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Herbivore-induced plant volatiles emitted by okra: Electroantennographic responses of *Earias vittella* F. and behavioral responses of its egg parasitoid, *Trichogramma chilonis* Ishii

Rakesh Kumar, Suresh Nebapure, Bishwajeet Paul, SR Sinha, RK Sharma and Rekha Kumawat

Abstract
The present investigation was aimed at identification of herbivore induced plant volatiles from okra and their possible implications in insect pest management through target specific attraction of natural enemies in okra agro-ecosystem. Findings of the present investigation revealed that mechanical damage and larval feeding of *Earias vittella* F. induced, albeit differently, the plant volatile production in okra. A total of 64 and 46 volatile compounds were identified from headspace compositions of okra plant and okra fruits, respectively. Mechanical damage and larval feeding of *Earias vittella* F. induced the plant volatile production in okra. A total of 64 and 46 volatile compounds were identified from headspace compositions of okra plant and fruits, respectively. Isoxazole, 5-chloro-4-(2-phenylethyl)- (8.01%), hexatriacontane (6.88%), tetratriacontane (4.32%), decane, 3,3,4-trimethyl- (4.11%), 3-hydroxypropyl palmitate, TMS derivative (2.92%), m-Ethylacetophenone (2.65%) and D-limonene (2.44%) were emitted in highest quantities from okra plant. While, mesitylene (10.01%), butanediol (5.51%), azulene (3.33%) and D-limonene (2.64%) were emitted in highest quantities from okra fruits. Shoot and fruit borer, *Earias vittella* antennae responded to the volatile stimulus by depolarization. Fresh fruits, mechanically damaged and undamaged plant elicited strong antennal responses from both sexes of *E. vittella.* In addition herbivore damaged fruits and mechanically damaged fruit plant elicited strong antennal (EAG) responses from *E. vittella* male only. In behavioural bioassay both sexes of egg parasitoid *Trichogramma chilonis* Ishii preferred headspace volatile blends from herbivore damaged plant, herbivore damaged fruit and larval excreta over the control. Whereas, volatile blends from mechanically damaged plants were only preferred by *T. chilonis* females. This is the first study which shows the importance of role of herbivore-induced plant volatiles in the indirect defense of okra against Shoot and fruit borer, *Earias vittella*.

Keywords: agro-ecosystem, okra, *earias vittella*, herbivore-induced plant volatiles, *Trichogramma chilonis* Ishii

Introduction
Plants have developed a multitude of direct and indirect defense mechanisms against insect herbivore (Piesik *et al*., 2011) [25]. Interestingly, plants can influence the natural enemies of herbivores by emitting behavior modifying volatile organic compounds. Thus, plant volatiles play a critical role in indirect defenses act via the attraction of organisms from an additional trophic level. When plants damaged by herbivores often produce a blend of volatiles and these volatile blends commonly referred to as herbivore-induced plant volatiles (HIPVs) (Kessler & Baldwin, 2001; Mumm & Dicke, 2010) [13, 20]. These HIPVs consist of a mixture of the so-called green-leaf volatiles (aldehydes, alcohols, and acetates), terpenes (monoterpenes, sesquiterpenes, homoterpenes) and aromatic compounds (Pichersky *et al*., 2006) [24]. The release of HIPV's may signal the presence of potential prey or hosts and therefore, can be exploited by natural enemies to locate the prey organism (Verkerk, 2004) [37]. The majority of herbivore-induced plant volatiles (HIPVs) can be classified in three major chemical groups based on their biosynthesis pathways or their known within-plant functions (Holopainen and Gershenzon, 2010) [9]. The terpenoids are the first dominant group of constitutively emitted HIPVs in many plant species and produced by the two separate pathways, one active in plastids (MEP) and one (MVA) in the cytosol (Loreto and Schnitzler, 2010; Maffei, 2010) [14, 16]. The volatile terpenoids include monoterpenes, homoterpenes and sesquiterpenes. Interestingly, the second group is lipoxygenase products better known as green leaf volatiles (GLVs).

Green leaf volatiles (GLVs) such as (Z)-3-hexenal and (Z)-3-hexenyl acetate released after mechanical or other destructive damage to cell membranes (Maffei, 2010; Holopainen, 2011) [16, 8]. The third group includes volatile aromatic compounds such as methyl salicylate and indole produced by the shikimate pathway and containing an aromatic ring (Maffei, 2010) [16]. In addition to these volatile groups, there are other volatiles too, that are specific to varying degrees such as order, genus or species. Interestingly, habitat management is an integral part of biological control of insect pests of agricultural crops. Hence, site specific utilization of HIPVs can play a pivotal role to direct target specific movement of natural enemies from their natural reservoirs. Thus, the present study was conducted to identify volatile compounds from okra plant and their possible functions involved in tritrophic (okra, *Abelmoschus esculentus*, Shoot and fruit borer (*Earias vittella*) and *Trichogramma chilonis*) interactions in agro-ecosystem.

Methods and Materials

Okra (*Abelmoschus esculentus* (L.) Moench) variety *Arka Anamika* was cultivated in polyhouse condition (30±5 ºC, 60% to 80% RH) at I.A.R.I., New Delhi. Individual okra seeds were grown in plastic pots (15×10 cm) filled with potting soil and any pesticide was not applied on these plants. To investigate the headspace volatile profile from Okra (*Abelmoschus esculentus* (L.) Moench) plant and fruits, 30 days old ten intact individual okra plants (*var*. *Arka Anamika*) were selected for headspace volatile collection from above ground portion of potted plants (stem, branches, and leaves). Headspace volatile collection from different treatments (undamaged, mechanically damaged and herbivory damaged okra plant and its fruits) was completed by dynamic headspace collection system described by Tholl *et al.* (2006) [35]. Ten fourth instar *Earias vittella* caterpillars were released on individual okra pant and allowed to feed for 12 hrs before volatile collection started. Caterpillars (and their frass) were removed from the herbivory damaged okra plant during volatile collection without causing any possible mechanical damage to the okra plant, whereas from the herbivory damaged okra fruit samples caterpillars were not removed because of possible mechanical damage to fruits caused by caterpillar removal. For mechanically damaged plant samples a single hole paper punch (4.5mm) was used to punch off 8 holes on each leaf of okra plant (Five leaves/plant) just before volatile collection and for mechanically damaged fruit samples fresh okra fruits were sliced in 1.5-2.0 cm pieces just before volatile collection. As a control, volatiles were collected from intact plant without any mechanical damage to plant and for fruit samples fresh fruits were used. Plant volatile collection was carried out in a day time (humidity: 75±5%, temperature: 25±2°C). Volatiles were collected using a dynamic push-pull system. Porapak Q adsorbent-filtered air was pumped into the glass made volatile collection chamber at a flow rate of 1.5 L min−1. At the same time, a portion of the air was pumped out of the volatile collection chamber with a second pump at a flow of 1L min−1. The outgoing air passed through a trap packed with 20 mg Porapak Q adsorbent (sigma-Aldrich) to retain the volatile compounds. Volatiles were collected after 12 hrs of herbivore release on the plants and fruits for 4 hrs. Volatile compounds were desorbed by eluting the filter twice with 200 µL of dichloromethane (DCM) containing nonyl acetate as an internal standard (10 ng µL-1).

Gas Chromatography–Mass Spectroscopy (GC–MS)

The separation and identification of okra plant headspace volatile compounds were done by using Shimadzu QP 2000 equipped with Rtx-5 ms column measuring 30× 0.25 mm composed of 95% dimethyl polysiloxane. Helium was used as carrier gas with flow rate 1 ml/min. One micro liter (1µl) volume of each sample was injected into injection port with temperature maintained at 230 ^{0}C. The initial temperature of oven temperature was programmed at 40 ºC for 4 min, and then it was increased to 220 ^{0}C with 5 ^{0}C ramping rate and hold for 2 min. Finally the temperature was increased to 270 ºC with ramping rate of 15 ºC for 1 min. The temperature for ion source was maintained at 200 ºC. Electron impact ionization (EII) with 70 eV was used for GC-MS analysis and data was evaluated by TIC (Total ion count) for identification and quantification of compounds. The spectrum of each volatile compound was compared with known stored data base of spectrum in GC–MS library (NIST14).

