase Microextraction; *Spodoptera litura*

INTRODUCTION

Plants have evolved various mechanisms to interact with the environment by releasing volatile organic compounds (VOCs) from leaves, flowers, and fruits into the atmosphere and roots into the soil. 1700 VOCs have been reported in over 90 plant families (Knudsen *et al*., 2006). Plant-emitted VOCs' composition and intensity can provide information on the plant's physiological status and its stresses. VOCs in general and herbivore-induced plant volatile compounds (HIPVs) were key mediators of information transfer (Kessler *et al*., 2019). When herbivores infest plants, they attract predators and parasitoids of the invading herbivores with a mixture of volatiles that provide information about their location, activity, and even developmental stages. Plant-emitted volatiles can directly act as phytophagous insect repellents (Piesik *et al*., 2010). Plant volatiles also act as a defence against pathogens. Some VOCs play important physiological roles in coping with abiotic

stress, such as high light intensity (Zuo *et al*., 2019). VOCs also facilitate intraplant or interplant communication by signalling information about impending danger, either to distant parts of the same plant or to neighbouring plants (Karban *et al*., 2014). VOCs also mediate interactions between plants and microorganisms (Arimura *et al*., 2009).

Chillies were also reported to produce different types of volatile compounds. 300 volatile compounds were detected in *Capsicum annuum*, *C. chinense* and *C. frutescens* (Burruezo *et al*., 2010). 87 volatile compounds from 34 varieties of chilli in Indonesia (Kirana *et al*., 2021). Volatile released by chillies include the chemical groups esters, alcohols, aldehydes, alkanes, sesquiterpenes, monoterpenes, and ketones. Stress due to herbivore infestation has been proven to affect the volatiles emitted by chilli plants. Chilli pepper (var. Japanese Chao tianjiao) emitted 217 volatile compounds after *Myzus persicae* infestation (Ali *et al*., 2022). Chilli cultivar MC11 emitted 20 volatile compounds after being infested with *Bemisia tabaci* adults (Mansour *et al*., 2015). Chilli genotype Hybrid green belt (SPHGB) emits 15 volatile compounds after *A. gossypii* infestation (da Costa *et al*., 2011). Chilli pepper volatiles are dominated by a mixture of α-pinene, decanal, and phthalic acid after being infested by *Myzus persicae* (Ali *et al*., 2022).

During the cultivation process, chilli plants were infested by more than one herbivore species, simultaneously or sequentially. *Spodoptera litura* and *Aphis gossypii* were the main chilli plant pests in Indonesia. Injury caused by phytophagous insects increases volatile emissions in plants (Santamaria *et al*., 2018). *S. litura* was a chewing insect, while *A. gossypii* was a piercing-sucking insect. The type of insect's mouth affects the synthesis of volatile compounds (Riddick, 2020). Phloem sap (aphids) did not cause an increase in corn volatile emissions, while injury caused by stem borer larvae increased volatile emissions (Turlings *et al*., 1998). Damage caused by the leaf-eating caterpillar *H. armigera* resulted in stronger induction than *A. gossypii*. Damage caused by chewing insects is related to the activation of jasmonic acid (JA), while damage caused by phloem sap insects is related to the activation of salicylic acid (SA) (Moran and Thompson, 2001).

Information regarding the semiochemicals produced by chilli plants when infested by *S. litura* and *A. gossypii* has not been widely reported in Indonesia. Likewise with the function of semiochemicals in their interactions with natural enemies. Plant volatile compounds caused by herbivores are often used as olfactory cues by natural enemies, thus having the potential to control agricultural pests. Manipulating volatile organic compounds can improve pest management and help reduce pesticide use (Piesik *et al*., 2010). The present research aimed to identify the volatile compounds released by chilli due to infested by chewing and sucking mouth-type insects.

