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Orientational Response of *Trichogramma Japonicum* to the Synomone from Rice Yellow Stem Borer-Damaged and Healthy Plants

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ABSTRACT

Olfactometer studies on the attraction of the egg parasitoid, Trichogramma japonicum to the synomones extracted from the YSB-damaged rice plants revealed that attraction was maximum in the order of YSB-damaged TN 1, YSB-damaged TKM 6, healthy TN 1 and healthy TKM 6. YSB-damaged TN 1 attracted 21.50 numbers of adult egg parasitoid as compared to 17.50 numbers in YSB-damaged TKM 6. The GC-MS analysis of hydrocarbon profile showed that in healthy TKM 6, 16 hydrocarbon compounds were identified. The hydrocarbon compounds n-Hexadecanoic acid, 9,12,15-Octa decatrienoic acid and 9-Octadecenoic acid had more peak area. Eighteen hydrocarbon compounds have been identified in YSB-damaged TKM 6. Two compounds namely n-Hexadecanoic acid and 9,12-Octa decadienoic acid had more peak area. 2-Methoxy-4-vinylphenol, Tetradecanal, Hexadecanoicacid, 9, 12-Octa decadienoic acid and Octadecanoic acid were present only in the YSB-damaged TKM 6 and not in healthy TKM 6. Three compounds Decane, 9,12,15-Octa decatrienoic acid and 9-Octadecenoic acid were detected only in healthy TKM 6 and not in YSB-damaged TKM 6. Twenty three hydrocarbon compounds were detected in the YSB-damaged TN 1. Two compounds n-Hexadecanoic acid and 9, 12-Octadecadienoic acid had more peak area. Healthy TN 1 had 13 hydrocarbon compounds. Seven compounds viz., Decane, Dodecanoic acid, 9,12,15-Octa decatrienoic acid, 9-octadecenoic acid, Docosane and 2 methyl hexacosane were present in healthy TN 1 and not in YSB-damaged TN 1.

1. Introduction

Stem borers are considered the most important rice pests, in particular *Scirpophaga incertulas* (Walker) and *S. innotata* (Walker) (Sigsgaard, 2000). Yellow stem borer (YSB), *S. incertulas* usually comprised more than 90 per cent of the borer population in rice, is a monophagous pest of paddy and caused nuisance to rainfed, low land and flood prone rice ecosystems (Deka and Barthakur, 2010).

Globally, YSB alone caused yield loss of 10 million tonnes and 50 per cent of the insecticides are used for its management in the rice ecosystem (Huesing and English, 2004).

In recent years, with the awareness of the high cost of insecticides and their negative impact on the environment, biological control by means of parasitoids has been used with varying degrees of success in many different areas (Perez and Cadapan, 1986). *Trichogramma japonicum* is an important egg parasitoid of YSB in southern India.

An intrinsic characteristic of egg parasitoids was that they attacked an immobile host stage, which, by itself, did not cause plant damage. In spite of that, recent data showed that host egg deposition and associated behaviours of parental females might determine a change in plant emission of volatile compounds, which consequently might act as host-induced synomones for the egg parasitoids.

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From an evolutionary point of view, both symbionts would have advantage from this "early alert" mechanism (Hilker and Meiners, 2006), as the egg parasitoids would use such highly detectable and reliable volatiles induced in plants soon after herbivore eggs are laid, whereas the plants would also increase their fitness by recruiting parasitoids which would attack the herbivore eggs before significant damage has occurred, *i.e*. before the herbivore egg hatched (Colazza*et al*., 2004a, Hilker and Meiners, 2006).

Most oviposition-induced synomones known so far are perceived by the parasitoids as olfactory stimuli (volatile synomones) (Meiners and Hilker 1997, 2000; Hilker*et al*., 2002, 2005; Colazza*et al*., 2004a, b), although there are few exceptions of chemical cues which acted after parasitoid's landing on the plant substrate, thus apparently being perceived through contact chemoreception (contact or short range synomones) (Fatouros *et al*., 2005a, 2007, Conti *et al*., 2006).

Our investigation aimed to explore the attraction of the egg parasitoid, *Trichogramma japonicum* to the synomone extracted from YSB-damaged and healthy plants of resistant TKM 6 and susceptible TN 1.