Electroantennography

The relative antennal receptivity of adult males and females of *Earias vittella* to plant volatiles emitted from undamaged, mechanically damaged and herbivory damaged okra plant and their fruit was compared by electroantennography (EAG). Ten replicates of both sexes were taken for EAG (M/S syntech, Germany) analysis. The antennal receptivity recording was conducted through EAG 2000 software (version 2.7c, Syntech, Germany). The antennae used for experimentation was excised at the base from moth's head without any physical damage by using micro-scissors and the part of distal antennal segment was carefully clipped of under 0.1 M electrolyte solution, for smooth electrical conduction between electrodes. "Parker, spectra 360" an electrical conductivity gel was used for fixing antennae onto electrodes. By using electrical conductivity gel the basal portion of antennae was connected onto indifferent electrode and the tip portion of antennae was fixed with recording electrode. The ideal electrical conductivity of antennae between electrodes was indicated by a stable base line having minimum fluctuations. Antennal responses were first recorded to select reference and then the headspace volatiles of okra plant/fruit were tested in a series (reference, control/solvent and plant volatiles collected from headspace of okra plant/fruit). In each test, 10 µl volume of extract was applied onto the filter paper (3X1 cm) and left for 10-15s for solvent evaporation. The filter paper with test stimuli was inserted inside the pasture pipette for air space saturation. The duration of stimulus onto antennal preparation was 0.3s. For the recovery of antenna, at least 1 min time interval was kept between subsequent stimulations. Over antennal preparation continuous air flow was maintained at 500 ml/min. The complete mixing of odour stimulus with continuous air flow was decasualized by injecting stimulus into the mixing tube through the side pore located at 10 cm distance from the antennal preparation. The response of the mechanically damaged plant volatiles (reference) was used to normalize the test stimuli. The solvent control and the reference compound were presented at the beginning and at the end of each trial.

Behavioral Bioassay

For behavioral bioassays against *Trichogramma chilonis* olfactometer experiments were conducted in a Y-tube olfactometer made up of transparent Plexiglas (3 cm ID; stem 10 cm, arms 8 cm; stem-arms angle 130º) with each arm

connected to a glass tube holding the odor source and filtered air 30 ml/min was drawn through the olfactometer by a pump. Headspace volatile blends were used for behavioral bioassays against *Trichogramma chilonis.* Whatman filter paper (2 × 2 cm) impregnated with 10 µl of test solution, left to dry for 10s, and introduced into one of the glass tube was used as the odor source. On the other arm of olfactometer, glass tube held untreated filter paper (control) of the same size. The olfactometer was disconnected from the glass tube and thoroughly washed with water, rinsed in 70% ethanol, and dried in an oven at 120ºC after every three runs. The apparatus was rotated 180◦ after five runs to exclude directional bias. Time to respond was standardized for 10 min to record maximum activity of *Trichogramma chilonis* for test compound and each insect was used only once. Insect behavior was recorded and responses were considered positive when insects traveled at least 4 cm along arm connected to test compounds. All experiments were conducted in day light at 28±2ºC and 60% relative humidity.

Results and Discussion

Volatile compounds identified in the okra plant headspace

A total of 64 volatile compounds were identified from headspace compositions of okra plant in different treatments (*viz*., undamaged plants, mechanically damaged and plants infested with larvae of *Earias vittella* Fab.). Headspace volatile profile of undamaged plants, mechanically damaged plants and plants infested with larvae of *Earias vittella* Fab., was made up of 33, 42 and 38 compounds, respectively (Table 1). Interestingly, present study showed that mechanically damaged plants and plants infested with larvae of *Earias vittella* induce, albeit differently, the plant volatile production in okra (Fig. 2&3). Emissions from mechanically damaged plants show a high degree of resemblance to volatile emissions from undamaged plants (Fig. 1&2). Whereas, *E. vittella* caterpillar feeding induces a blend of volatiles in okra that was different from undamaged and mechanically damaged okra plant. A detailed survey of the literature shows that no previous study of the herbivore induced plant volatile's components of okra infested with *E. vittella* has been reported. Our findings are in agreement with Rodriguez-Saona *et al.,* (2013) [29] who reported that the volatile profiles from herbivore feeding was different from volatiles induced by methyl jasmonate treatment in cranberry plants. They also reported variability in emissions from mechanically damaged plants to undamaged plant but differences were found non-significant. Our findings corroborates with the findings of Jennifer and Macleod (1990) [11]; Marius *et al.* (1999) [17] Bhagat and Bakthavatsalam (2012) [2] and Rigsby *et al*. (2017) [28] who reported the production of these volatile compounds in different plant species. Interestingly, in the present study out of 64 volatile compounds 28 compounds *viz*., Silanediol, dimethyl-, 3-Decene, 2,2-dimethyl-, p-Xylene, 1-Butanol, 3-methyl-, carbonate (2:1), Decane, 3, 3, 4-trimethyl-, 5-Hepten-2-one, D-Limonene, Pentane, 1-(2-butenyloxy)-, (E)-, Ethanone, 1-(3-butyloxiranyl)-, 2-Octanone, 1-nitro-,2-Pentanone, 3-ethyl-, Benzaldehyde, 4-ethyl-, Azulene, Dodecanal, alpha.-Thujenal, 4-(1-Hydroxyethyl) benzaldehyde, m-Ethylacetophenone, Hexadecane, Heneicosane, 2-Methyltricosane, Hexanedioic acid, mono (2-ethylhexyl) ester, Isoxazole, 5-chloro-4-(2-phenylethyl)-, 3-Hydroxypropyl palmitate, TMS derivative, Tetratriacontane, Hexatriacontane, Fumaric acid, octyl tetradecyl ester, 2-Methylhexacosane and Hexadecanoic acid, 2- hydroxy-1 (hydroxymethyl) ethyl ester were significantly induced by herbivore (*E. vittella*) feeding from okra plants compared with undamaged plants (fig.3). These findings are also in agreement with Rodriguez-Saona *et al.* (2013) [29] who reported significant induction of volatile production in cranberry plants by herbivore feeding. The emission of plant volatiles induced by herbivore feeding was also observed by Vet and Dicke (1992) [38]; Ninkovic *et al.* (2001) [21]; Van den Boom *et al.* (2004) [36]; Blande *et al.* (2007) [3]; Blande *et al.* (2010); Hare (2011) [7]. In the present study, 17 volatiles *viz*., Acetic acid, butyl ester, o-Xylene, Nonane, Octanol, Eucalyptol, Decane, 3,7- dimethyl-, (+)-4-Carene, Undecane, (+)-2-Bornanone, Isoborneol, Dodecane, Nonane,1-iodo-, Heptadecane, 2,6,10,14-tetramethyl-,2,4-Di-tert-butylphenol, Eicosane and Hexatriacontane were significantly induced by mechanical damage to okra plant (Table 1). Our findings are similar to those of Mattiacci *et al.* (1995) [18]; Halitschke *et al.* (2001) [6]; Schmelz *et al*. (2001 & 2003) [32, 33], who reported the emission of plant volatiles induced by mechanical injury or damage. Present findings are in agreement with Philip *et al.* (1994) [23] who reported production of significantly greater amounts of volatile compounds in herbivore damaged cotton plants *viz*., (Z)-3-hexenyl acetate, hexenyl acetate, (E)-β-ocimene, (3E)-4,8-dimethyl-1,3,7-nonatriene, (Z)-3-hexenyl butyrate, (E)-2-hexenyl butyrate, (Z)-3-hexenyl 2-methylbutyrate, (E)-2-hexenyl 2- methylbutyrate, and indole. Likewise, Saad *et al.* (2015) [31] reported Limonene, β-2-Carene, naphthalene, Nonanal, Decanal, Octanal, 5-Hepten-2-one,6-methyl, Hexadecanoic acid, Eicosane, Tetradecane, Hexacosane, Undecane, Dodecane, Pentadecane and Decane from pre-infested (by aphid) chilli plants through headspace sampling. Similarly, Vijaya *et al.* (2018) [39] reported several long chain hydro carbon compounds released from *Spodoptera litura* damaged Chilli, *Capsicum annuum* L. plants, identified as Ethyl acetate, Tetradecane, Dodecanoic acid, Pentacosane, Hexacosane, Heptacosane, Triacontane.