MATERIALS AND METHODS

Chilli plants preparation and insect rearing

Preparation of chilli plants and rearing *A. gossypii*, and *S. litura* were in the greenhouse, Department of Plant Protection, IPB University, Bogor, West Java. The chilli used were the Dumay variety. Chilli seeds were planted in polybags (5 cm x 15 cm) until 21 days old. The seedlings were then planted in polyethylene terephthalate (PET) pots. Pest-free conditions (Vijaya *et al*., 2018). Chilli plants were cultivated until six weeks old and were at the nine- or ten-leaf stage (Saad *et al*., 2015). The plants were used for insect rearing and volatile trapping. The Aphid species used in this experiment was *A. gossypii*. Aphid rearing following the method Venkanna and Suroshe (2023). Insect rearing was done by infesting wingless *A. gossypii* on six-week-old chilli plants. Chilli plants that have been infested with *A. gossypii* were placed in insect cages (100 cm x 100 cm x 100 cm) under controlled greenhouse conditions (24–30°C; 70–93% relative humidity [RH]). The aphids were provided with new chilli plants weekly (Saad *et al*., 2015). After a month, aphids emerged in hundreds and were used for subsequent experiments (Sharma *et al*., 2017). *S. litura* larvae were collected from chilli fields in Cianjur regency, West Java, Indonesia. Larvae of *S. litura* were kept in plastic tubs (25 cm diameter) and were provided with fresh chilli leaves in the laboratory under controlled room conditions (22–29°C, RH 82–98%). The larvae were reared individually until pupation (Azam *et al*., 2016). Male and female pupae were separated and put in different cages for eclosion. The adults were fed with 10% honey water (Wei *et al*., 2023). Eggs laid by the mature were reared until they hatched, and larvae were reared, using chilli leaves as food, until instar IV. *The S. litura used in this experiment was instar III,* the most destructive phase (Kundu *et al*., 2018).

Trapping volatile compounds on chilli plants undamaged

Trapping of volatile compounds using the headspace method. The VOCs emitted by chilli plants were collected using a static-headspace sampling device with a solid-phase microextraction (SPME) (Saad *et al*., 2015). Trapping used one six-week-old chilli plants planted in a PET pot. Test plants were covered with PET plastic (30 cm x 30 cm). The PET plastic was sufficiently sealed to contain volatile gases and prevent leakage. The PET used has no small holes or leaks that may affect the capture of volatile compounds. In this study, we also used a control, i.e., an empty experiment (with PET enclosure without plants), to ensure that the PET material did not release or absorb volatile

compounds. A Solid Phase Micro Extraction (SPME) holder was installed in the cover hole. The SPME fiber used in this experiment was Supelco (Sigma-Aldrich, Bellefonte, PA, USA) divinylbenzene / carboxen / polydimethylsiloxane (DVB/CAR/PDMS) 2 cm with a polymer thickness of 50/30 µm. The trapping of volatile compounds lasted for 60 minutes (Saad *et al*., 2015). SPME fiber containing undamaged chilli plants volatiles was injected into the GC-MS.

Trapping volatile compounds on chilli plants infested by *Aphis gossypii*

Volatile capture using chilli plants aged six weeks after planting (Hegde *et al*., 2011). Two hundred individuals of *A. gossypii* of mixed stages were infested on chilli plants (Xu *et al*., 2023; Schettino *et al*., 2017). Aphids were released and allowed to stand for 1 hour before trapping (Hegde *et al*., 2011). *A. gossypii* was infested evenly on bud, young, and old leaves. All insects remained on the plants during experiments (Schettino *et al*., 2017). The *A. gossypii* -infested chilli plants were covered with PET plastic (Kigathi *et al*., 2019). Before covering the surface of the planting medium, it was covered using aluminum foil to reduce the release of pot and soil-derived volatiles (da Costa *et al*., 2011). The SPME holder installation process followed the previous experiments. In control plants, *A. gossypii* was not added (Ren *et al*., 2010). *A. gossypii* volatiles were also trapped as a control.

Trapping volatile compounds on chilli plants infested by *Spodoptera litura*

Volatiles were collected as described previously by Du *et al*. (2022); Paré and Tumlinson (1998) with some modifications. *S. litura* was infested six hours before volatile trapping (Paré and Tumlinson, 1998). *S. litura* was starved for seven hours before infesting in chilli plants (Kundu *et al*., 2018). Larvae of third instars were used (Gouinguené *et al*., 2003). Larvae of *S. litura* were placed randomly on the leaves (Sun *et al*., 2021). The *S. litura*-infested chilli plants were covered with PET plastic (Kigathi *et al*., 2019). The volatile collections began when insects fed on the leaves (Sun *et al*., 2021). The SPME holder installation process followed the previous experiments. Fibers were exposed to the sample headspace for 60 min (Du *et al*., 2022). Five larvae were placed on a plant and were allowed to feed continuously while plants were in the volatile collection apparatus (Paré and Tumlinson, 1998; Sun *et al*., 2021). For the control plants, no larva was added (Sun *et al*., 2021).