2. Material and Methods

2.1. Extraction of Synomone from YSB-Damaged and Healthy Plants

Plant samples were collected from YSB-damaged TKM 6 and TN 1 as well as from the healthy plants of TKM 6 and TN 1. The collected plant samples were pooled and the final volume was reduced to 10 g by quartering method. Ten grams of each sample was immersed overnight in 100 ml of distilled hexane. The hexane then filtered through Whatman No. 1 filter paper. Anhydrous sodium sulphate @ 1g/10g sample was added to the filtrate and kept for 2 h for dehydration. The filtrate was then passed through silica gel (60-120 mesh) column. The hexane solvent was allowed to evaporate and the left over residue was collected by rinsing the container with a small quantity of HPLC grade hexane (Merck) and stored in separate vials in a refrigerator for bioassay studies.

2.2. Orientation Response of the *Trichogramma Japonicum*

Four-way olfactometer was used to test the orientation response of the *Trichogramma japonicum,* towards the synomone and were similar to the one described by Vet *et al*., (1983). Non-absorbent, transparent acrylic sheet of 2 mm thickness was used for constructing the olfactometer. The design consisted of a central rectangular chamber (25 x 25 cm base and 15 cm height) with four arms extending from the four sides. Acrylic sheet of 25 x 25 cm formed the base plate. From the centre of each face of the chamber, at 50 mm height, a 50 mm square hole was cut. Into this, a 15 cm tube, which formed the odour arm, was inserted.

A small acrylic rim at the connecting end provided support to keep the tube in position. This also prevented the tube from being pushed inside. The distal end of the odour arm was closed with a removable acrylic sliding door. An acrylic support at the distal end kept the rectangular tube in position. The top of the central chamber was closed with an acrylic lid (20 x 20 mm) having a retaining rim. This lid was made removable and had a central 5 mm hole to allow air movement.

A small axial flow fan (DC 12V, 3.5 cm dia) was installed at each of the four distal openings of the arms to provide uniform airflow. The axial fan was screwed on to an acrylic sheet and housed on a wooden base in such a way to direct the air flow towards the inside of olfactometer. The odour source was kept at the distal end of each arm. This setup allowed the insects to receive airflow bathing the olfactory cues from the odour source and travel upwind in response to the volatiles. Humidified and purified air was passed into each of the olfactometer arms at 200 ml/min while the vacuum pump was set at 800 ml/min to avoid a mix up of volatiles in the chamber.

Before the beginning of the assays, the system was cleaned with pure ethanol and rinsed with distilled water. Synomone extracted from YSB-damaged TN 1, YSB-damaged TKM 6, healthy TN 1 and healthy TKM 6 samples formulated in hexane were delivered @ 10 micro liters using micro pipette. Each sample were smeared on a strip of Whatman No. 1 filter paper separately and solvent was allowed to evaporate from the filter paper for about 10 sec before insertion into a 25 ml airtight glass flask linked by plastic tube to the four olfactometer arms. One of the arms was kept as control. The olfactometer system was placed at room temperature. The adult egg parasitoids were released at the centre of the olfactometer and it was covered with the black cloth. The egg parasitoids were closely observed for its movement towards the odour source.

2.3. Identification of the Hydrocarbons in the Synomone Using GC-MS

The hexane extracts from YSB-damaged resistant TKM 6, YSB-damaged susceptible TN 1, healthy TKM 6 and healthy TN 1 were analysed on a GC MS-QP 2010 Plus (Shimadzu, Kyoto, Japan) mass selective detector (70 eV) equipped with a 10 : 1 split injector. The gas chromatograph was equipped with 30 m fused silica capillary column having 0.25 mm ID and 0.25 µm film thickness run in constant flow mode (1.0 ml/min helium). Oven temperature programmed at 60°C (1 min hold) to 100°C at 5°C/min rate (1 min hold), then to 220°C at 10°C/min rate (5 min hold) and then to 240°C at 50°C/min rate (8 min hold). Injector temperature was set at 275°C. One microlitre of the extract was injected using auto-sampler into the gas

chromatography–mass spectrometry (GC–MS) system for analysis. Injections were done in split 10: 1 mode. Shimadzu GC-MS Lab solution software was used for the analysis of compounds in the extracts. Injected sample was separated into various constituents with different retention time which were detected by mass spectrophotometer. The compounds separated were identified using standard NIST mass spectral (NIST MS 2) library. The chromatogram a plot of intensity against retention time was recorded by the software attached to it.