In present study, variation in the emission of plant volatiles at different plant stages was observed. A total of 46 volatile compounds were also identified from fresh fruits (Fig 4), mechanically damaged fruits (Fig 5), herbivore damaged fruits (Fig 6) and larval excreta (Fig 7) with highest number of volatile compounds (26) were identified in headspace of mechanically damaged fruits followed by 24, 22 and 20 volatile compounds from larval excreta, herbivore damaged fruits and fresh fruits, respectively (Table 2). Twelve out of 46 volatiles *viz*., Butanediol, 3-Hexanol, 2-methyl-,(1R)-2,6,6- Trimethylbicyclo [3.1.1] hept-2-ene, Ethanone, 1-(1-methylcyclohexyl)-, 3-Carene, D-Limonene, Eucalyptol, Azulene, m-Ethylacetophenone, 2,6,10-Trimethyltridecane, Tetradecane and Eicosane were significantly induced by herbivore damage from okra fruits compared with undamaged fruits, with Mesitylene (10.01%), Butanediol (5.51%), Azulene (3.33%) and D-Limonene (2.64%) emitted in highest quantities ($\alpha<0.05$). Present findings conform to those made by Zhu and Park (2005) [40] and Ameye *et al.* (2017) [1], who observed the differences between plant volatile profiles at different plant stages.

The present findings happen to be closely associated with the observations of Srivastava *et al.* (2004) [34], who reported Heneicosane in the vegetative phase and Heneicosane and tricosane in the flowering phase in the hexane extract of chickpea.

Table 1: Volatile compounds identified in the okra plant headspace

S.N	Compound Name	Concentration (Area %) of HIPVs collected from okra plant headspace (Mean±SE)		
		Undamaged plant	Mechanically damaged plant	Herbivory damaged plant
1	Silanediol, dimethyl- (C2)	nd	nd	2.03 ±0.41[a]
2	Acetic acid, butyl ester	0.71 ±0.02[b]	0.89 ±0.04[a]	0.73 ±0.03[b]
3	3-Decene, 2,2-dimethyl-, (E)-	nd	nd	1.77±0.11 [a]
4	p-Xylene	nd	nd	0.95 ±0.03[a]
5	o-Xylene	2.29±0.30 [b]	3.33±0.10 [a]	nd
6	Nonane, 2-methyl-	1.17±0.03 [a]	0.70±0.02 [b]	0.52 ±0.04[C]
7	Nonane	nd	1.6 ±0.11[a]	nd
8	1-Butanol, 3-methyl-, carbonate (2:1)	nd	nd	0.54 ±0.04[a]
9	Decane, 3,3,4-trimethyl-	nd	nd	4.11±0.06 [a]
10	5-Hepten-2-one, 6-methyl-	nd	nd	0.74±0.02 [a]
11	Mesitylene	0.82 ±0.03[b]	1.27 ±0.01[a]	1.18 ±0.04[a]
12	Octanol	0.23 ±0.04[b]	0.45±0.06 [a]	nd
13	3-Carene	1.79±0.08[a]	0.69 ±0.02[b]	0.70 ±0.04[b]
14	Heptane, 2,5,5-trimethyl-	0.4±005[a]	0.46 ±0.03[a]	nd
15	D-Limonene	0.92±0.05[b]	1.18±0.10[b]	2.44±0.16 [a]
16	Eucalyptol	0.68±0.05[b]	1.02±0.02[a]	nd
17	Ethanone, 1-(3-butyloxiranyl)-	0.44 ±0.02[b]	0.53±0.04[b]	1.83±0.11 [a]
18	Pentane, 1-(2-butenyloxy)-, (E)-	nd	nd	0.43±0.04 [a]
19	2-Octanone, 1-nitro-	nd	nd	0.74±0.03 [a]
20	1-Iodo-2-methylnonane	0.84±0.04 [a]	0.73±0.04 [a]	nd
21	2-Pentanone, 3-ethyl-	nd	nd	0.83±0.05 [a]
22	.gamma.-Terpinene	2.09 ±0.08[a]	0.93±0.05[b]	nd
23	Decane, 3,7-dimethyl-	nd	0.36±0.03[a]	nd
24	Nonane, 5-(2-methylpropyl)-	0.31 ±0.04[a]	0.30 ±0.02[a]	nd
25	Acetophenone	0.49±0.05 [a]	0.45±0.03[a]	nd
26	2-Carene	1.2±0.18 [a]	1.23±0.01[a]	nd
27	(+)-4-Carene	nd	0.82 ±0.08[a]	nd
28	Undecane	1.52 ±0.09[b]	2.27±0.2 [a]	2.47±0.23 [a]
29	Nonanal	1.28 ±0.08[a]	1.19±0.13 [ab]	0.89±0.03 [bc]
30	(+)-2-Bornanone	nd	0.51±0.06 [a]	nd
31	Isoborneol	nd	0.28±0.03 [a]	nd
32	Benzaldehyde, 4-ethyl-	0.56 ±0.03[b]	0.49±0.03[b]	0.89 ±0.04[a]
33	Azulene	nd	nd	1.1 ±0.16[a]
34	Naphthalene	3.72±0.24 [a]	3.86±0.18 [a]	3.80±0.16[a]
35	Dodecane	1.17 ±0.03[b]	1.66 ±0.15[a]	0.7±0.07 [c]
36	Dodecanal	nd	nd	0.33 ±0.04[a]
37	Decanal	0.64±0.03 [a]	0.63±0.04 [a]	nd
38	.alpha.-Thujenal	nd	nd	0.58 ±0.02[a]
39	4-(1-Hydroxyethyl)benzaldehyde	0.21 ±0.02[b]	0.25±0.03[b]	0.82 ±0.05[a]
40	Nonane, 1-iodo-	nd	0.45 ±0.04[a]	nd
41	2-Isopropyl-5-methylphenyl 2-methylbutanoate	0.55 ±0.03[a]	0.51±0.02[a]	nd
42	m-Ethylacetophenone	1.1±0.03 [b]	1.28±0.05 [b]	2.65±0.08 [a]
43	Nonane, 5-butyl-	0.36±0.06 [a]	0.33±0.03[a]	nd
44	Heptadecane, 2,6,10,14-tetramethyl-	nd	0.76 ±0.03[a]	nd
45	Tetradecane	0.55 ±0.02[a]	0.62 ±0.03[a]	nd
46	Pentadecane	0.45±0.03 [a]	0.43±0.03[a]	nd
47	Hexadecane	nd	nd	0.36±0.02 [a]
48	2,4-Di-tert-butylphenol	nd	0.43±0.02 [a]	nd
49	Heptadecane	0.37±0.03 [a]	nd	nd
50	Heneicosane	0.33 ±0.03[b]	0.29±0.02[b]	1.68 ±0.13[a]
51	Eicosane	0.36 ±0.03[b]	0.4 ±0.05[a]	nd
52	n-Nonadecanol	nd	0.18±0.01[a]	0.2 ±0.02[a]
53	2-Methyltricosane (C24)	nd	nd	2.36 ±0.07[a]
54	11-Methyltricosane (C24)	0.89 ±0.04[a]	0.73±0.03[b]	nd
55	Hexanedioic acid, mono (2-ethylhexyl) ester (C22)	nd	nd	1.45±0.10 [a]
56	Hexatriacontane (C36)	nd	0.94 ±0.07[a]	nd
57	n-Nonadecanol-1 (C19)	0.2±0.06 [a]	0.22±0.05[a]	nd
58	Isoxazole, 5-chloro-4-(2-phenylethyl)-	nd	nd	8.01±0.65 [a]
59	3-Hydroxypropyl palmitate, TMS derivative	nd	nd	2.92±0.08 [a]
60	Tetratriacontane	nd	nd	4.32±0.12 [a]
61	Hexatriacontane (C36)	nd	nd	6.88±0.13 [a]
62	Fumaric acid, octyl tetradecyl ester	nd	nd	1.93 ±0.03[a]
63	2-Methylhexacosane (C27)	nd	nd	2.36 ±0.03[a]
64	Hexadecanoic acid, 2-hydroxy-1-	nd	nd	1.07 ±0.04[a]