Identification and quantification of volatile compounds

The identification and quantification of each volatile compound refer to the modified method of Saad *et al*. (2015) with some modifications. SPME fibers from each treatment were injected into a GC-MS device (GC 789A, MS 5975C inert XL EI/CI MSD Agilent Technologies Inc., Santa Clara, USA). The SPME device was left for 15 minutes to release all the volatile compounds contained in the fiber into the column. Analytes were separated using a DB-5 MS capillary column (5% diphenyl, column length 30 m x 0.25 mm I.D; film thickness 0.25 mm). Operating conditions were splitless, injector at 240^0C with helium carrier gas at a constant flow rate of 0.8 ml/min. The sample running time is 29.167 minutes. The oven temperature setting starts at 40^0C, then increases 6^0C/minute to 155^0C, then increases 25^0C/minute to 280^0C, and holds at that temperature for 5 minutes. The mass spectrum was recorded at 70 eV electron impact mode (source at 200^0C, transfer line at 250^0C), and the scanned mass range was 29 to 550 m/z. Detected peaks were identified from their retention data and by comparing the obtained mass spectra with a spectral library. The retention index was determined using standard retention times of n-alkanes (from C_9 to C_{40}, 10 µg/ml in n-hexane) and compared with literature values. The relative quantities of each volatile compound were estimated based on its peak area shown by mass spectrometry.

RESULTS AND DISCUSSION

Volatile composition emitted by chilli plants

The number of volatile compounds released by undamaged chilli plants were 34 compounds. The number was more than the chilli plant infested by *A. gossypii* and *S. litura*. The total volatiles emitted by chilli plants infested by *A. gossypii* were 24 compounds, and chilli plants infested by *S. litura* emitted 27 compounds. Naturally, some plants emit various volatiles without triggers (Dudareva *et al*., 2004). The difference in the amount of volatiles emitted by undamaged chilli plants compared to those infected by *A. gossypii* and *S. litura* was thought to be caused by changes in metabolic and physiological priorities. Insect herbivore infestations trigger defence responses that focus resources on producing specific defence compounds, reducing the diversity of volatiles. Undamaged plants can produce a variety of volatile compounds for various ecological and physiological functions under optimal conditions (Steglińska *et al*., 2022). In undamaged chilli plants, volatile compounds were not identified in chillies infested by *A. gossypii* and *S. litura*, namely decanal, cardene, and 2,5-dimethylstyrene. This shows that the volatile profile of chilli plants had changed in response to infested by *A. gossypii* and *S. litura*.

No specific compounds were produced by the chilli plants infested by *A. gossypii* and *S. litura* (Fig. 1). Silva *et al.* (2017) found that quantitative changes were