3. Result

3.1. Efficacy of Synomone for the Attraction of Egg Parasitoid

Attraction of the adult egg parasitoid to the synomones extracted from the YSB-damaged TKM 6 and YSB-damaged TN 1 was compared with the healthy TKM 6 and healthy TN 1 and result furnished in Table 1. The mean number of parasitoids attracted was more in YSB-damaged TN 1 plant extract and it significantly differed from others. It attracted 21.50 numbers of adult as compared to 17.50 numbers in YSB-damaged TKM 6 plant extract. YSB-damaged TN 1 attracted 1.72 times more adult egg parasitoids compared to the healthy TKM 6. YSB-damaged plant extracts attracted more number of parasitoids than the healthy plant extracts irrespective of the levels of plant resistance.

Table 1. *Efficacy of synomone on the attraction of egg parasitoid,T. japonicum*

Treatment	No. of parasitoids attracted (at hours after release)		Mean*
	2	4	
TKM 6 YSB damaged plant extract	13.00	22.00	17.50 (4.15)[b]
TKM 6 healthy plant extract	10.00	15.00	12.50 (3.52)[d]
TN 1 YSB damaged plant extract	16.00	27.00	21.50 (4.60)[a]
TN 1 healthy plant extract	11.00	18.00	14.50 (3.78)[c]

	SEd	CD (0.05)	CD (0.01)
Treatment	0.04049	0.08294	0.11189
Time	0.02164	0.04433	0.05981
Treatment x time	0.05726	0.11729	0.15824

The adult egg parasitoid attracted was maximum to the synomone extract in the order of YSB-damaged TN 1, YSB-damaged TKM 6, healthy TN 1 and healthy TKM 6. The adult egg parasitoid attracted was 27, 22, 18 and 15 numbers at four hours after release to YSB-damaged TN 1, YSB-damaged TKM 6, healthy TN 1 and healthy TKM 6 respectively. YSB-damaged TKM 6 had 1.40 times more attraction of the adult egg parasitoid compared to the healthy TKM 6. Similarly, YSB-damaged TN 1 had 1.48 times more attraction of the adult egg parasitoid compared to the healthy TN 1.

3.2. Identification of the Hydrocarbons in the Synomones Using GC–MS

3.3. YSB-Damaged and Healthy TKM 6

Eighteen hydrocarbon compounds have been identified in YSB-damaged TKM 6 *viz*., 1-Nonen-4-ol, 2-Methoxy-4-vinylphenol, Dodecanoic acid, Tetradecanal, 2-Cyclohexen-1-one, Hexadecanal, Tetradecanoic acid, Pentadecanoicacid, n-Hexadecanoic acid, Hexadecanoic Acid, 9,12-Octa decadienoic acid, Octadecanoic acid, Docosane, Tricosane, Pentacosane, Tetracontane, Octacosane and Squalene (Table 2, Appendix 1). Two compounds namely n-Hexadecanoic acid and 9,12-Octa decadienoic acid had more peak area.2-Methoxy-4-vinylphenol, Tetradecanal, Hexadecanoic Acid, 9, 12-Octa decadienoicacid and Octadecanoic acid were present only in the YSB-damaged TKM 6 and not in healthy TKM 6 (Table 2).

In healthy TKM 6, 16 hydrocarbon compounds were identified (Appendix 1). They are 1-Nonen-4-ol, Decane, Dodecanoic acid, 2-Cyclohexen-1-one, Hexadecanal, Tetradecanoic acid, Pentadecanoic acid, n-Hexadecanoic acid, 9,12,15-Octa decatrienoic acid, 9-Octadecenoic acid, Docosane, Tricosane, Pentacosane, Tetracontane, Octacosane and Squalene (Table 2). Three compounds Decane, 9,12,15-Octa decatrienoic acid and 9-Octadecenoic acid were detected only in healthy TKM 6 and not in YSB-damaged TKM 6. The hydrocarbon compounds n-Hexadecanoic acid, 9,12,15-Octa decatrienoic acid and 9-Octadecenoic acid had more peak area in healthy TKM 6.

3.4. YSB-Damaged and Healthy TN 1

Seventeen hydrocarbon compounds that is 1-Nonen-4-ol, Nonane, Hexadecane, Pentadecanal, Tetradecanoic acid, Heneicosane, Pentadecanoic acid, 9, 17-Octadecadienal, cis-9-Hexadecenoic acid, n-Hexadecanoic acid, 9,12-Octadecadienoic acid, Phytol acetate, Tricosane, Pentacosane, Eicosane, Tetracontane, Octacosane were detected in the YSB-damaged TN 1 (Table 3, Appendix I1). Two compounds n-Hexadecanoic acid and 9, 12-Octadecadienoic acid had more peak area. Nonane, Hexadecane, Pentadecanal, Tetradecanoic acid, Heneicosane, Pentadecanoic acid, 9,17-Octadecadienal, cis-9-Hexadecenoic acid, 9,12-Octadecadienoic acid, Phytol acetate and Eicosane were present only in the YSB-damaged TN 1 and not in the healthy TN 1.