	(hydroxymethyl)ethyl ester (C19)			
Total compounds		33	42	38

* For each compound, different letters indicate significant differences between the samples (ANOVA followed by the Tukey's HSD test, $\alpha < 0.05$)

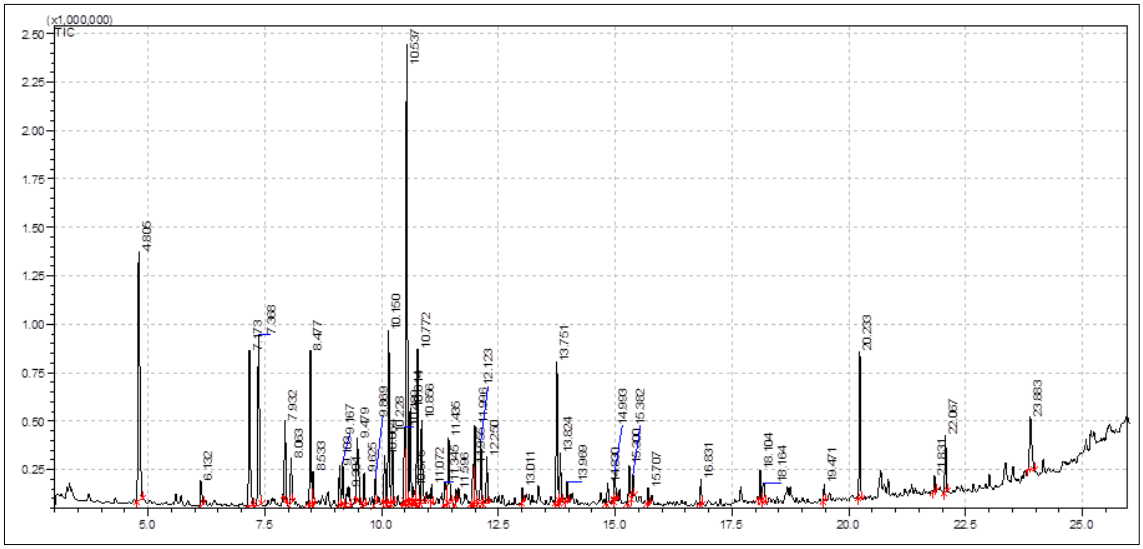

Fig 1: GC-MS chromatogram of volatile compounds emitted from undamaged okra plant headspace

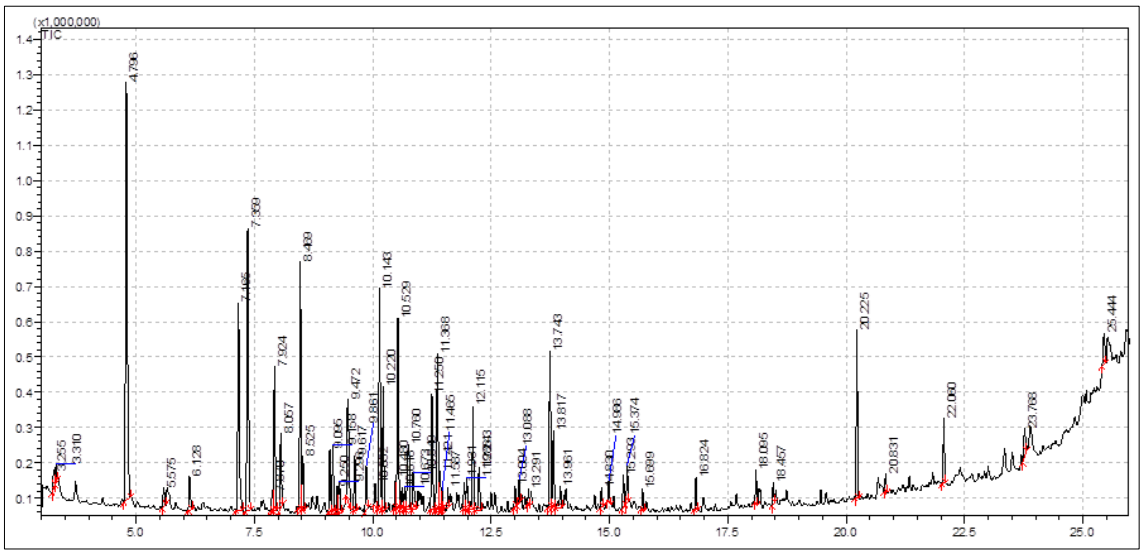

Fig 2: GC-MS chromatogram of volatile compounds emitted from mechanically damaged okra plant headspace

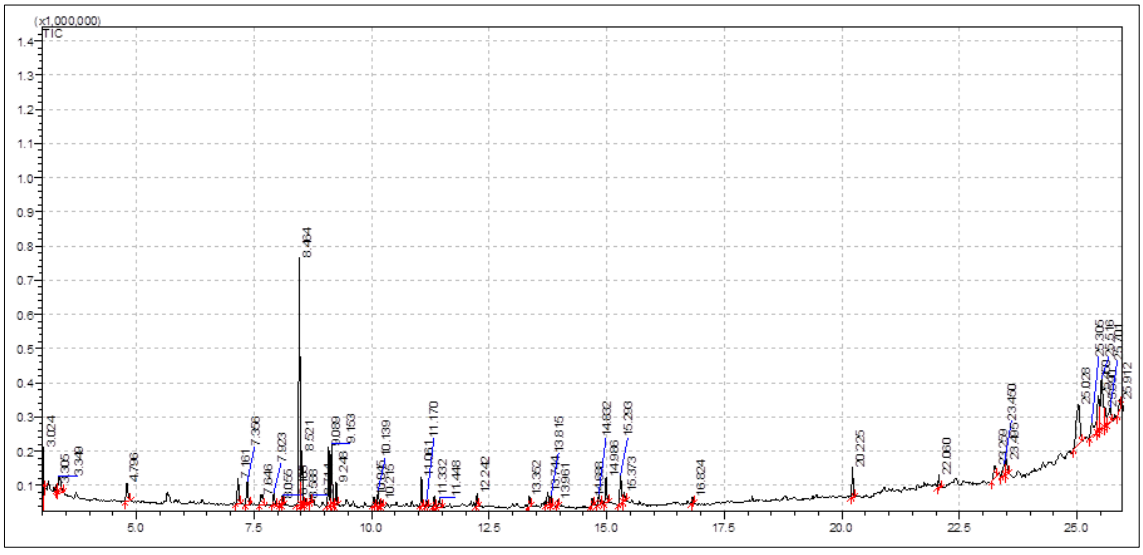

Fig 3: GC-MS chromatogram of volatile compounds emitted from herbivore (*E. vittella* Fab.) damaged okra plant headspace