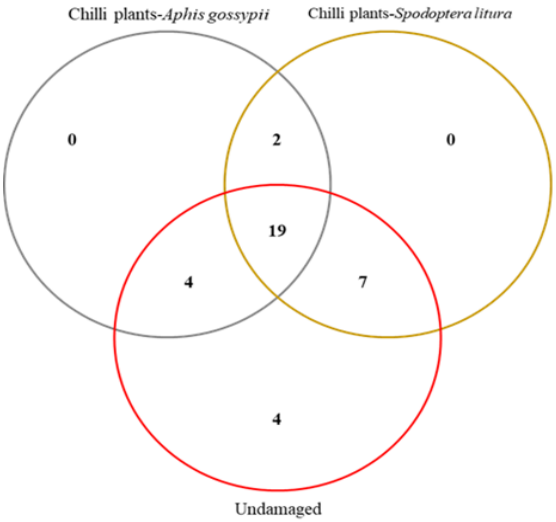

Fig. 1. *Composition of the number of volatiles emitted by chilli plants*

more common than qualitative changes in plant volatiles infested by herbivores. Plants may rely on quantitative changes in the profile of existing volatile compounds as an adaptive response (Kigathi *et al.,* 2019). Several compounds were only found in undamaged chilli plants and infested by *A. gossypii*, namely prenitol, dodecane, 4-methyl-, 3-ethyl-3-methylheptane, o-xylene, 3-ethyl-. The compounds remained present or increased in proportion in plants infested by *A. gossypii*, indicating that these volatiles played a role in plant defense mechanisms. Some compounds were only found in undamaged chilli plants and infected with *S. litura*, namely cis-3-Hexenyl acetate; m-Xylene; o-Xylene; 3-Ethyltoluene; acetophenone; 2-ethyltoluene; Mesitylene; and Benzene, 1,3-dichloro-. Most of the identified compounds are not reported as chewing-type induced volatiles. Each plant species could synthesize a unique set of volatile compounds.

Volatile compounds emitted by chilli plants infested by *Aphis gossypii*

Eucalyptol (12.92%), 1,2-dimethylindane (12.22%), 4-methyl-1-undecene (6.35%), 2-methyl-1-penten-3-ol (6.15%), p-xylene, 2-ethyl- (5.55%) was the compound with the highest proportion (Table1, Fig. 2). Eighty-seven volatiles were identified in 34 varieties of chilli plants damaged by *Thrips* spp, *S. litura*, and *B. dorsalis*, one of which was eucalyptol (Kirana *et al*., 2021). Chilli plants infested by *M. persicae* also emit eucalyptol (Saad *et al*., 2015). Infestation of *A. gossypii* in chilli plants triggers an increase in the proportion of release of several volatile. These volatile compounds were eucalyptol, 2-methyl-1-penten-3-ol, 3-octen-2-ol, 4-methyl-1-undecene, 1-decanol, 2-ethyl-,dodecane, 4-methyl-, 3 -ethyl-3-methylheptane, p-xylene, 2-ethyl-, benzene, 1-

Table 1. Compounds identified and relative peak area (%) in the GC chromatogram detected in chilli infested by *Aphis gossypii*

Group	No.	Compound	Relative peak area (%)
Alkenes	1.	4-Methyl-1-undecene	6.35
	2.	2-Methyl-1-penten-3-ol	6.15
	3.	3-Octen-2-ol	4.47
Alcohol	4.	1-Decanol, 2-ethyl-	3.42
Sugar alcohol	5.	Prehnitol	2.53
Aliphatic hydrocarbons	6.	2-Methyldecane	1.35
	7.	Undecane	4.56
	8.	Undecane, 5,7-dimethyl-	2.43
	9.	Dodecane, 4-methyl-	4.94
	10.	3-Ethyl-3-methylheptane	4.71
	11.	Undecane, 3,5-dimethyl-	1.37
	12.	Undecane, 3,7-dimethyl-	2.01
	13.	Undecane, 6-ethyl-	1.31
	14.	Tridecane, 5-methyl-	2.06
	15.	Hexane, 3,3-dimethyl-	1.48
	16.	Tetradecane, 4-methyl-	1.90
	17.	Cetene	1.23
Aromatic hydrocarbons	18.	p-Xylene, 2-ethyl-	5.55
	19.	3,5-Diethyltoluene	3.90
	20.	o-Xylene, 3-ethyl-	3.49
	21.	Benzene, 1-ethyl-2,4,5-trimethyl-	4.34
	22.	Cumene, 3,5-dimethyl-	5.31
	23.	1,2-Dimethylindane	12.22
Terpenoids	24.	Eucalyptol	12.92

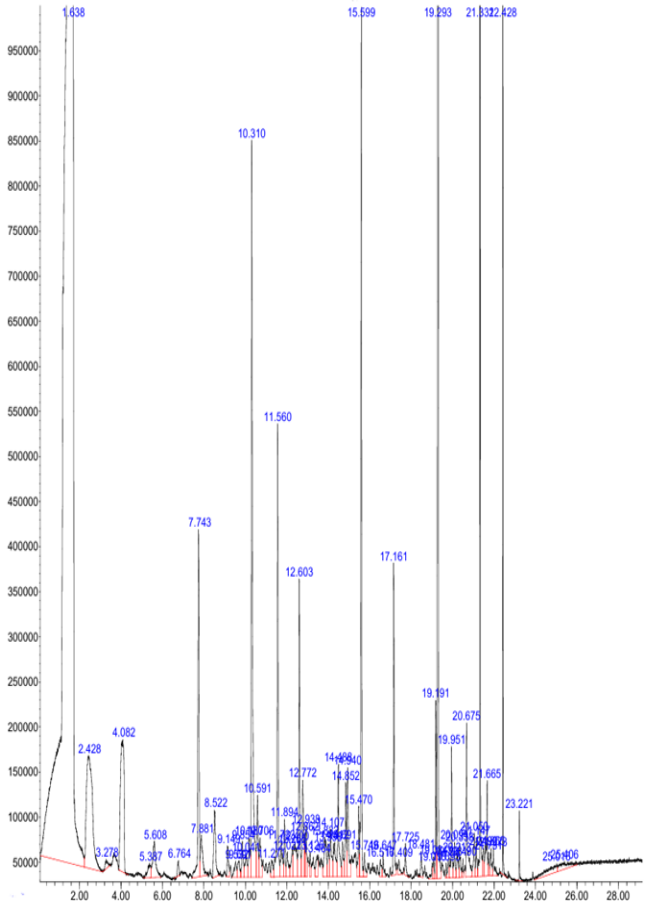

Fig. 2. *Chromatographic profile of chilli plants infested by Aphis gossypii*

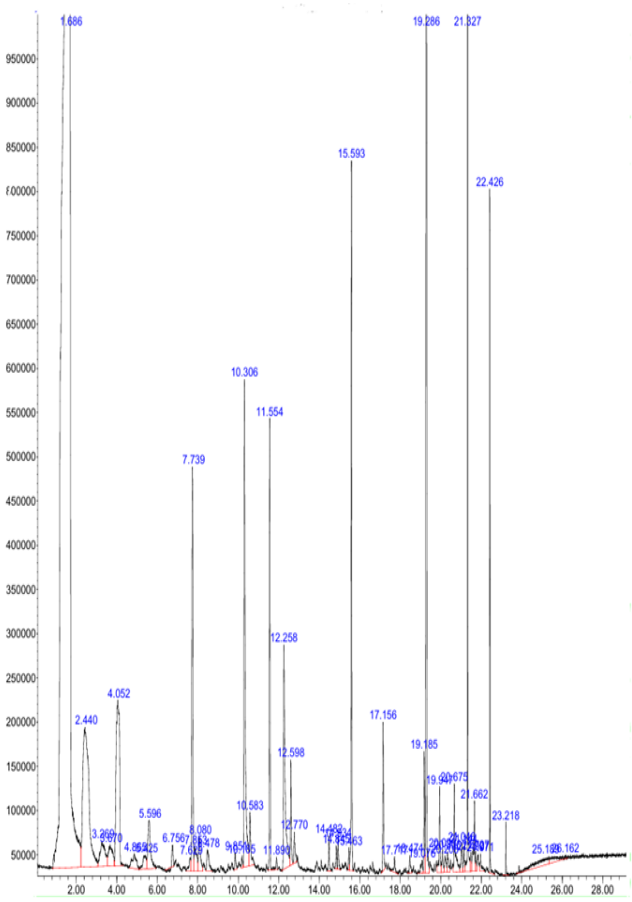

Fig. 3. *Chromatographic profile of chilli plants infested by Spodoptera litura*

ethyl-2,4,5-trimethyl, cumene and 3,5-dimethyl-. Eucalyptol had the highest increase, 0.01% in undamaged plants, to 12.92% in plants infested by *A. gossypii*. Eucalyptol was thought to have an important function in the defense strategy of chilli plants. Rodriguez-Saona and Frost, (2010) reported that high emissions can directly impact herbivores as repellents. In addition, a high proportion of eucalyptol can be a strong signal for predators or parasitoids of *A. gossypii*. *A. gossypii* infestation has been reported to trigger an increase in plant-released volatiles. Damage to cotton plants caused by *A. gossypii* increases heptane, 3-hexenal, and α-copaene compounds (Yasa *et al*., 2024).