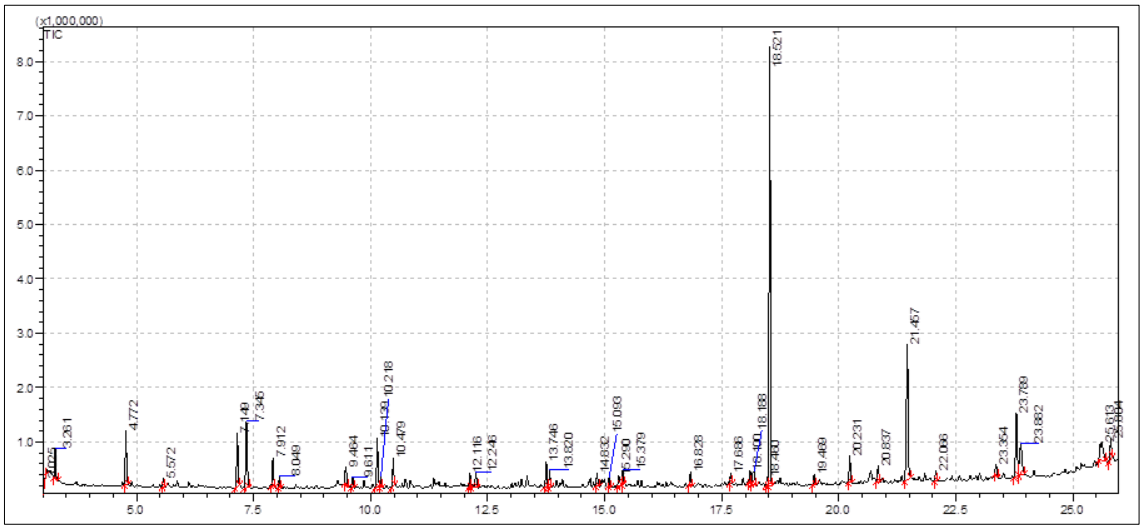

Fig 4: GC-MS chromatogram of volatile compounds emitted from fresh okra fruits

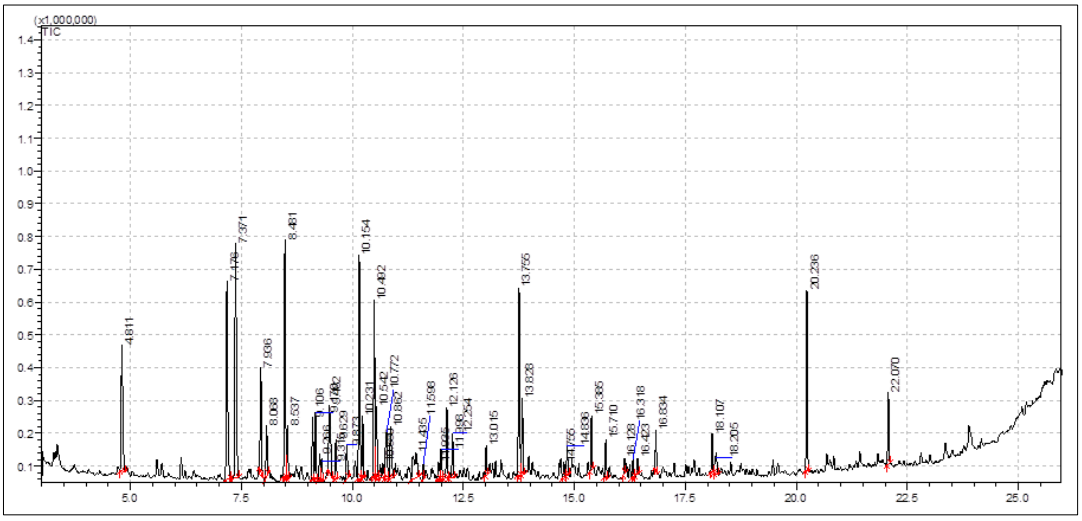

Fig 5: GC-MS chromatogram of volatile compounds emitted from mechanically damaged okra fruit

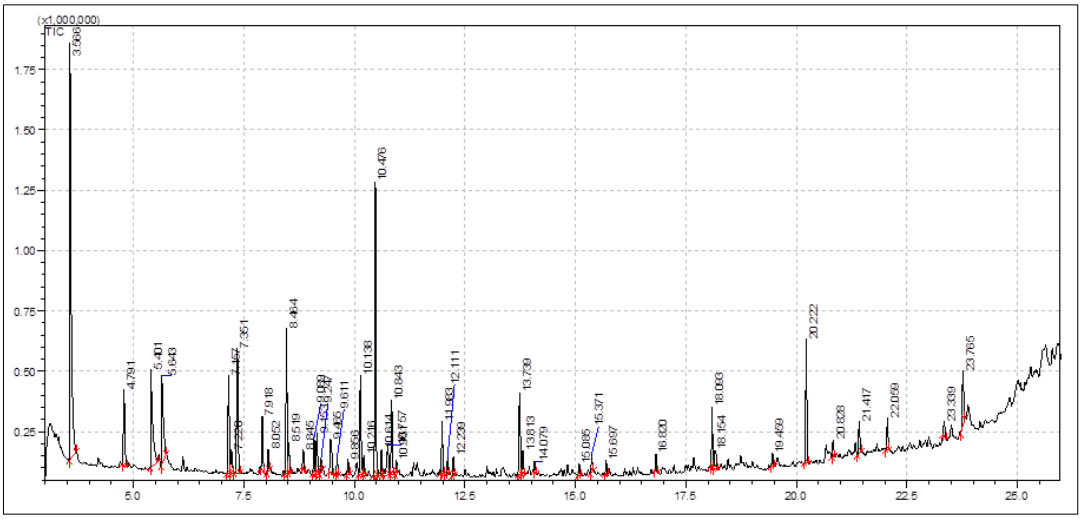

Fig 6: GC-MS chromatogram of volatile compounds emitted from herbivore (*E. vittella* Fab.) damaged okra fruits

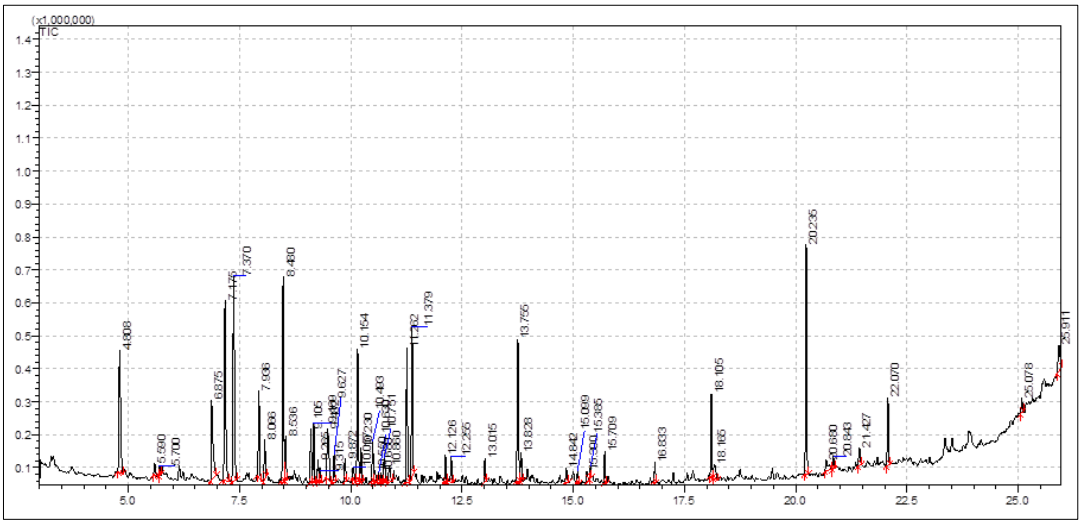

Fig 7: GC-MS chromatogram of volatiles emitted from larval excreta of *E. vittella*