Volatile compounds emitted by chilli plants infested by *Spodoptera litura*

o-xylene (11.77%), mesitylene (7.99%), 3-ethyltoluene (7.58%), acetophenone (6.52%), cis-3-hexenyl acetate (6.19%) were the volatiles with the highest proportion compared to other compounds in chilli plants infested by *S. litura* (Table 2, Fig. 3). Infestations of chewing insects can trigger higher volatile release. The proportions of the volatile compounds in the chilli plants that increase after an infestation by *S. litura* are m-xylene, o -xylene, and mesitylene. Increased volatile release due to the induction of chewing insects has been reported in several other plants. Röse and Tumlinson (2004) reported an increase in the release of seven main volatile compounds in cotton plants after an infestation of *Helicoverpa zea*. Piesik *et al*. (2010) found that *Oelema melanopus* infestation caused wheat, barley, and oats to release higher volatile compounds. Chewing insects infest and cause physical damage to plant tissue, triggering metabolic pathways that increase the production and release of volatile compounds as a defense mechanism.

Volatile compounds emitted by undamaged chilli plants

The five volatile compounds with the highest proportion were cis-3-hexenyl acetate (21.22%), o-xylene (11.19%), 3-ethyltoluene (7.57%), acetophenone (7.38%), and p-xylene, 2-ethyl- (4.88%) (Table 3, Fig. 4). Cis-3-hexenyl acetate was also reported in cotton with a relatively high proportion of 14% (Röse and Tumlinson 2004). In addition, Röse and Tumlinson (2004) detected a high proportion of the compound cis-3-Hexenyl acetate 24 hours after *H. zea* feeding activity. According to Smith and Beck (2015), cis-3-hexenyl acetate is often emitted by undamaged plants. Healthy plants emit low-level volatiles and are released rapidly in response to insect-feeding activity and mechanical

Table 2. Identified compounds and relative peak area (%) on GC chromatogram detected in chilli plants infested by *Spodoptera litura*

Group	No.	Compound	Relative peak area (%)
Alkenes	1.	4-Methyl-1-undecene	3.52
Alcohol	2.	3-Octen-2-ol	3.81
	3.	2-Methyl-1-penten-3-ol	1.57
	4.	1-Decanol, 2-ethyl-	2.56
Ester	5.	cis-3-Hexenyl acetate	6.19
Aliphatic hydrocarbons	6.	2-Methyldecane	2.59
	7.	Undecane	5.97
	8.	Undecane, 5,7-dimethyl-	1.56
	9.	Undecane, 3,5-dimethyl-	1.47
	10.	Undecane, 3,7-dimethyl-	2.01
	11.	Undecane, 6-ethyl-	1.10
	12.	Tridecane, 5-methyl-	2.24
	13.	Hexane, 3,3-dimethyl-	1.58
	14.	Tetradecane, 4-methyl-	2.13
	15.	Cetene	2.20
Aromatic hydrocarbons	16.	m-Xylene	6.02
	17.	o-Xylene	11.77
	18.	3-Ethyltoluene	7.58
	19.	Acetophenone	6.52
	20.	2-Ethyltoluene	4.16
	21.	Mesitylene	7.99
	22.	p-Xylene, 2-ethyl-	3.05
	23.	3,5-Diethyltoluene	2.63
	24.	Benzene, 1-ethyl-2,4,5-trimethyl-	2.81
	25.	Cumene, 3,5-dimethyl-	2.80
	26.	1,2-Dimethylindane	1.53
Terpenoids	27.	Eucalyptol	2.63

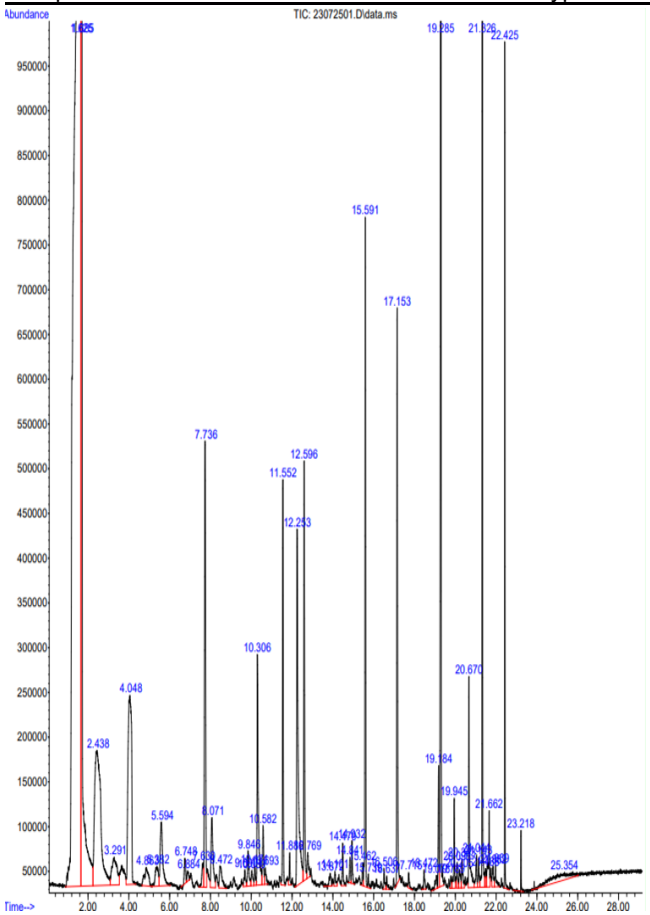

Fig. 4. *Chromatographic profile of undamaged chilli plants*

damage. Plant production of volatile compounds does not always depend on the presence of stress or damage but can also occur naturally and in significant quantities (Ninkovic *et al.*, 2021).

Conclusion

The mouthparts of insect herbivores affect the composition and proportion of volatiles emitted by chilli plants. Infestations by herbivores with different mouthparts trigger the synthesis of new compounds that were not naturally emitted. Undecane was the only specific compound produced by chilli plants infested by *A. gossypii* and *S. litura*. The effect of herbivore Infestations with different mouthparts triggers an increase in the proportion of several volatile compounds, especially those that function as defences. The highest proportion of compounds in chilli plants infested by *A. gossypii* was eucalyptol (12.92%), while chilli plants infested by *S. litura* were o-Xylene (11.77%). These volatiles function as anti-microbial, anti-herbivores, and attractants.

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Table 3. Identified compounds and relative peak areas (%) on GC chromatograms detected in undamaged chilli plants

Group	No.	Compound	Relative peak area (%)
Aldehydes	1.	Decanal	6.70
Alkenes	2.	4-Methyl-1-undecene	1.05
Alkohol	3.	3-Octen-2-ol	1.56
	4.	2-Methyl-1-penten-3-ol	1.28
	5.	1-Decanol, 2-ethyl-	1.04
Ester	6.	cis-3-Hexenyl acetate	16.67
Sugar alcohol	7.	Prehnitol	0.98
Aliphatic hydrocarbons	8.	2-Methyldecane	0.93
	9.	Undecane, 5,7-dimethyl-	0.68
	10.	Dodecane	1.84
	11.	3-Ethyl-3-methylheptane	1.62
	12.	Undecane, 3,5-dimethyl-	0.65
	13.	Undecane, 3,7-dimethyl-	1.07
	14.	Undecane, 6-ethyl-	0.80
	15.	Tridecane, 5-methyl-	1.55
	16.	Hexane, 3,3-dimethyl-	0.61
	17.	Tetradecane, 4-methyl-	1.09
	18.	Cetene	1.24
Aromatic hydrocarbons	19.	m-Xylene	3.31
	20.	o-Xylene	8.79
	21.	3-Ethyltoluene	5.95
	22.	Acetophenone	5.80
	23.	2-Ethyltoluene	3.57
	24.	Mesitylene	3.49
	25.	Benzene, 1,3-dichloro-	3.72
	26.	p-Xylene, 2-ethyl-	3.83
	27.	3,5-Diethyltoluene	1.71
	28.	2,5-Dimethylstyrene	2.15
	29.	o-Xylene, 3-ethyl-	1.53
	30.	Benzene, 1-ethyl-2,4,5-trimethyl-	1.48
	31.	Cumene, 3,5-dimethyl-	2.45
	32.	1,2-Dimethylindane	1.98
Calcium	33.	Cardene	8.89
Terpenoids	34.	Eucalyptol	0.01

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Conflict of interest

The authors declare that they have no conflict of interest.

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