Table 2: Volatile compounds identified in the okra fruit headspace

S.N.	Compound name	Concentration (Area %) of HIPVs collected from okra fruit headspace (Mean±SE)			Larval excreta
		Fresh fruit	Mechanically damaged fruit	Herbivory damaged fruit	
1	2-Trifluoroacetoxydodecane	0.46±0.03a	0.43±0.02a	nd	nd
2	Hexanal	nd	nd	nd	0.27±0.02a
3	2-Pentanone, 4-hydroxy-4-methyl-	nd	nd	nd	3.92±0.08a
4	Butanediol	nd	nd	5.51±0.18a	nd
5	Ethylbenzene	3.57±0.10b	7.39±0.10a	3.87±0.05b	7.45±0.07a
6	3-Hexanol, 2-methyl-	nd	nd	0.94±0.03a	nd
7	o-Xylene	1.82±0.05b	3.22±0.11a	1.88±0.07b	3.03±0.03a
8	Nonane, 2-methyl-	0.72±0.05b	1.54±0.04a	0.72±0.01b	nd
9	(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	nd	nd	0.66±0.04a	nd
10	1-Heptanol, 2-propyl-	nd	nd	nd	1.33±0.04a
11	Ethanone, 1-(1-methylcyclohexyl)-	nd	nd	1.66±0.10a	nd
12	5-Hepten-2-one, 6-methyl-	nd	nd	nd	0.45±0.02a
13	Mesitylene	0.69±0.03b	1.24±0.03a	0.58±0.05b	nd
14	3-Carene	1.73±0.04c	5.28±0.11b	10.01±0.04a	1.45±0.07c
15	Undecane	0.87±0.04b	1.69±0.06a	0.45±0.08c	0.790.04c
16	Nonane,2,6-dimethyl-	nd	0.9±0.04a	nd	0.39±0.03b
17	1,3,5-Cycloheptatriene, 3,7,7-trimethyl-	nd	2.29±0.29a	1.72±0.11b	nd
18	D-Limonene	nd	2.04±0.31b	2.64±0.08a	0.78±0.07c
19	Eucalyptol	nd	nd	0.43±0.02a	nd
20	Nonane, 5-(2-methylpropyl)-	nd	0.38±0.01a	nd	nd
21	Nonanal	1.24±0.08b	1.62±0.07a	0.69±0.11c	1.04±0.1b
22	Azulene	nd	nd	3.33±0.10a	nd
23	Naphthalene	1.88±0.24b	6.36±0.12a	nd	5.53±0.33a
24	Octane, 2,3,7-trimethyl-	nd	nd	nd	1.07±0.3
25	Dodecane	1.02±0.08b	2.28±0.06a	0.91±0.08b	nd
26	Dodecane, 4-methyl-	0.9±0.02a	0.41±0.04b	nd	nd
27	Nonane, 1-iodo-	nd	nd	nd	0.57±0.03a
28	Nonane, 5-methyl-5-propyl-	nd	nd	nd	0.38±0.02a
29	Nonane, 5-butyl-	0.55±0.03a	nd	nd	nd
30	Undecane, 2,10-dimethyl-	nd	0.86±0.04a	nd	nd
31	m-Ethylacetophenone	0.86±0.06b	0.93±0.02b	1.22±0.01a	0.46±0.03c
32	2,6,10-Trimethyltridecane	nd	nd	0.34±0.02a	nd
33	Tetradecane (C14)	0.78±0.03b	1.01±0.02ab	1.10±0.05a	0.61±0.02b
34	Pentadecane	0.78±0.09a	1.39±0.07b	nd	0.3±0.01c
35	Heptadecane	0.62±0.03a	0.60±0.02a	0.42±0.03b	0.74±0.02a
36	Heptadecane, 2,6,10,15-tetramethyl-	nd	1.02±0.08a	Nd	nd
37	Tridecane, 6-methyl-	nd	0.42±0.04a	nd	nd
38	Tridecane, 2-methyl-	nd	0.46±0.02a	nd	nd
39	2-Bromo dodecane	nd	0.39±0.05a	nd	nd
40	Triacontane, 1-iodo-	nd	nd	nd	0.41±0.03a
41	Tetradecanoic acid(C14)	2±0.03a	2.55±0.1a	1.29±0.08b	0.78±0.01b
42	n-Nonadecanol-1	nd	nd	nd	0.49±0.01a
43	Hexadecanoic acid, 2-hydroxy-1-	nd	nd	nd	1.56±.05a

	(hydroxymethyl)ethyl ester				
44	Eicosane (C20)	0.78±0.04b	0.85±0.05b	1.16±0.02a	0.9±0.1ab
45	Benzothiazole, 2-(2-hydroxyethylthio)-	3.93±0.05a	nd	nd	nd
46	cis-9-Hexadecenal	3.07±0.06a	nd	nd	nd
Total compounds		20	26	22	24

* For each compound, different letters indicate significant differences between the samples (ANOVA followed by the Tukey's HSD test, $\alpha < 0.05$)

Electroantennographic responses of *E. vittella* Fab. (Lepidoptera: Noctuidae) to headspace volatiles

The EAG responses of *E. vittella* antennae evoked by the headspace volatile compounds of okra plant and fruits were negative, thereby indicated that the olfactory receptors contributing to them and mostly responded to the volatile stimulus by depolarization (Fig.8). In male antennae of *E. vittella* significantly higher responses were elicited to headspace volatiles from herbivore damaged fruits, fresh fruit, mechanically damaged plant, mechanically damaged fruit and undamaged plant. On the contrary, in female antennae of *E. vittella* significantly higher responses were elicited to headspace volatiles from mechanically damaged plant volatiles, fresh fruit, and undamaged plant volatiles (Table 3). The present findings are in close agreement with the findings of Jonsson and Anderson (1999) [12] who reported that the female *S. littolaris* have receptor neurons that would make it possible to discriminate between damaged and undamaged plants using volatile signals. Likewise, Molnar *et al.* (2015) [19] studied the headspace of maize plants, to which gravid females orientated in a wind tunnel, the antennae of female moths (*Ostrinia nubilalis*) consistently responded to two maize volatiles, nonanal and decanal. The present results are in conformity to the findings of Huang *et al,* (2009) [10], who reported the electroantennographic differences between female and male Asian corn borer antennae in response to larvae-induced maize volatiles; female responded to (E)-2-hexenal, nonanal, (Z)-3-hexen-1-ol and three unknown compounds while the male only responded to (E)-2-hexenal, nonanal and one unknown compound. Likewise, Pinto-Zevallos *et al.* (2016) [26] reported compounds emitted by maize upon feeding by *Spodoptera frugiperda* Walker (Lepidoptera: Noctuidae) elicited a response in the antennae of *S. frugiperda* females (virgin and mated).

Behavioral response of egg parasitoid, *Trichogramma chilonis* Ishii to headspace volatiles

It is evident from detailed survey of the literature that no previous study to investigate the responses of natural enemies of okra agro-ecosystem to the herbivore induced plant volatile's components emanating from the okra plant. The results obtained from y-tube olfactometer revealed that the male *T. chilonis* adults preferred headspace volatile blends from herbivore damaged plant, herbivore damaged fruit and larval excreta over the control, while undamaged plant, mechanically damaged plants, fresh fruits and mechanically damaged fruits failed to elicit significant responses (Table 4). Similarly, significantly higher responses of *T. chilonis* Ishii females were recorded from mechanically damaged plants, herbivore damaged plant, herbivore damaged fruit and larval excreta over the control. In contrast, undamaged plant, fresh fruits and mechanically damaged fruits failed to elicit significant responses (Fig. 9). Present findings conform the findings of Raghava *et al*. (2010) [27], who reported that herbivore induced volatiles of tomato cultivars triggered a distinct cultivar specific olfactory response in *T. chilonis*. Similarly, Romeis *et al.* (1997) [30] reported volatiles emitted by sorghum and pigeon pea plants elicited a behavioral response from *Trichogramma chilonis* females and response varied depending on the growth stage of the plant. The present findings are in close agreement with the findings of Peñaflor *et al*. (2011) [22] who reported that the generalist egg parasitoid *T. pretiosum* was attracted by volatiles from freshly-damaged plants 0–1 and 2–3 h after regurgitant treatment. The specialist *T. remus* on the other hand was attracted only to volatiles emitted from fresh and old damage after associating these volatiles with oviposition. Various studies demonstrated that natural enemies of herbivorous arthropods use specific compounds from complex herbivore-induced volatile blends, in the selective foraging behavior (De Moraes, 1998; Boer *et al.*, 2004) [5]. Likewise, Lou *et al.* (2005) [15] found *Anagrus nilaparvatae* was attracted to volatiles released from *N. lugens*-infested plants. On the contrary, there was no attraction to volatiles from undamaged plants, artificially damaged plants, or volatiles from *N. lugens* nymphs, female adults, eggs, honeydew, and exuvia. It can be concluded that emission of volatile compounds from okra was greatly influenced by mechanical and herbivore damage to the plant. Although, okra plants emit blends of volatile compounds that are complex. These volatile compounds are used as key information source by many plant-associated organisms to find out their potential host in a complex ecosystem. The findings of present investigation also revealed the variation in the inducibility of plant volatile production in response to mechanical and herbivory damage. Herbivory induced plant volatiles produced by okra plant were qualitatively and quantitatively distinct from undamaged and mechanically damaged okra. These variations in the HIPV profile can be exploited by the insect pests and their natural enemies in okra agro-ecosystem. *E. vittella* and *T. chilonis* responded to headspace volatiles from okra plant, albeit differently. These findings suggest that volatiles emanating from okra can be exploited by predators and parasitoids for their prey finding in okra agro-ecosystem.

Table 3: Electroantennogram (EAG) response of *Earias vittella* Fab. (Male and Female) to plant volatiles collected from headspace of different treatments (see methodology for more details of treatments)

Treatment	Number of observations (N)	Normalized relative EAG responses (%)				EAG responses (-mV)			
		Male		Female		Male		Female	
		Mean	(±SE)	Mean	(±SE)	Mean	(±SE)	Mean	(±SE)
Undamaged plant (UDP)	10	100.30^{c}	0.30	104.60^{b}	2.05	-0.479^{abc}	0.04	-0.61^{ab}	0.03
Mechanically damaged plant (MDP)	10	138.50^{b}	2.92	135.20^{a}	5.93	-0.578^{a}	0.05	-0.684^{a}	0.03
Herbivory damaged plant (HDP)	10	68.30^{d}	3.67	71.60^{cd}	2.55	-0.356^{c}	0.03	-0.535^{b}	0.04
Fresh fruit (FF)	10	101.50^{c}	6.11	106.30^{b}	8.23	-0.586^{a}	0.03	-0.62^{ab}	0.03
Mechanically damaged fruit(MDF)	10	137.70^{b}	5.83	44.00^{e}	4.49	-0.523^{ab}	0.04	-0.26^{d}	0.02
Herbivory damaged fruit(HDF)	10	169.20^{a}	4.76	53.30^{de}	1.80	-0.621^{a}	0.02	-0.37^{cd}	0.02
Larval excreta(EXHS)	10	81.60^{d}	3.50	92.50^{bc}	6.04	-0.365^{bc}	0.04	-0.39^{c}	0.03

* Values in each column with no letter in common are significantly different for both normalized relative EAG response and EAG responses (-mV), separately (ANOVA followed by the Tukey's HSD test, $\alpha < 0.05$)

Table 4: Behavioral responses of *Trichogramma chilonis* Ishii to headspace volatiles in Y-tube olfactometer

S. N.	Odour Source	Male response (mean ±SE)			Female response (mean ±SE)		
		Source	Control	P value	Source	Control	P value
1.	Undamaged plant	14±0.10^{a}	11±0.10^{a}	0.56	13±0.10 ^{a}	12±0.10 ^{a}	0.85
2.	Mechanically damaged plant	17±0.09^{a}	8±0.09^{a}	0.07	18±0.09^{a}	7±0.09 ^{b}	0.03
3.	Herbivore damaged plant	21±0.07^{a}	4±0.07^{b}	0.0001	19±0.09 ^{a}	6±0.9 ^{b}	0.006
4.	Fresh fruits	13±0.10^{a}	12±0.10^{a}	0.85	15±0.1^{a}	10±0.1^{a}	0.32
5.	Mechanically damaged fruits	16±0.10^{a}	9±0.10^{a}	0.17	17±0.10^{a}	8±0.10^{b}	0.07
6.	Herbivore damaged fruits	19±0.09^{a}	6±0.09^{b}	0.006	20±0.08^{a}	5±0.08^{b}	0.001
7.	Larval Excreta	20±0.08^{a}	5±0.08^{b}	0.001	21±0.07 ^{a}	4±0.07 ^{b}	0.0001

* For each odour source, different letters indicate significant differences from control (Paired t test, N=25).

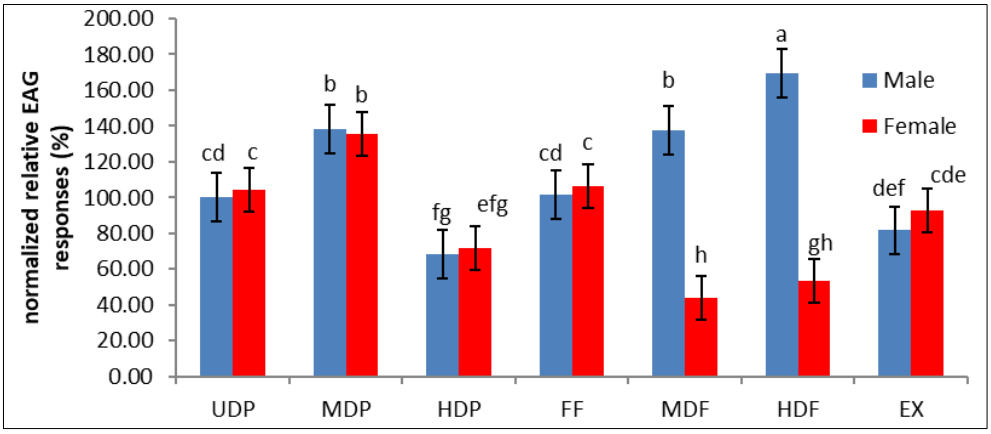

Fig 8: Electroantennogram (EAG) response of *Earias vittella* Fab. (Male and Female) to plant volatiles collected from headspace of different treatments. Mean (± standard error) normalized relative EAG responses (%) with no letter in common are significantly different (ANOVA followed by the Tukey's HSD test, N=10, $\alpha < 0.05$). Code for each treatment: UDP- undamaged plant, MDP- mechanically damaged plant, HDP- herbivory damaged plant, FF- Fresh fruit/undamaged Fruit, MDF- mechanically damaged fruit, HDF- herbivory damaged fruit, EX- Larval excreta

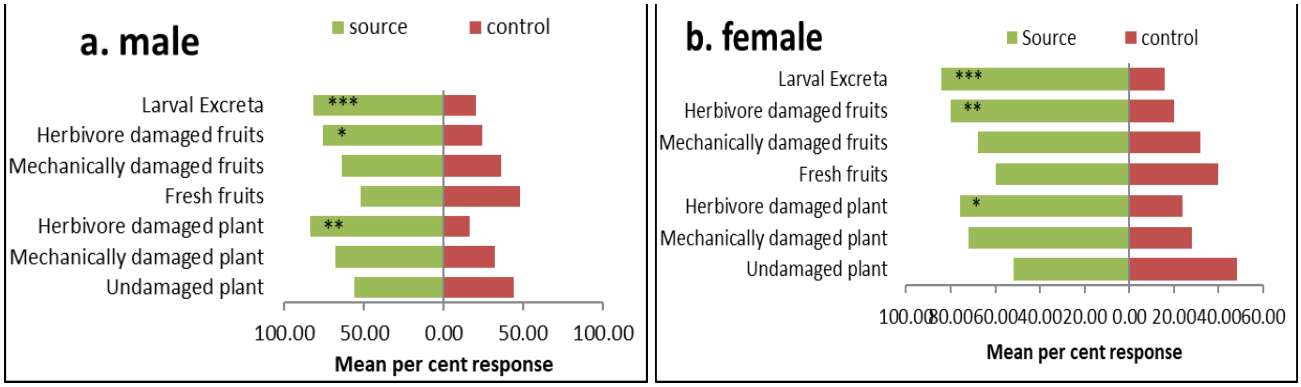

Fig 9: Behavioral responses (mean per cent response) of *Trichogramma chilonis* Ishii (a) male and (b) female to headspace volatiles in Y-tube olfactometer. Asterisks indicate significant differences over the control (Paired t test: N = 25, *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$, ****$P \leq 0.0001$)

References

1. Ameye M, Allmann S, Verwaeren J, Smagghe G, Haesaert G, Schuurink RC, *et al*. Green leaf volatile production by plants: A Meta analysis. New Phytologist.

2017;220(3):666-683.
2. Bhagat D, Bakthavatsalam N. Influence of rice cultivars on the parasitization efficiency of *Trichogramma chilonis* Ishii and *Trichogramma japonicum* Ashmead. Journal of Biological Control. 2012;26(4):329-333.
3. Blande JD, Korjus M, Holopainen JK. Foliar methyl salicylate emissions indicate prolonged aphid infestation on silver birch and black alder. Tree Physiology. 2010;30(3):404-416.
4. Boer DJG, Dicke M. The role of methyl salicylate in prey searching behavior of the predatory mite *Phytoseiulus persimilis*. Journal of Chemical Ecology. 2004;30(2):255-271.
5. De Moraes CM, Lewis WJ, Pare PW, Alborn HT, Tumlinson JH. Herbivore-infested plants selectively attract parasitoids. Nature. 1998;393(6885):570-573.
6. Halitschke R, Schittko U, Pohnert G, Boland W, Baldwin IT. Molecular interactions between the specialist herbivore *Manduca sexta* (Lepidoptera, Sphingidae) and its natural host *Nicotiana attenuata*. III. Fatty acid-amino acid conjugates in herbivore oral secretions are necessary and sufficient for herbivore-specific plant responses. Plant Physiology. 2001;125:711-717.
7. Hare JD. Ecological role of volatiles produced by plants in response to damage by herbivorous insects. Annual Review of Entomology. 2011;56:161-180.
8. Holopainen JK. Can forest trees compensate for stress-generated growth losses by induced production of volatile compounds? Tree Physiology. 2011;31:1356-1377.
9. Holopainen JK, Gershenzon J. Multiple stress factors and the emission of plant VOCs. Trends Plant Science. 2010;15:176-184.
10. Huang C, Feng-Ming Yan John A, Byers Rong-Jiang Wang, Chong-Ren Xu. Volatiles induced by the larvae of the Asian corn borer (*Ostrinia furnacalis*) in maize plants affect behavior of conspecific larvae and female adults. Insect Science. 2009;16:311-320.
11. Jennifer MA, Macleod G. Volatile components of okra. Phytochemistry. 1990;29:1201-1207.
12. Jonsson M, Anderson P. Electrophysiological response to herbivore induced plant volatiles in the moth *Spodoptera littolaris*. Physiological Entomology. 1999;24:377-385.
13. Kessler A, Baldwin IT. Defensive function of herbivore-induced plant volatile emissions in nature. Science. 2001;291:2141-2144.
14. Loreto F, Schnitzler JP. Abiotic stresses and induced BVOCs. Trends Plant Science. 2010;15:154-166.
15. Lou YG, Ma B, Cheng JA. Attraction of the parasitoid *Anagrus nilaparvatae* to rice volatiles induced by the rice brown plant hopper *Nilaparvata lugens*. Journal of Chemical Ecology. 2005;31:2357-2372.
16. Maffei ME. Sites of synthesis, biochemistry and functional role of plant volatiles. South African Journal of Botany. 2010;76:612-631.
17. Marius Camciuc, Gérard Vilarem, Antoine Gaset, Jean Marie Bessière. Volatile Constituents of the Seed Teguments of *Abelmoschus esculentus* (L.) Moench. Journal of Essential Oil Research. 1999;11:545-552.
18. Mattiacci L, Dicke M, Posthumus MA. Beta-glucosidase—an elicitor of herbivore-induced plant odor that attracts host-searching parasitic wasps. National Academy of Science USA. 1995;92:2036-2040.
19. Molnár BP, Tóth Z, Fejes-Tóth A, Dekker T, Kárpáti Z. Electrophysiologically-active maize volatiles attract gravid female European corn borer, *Ostrinia nubilalis*. Journal of chemical ecology. 2015;41(11):997-1005.
20. Mumm R, Dicke M. Variation in natural plant products and the attraction of bodyguards involved in indirect plant defense. Canadian Journal of Zoology. 2010;88:628-66.
21. Ninkovic V, Alnasi AL, Petterson J. The influence of aphid-induced plants volatiles on ladybird beetle searching. Biological Control. 2001;21:191-195.
22. Peñaflor MFGV, Erb M, Miranda LA, Werneburg AG, Bento JMS. Herbivore-induced plant volatiles can serve as host location cues for a generalist and a specialist egg parasitoid. Journal of Chemical Ecology. 2011;37:1304-1313.
23. Philip JM, Turlings CJ, Adron John Loughrin, James, TP, Tumlinson H. Herbivore-induced volatile emissions from cotton (*Gossypium hirsutum* L.) seedlings. Journal of Chemical Ecology. 1994;20(12):3039-3050.
24. Pichersky E, Noel JP, Dudareva N. Biosynthesis of plant volatiles: nature's diversity and ingenuity. Science. 2006;311:808-811.
25. Piesik D, Pańka D, Delaney KJ, Skoczek A, Lamparski R, Weaver DK. Cereal crop volatile organic compound induction after mechanical injury, beetle herbivory (*Oulema* spp.) or fungal infection (*Fusarium* spp.). Plant Physiology. 2011;168:878-886.
26. Pinto-Zevallos DM, Strapasson P, Zarbin PHG. Herbivore-induced volatile organic compounds emitted by maize: Electrophysiological responses in *Spodoptera frugiperda* females. Phytochemistry Letters. 2016;16:70-74.
27. Raghava T, kumar PR, Hegde R, Kush A. Spatial and temporal volatile organic compound response of select tomato cultivars to herbivory and mechanical injury. Plant Science. 2010;179(5):520-526.
28. Rigsby CM, McCartney NB, Herms DA, Tumlinson JH, Cipollini D. Variation in the volatile profiles of black and manchurian ash in relation to emerald ash borer oviposition preferences. Journal of Chemical Ecology. 2017;43:831-842.
29. Rodriguez-Saona CR, Polashock J, Malo EA. Jasmonate-mediated induced volatiles in the American cranberry, *Vaccinium macrocarpon*: from gene expression to organismal interactions. Frontiers in Plant Science. 2013;4:115.
30. Romeis J, Shanower TG, Zebitz CPW. Volatile Plant Infochemicals Mediate Plant Preference of *Trichogramma chilonis*. Journal of Chemical Ecology. 1997;23:2455-2465.
31. Saad K, Mohamad Roff M, Hallett R, *et al*. Aphid-induced Defences in Chilli Affect Preferences of the Whitefly, *Bemisia tabaci* (Hemiptera: Aleyrodidae). Scientific Reports. 2015;5:13697.
32. Schmelz EA, Alborn HT, Tumlinson JH. The influence of intact plant and excised-leaf bioassay designs on volicitin- and jasmonic acid induced sesquiterpene volatile release in *Zea mays*. Planta. 2001;214:171-179.
33. Schmelz EA, Alborn HT, Tumlinson JH. Synergistic interactions between volicitin, jasmonic acid and ethylene mediate insect-induced volatile emission in *Zea mays*. Physiologia Plantarum. 2003;117:403-412.
34. Srivastava M, Paul AVN, Singh AK, Dureja P. Synomonal effect of chickpea varieties on the egg parasitoid, *Trichogramma chilonis* Ishii

(Trichogrammatidae: Hymenoptera). Indian Journal of Entomology. 2004;66:332-338.
35. Tholl D, Boland W, Hansel A, Loreto F, Rose US, Schnitzler JP. Practical approaches to plant volatile analysis. The Plant Journal. 2006;45:540-560.
36. Van Den Boom CEM, Van Beek TA, Posthumus MA, De Groot A, Dicke M. Qualitative and quantitative variation among volatile profiles induced by *Tetranychus urticae* feeding on plants from various families. Journal of Chemical Ecology. 2004;30:69-89.
37. Verkerk RHJ. Manipulation of tritrophic interactions for IPM, In: Integrated Pest Management: Potential, Constraints and Challenges, Eds. O. Koul, G.S. Dhaliwal & G.W. Cuperus, CABI, UK. 2004, 55-71.
38. Vet LEM, Dicke M. Ecology of infochemical use by natural enemies in a tritrophic context. Annual Review of Entomology. 1992;37:141-172.
39. Vijaya M, Rani PU, Rajna S. Induced indirect defense in chilli plant, *Capsicum annuum* L. due to feeding stress caused by herbivore, *Spodoptera litura* F. Journal of Entomology and Zoology Studies. 2018;6(2):1264-1270.
40. Zhu J, Park KC. Methyl salicylate, a soybean aphid-induced plant volatile attractive to the predator *Coccinella septempunctata*. Journal of Chemical Ecology. 2005;31:1733-1746.