Synomone extracted from healthy TN 1 had 13 hydrocarbon compounds (II) namely, 1-Nonen-4-ol, Decane, Dodecanoic acid, 9,12,15- Octadecatrienoic acid, n-Hexadecanoicacid,

9,12,15-Octa decatrienoic acid, 9-octadecenoic acid, Docosane, Tricosane, 2 methylhexacosane, Pentacosane, Tetracontane, Octacosane. Seven compounds *viz*., Decane, Dodecanoic acid, 9,12,15-Octa decatrienoic acid, 9-octadecenoic acid, Docosane and 2 methyl hexacosane were present in healthy TN 1 and not in YSB-damaged TN 1 (Table 3).

Table 2. *Hydrocarbon profile in the YSB-damaged and healthy rice plants of TKM 6*

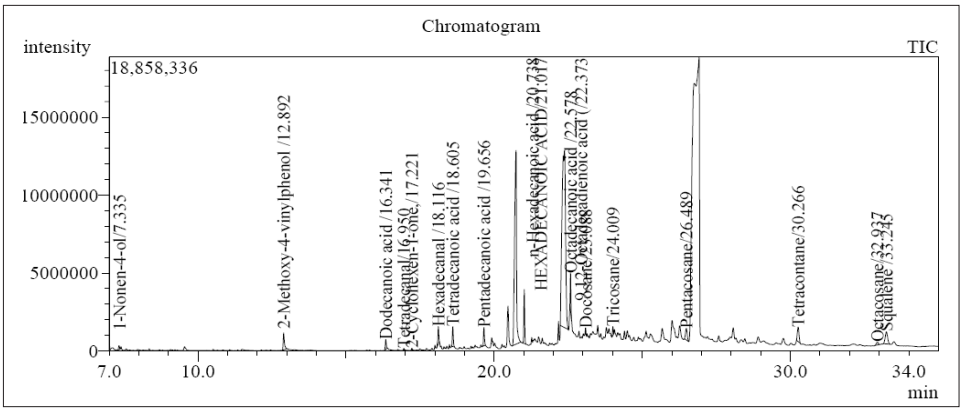
S. No	TKM 6 YSB-damaged plants			TKM 6 healthy plants		
	Retention time	Area	Compounds	Retention time	Area	Compounds
1	7.335	578082	1-Nonen-4-ol	7.334	372630	1-Nonen-4-ol
2	-	-	-	7.800	63145	Decane
3	12.892	3071099	2-Methoxy-4-vinylphenol	-	-	-
4	16.341	1431596	Dodecanoic acid	16.343	384585	Dodecanoic acid
5	16.950	225280	Tetradecanal	-	-	-
6	17.221	219408	2-Cyclohexen-1-one	17.222	224421	2-Cyclohexen-1-one
7	18.116	2444981	Hexadecanal	18.124	192157	Hexadecanal
8	18.605	2873418	Tetradecanoic acid	18.600	681527	Tetradecanoic acid
9	19.656	2574960	Pentadecanoic acid	19.652	171382	Pentadecanoic acid
10	20.738	56738331	n-Hexadecanoic acid	20.683	17872429	n-Hexadecanoic acid
11	-	-	-	20.738	56738331	9,12,15-Octa decatrienoic acid
12	21.017	6636181	HEXADECANOIC ACID	-	-	-
13	-	-	-	21.017	6636181	9-OCTADECENOIC ACID
14	22.373	94296023	9,12-Octadecadienoic acid	-	-	-
15	22.578	3458805	Octadecanoic acid	-	-	-
16	23.088	601365	Docosane	23.083	806291	Docosane
17	24.009	967602	Tricosane	24.007	1470180	Tricosane
18	26.489	2881520	Pentacosane	26.499	800889	Pentacosane
19	30.266	3554618	Tetracontane	30.260	1460286	Tetracontane
20	32.937	806657	Octacosane	32.954	226119	Octacosane
21	33.245	4322482	Squalene	33.244	2212602	Squalene
	Mean	87682408		**Mean**	60482169	

Table 3. *Hydrocarbon profile in the YSB-damaged and healthy rice plants of TN 1*

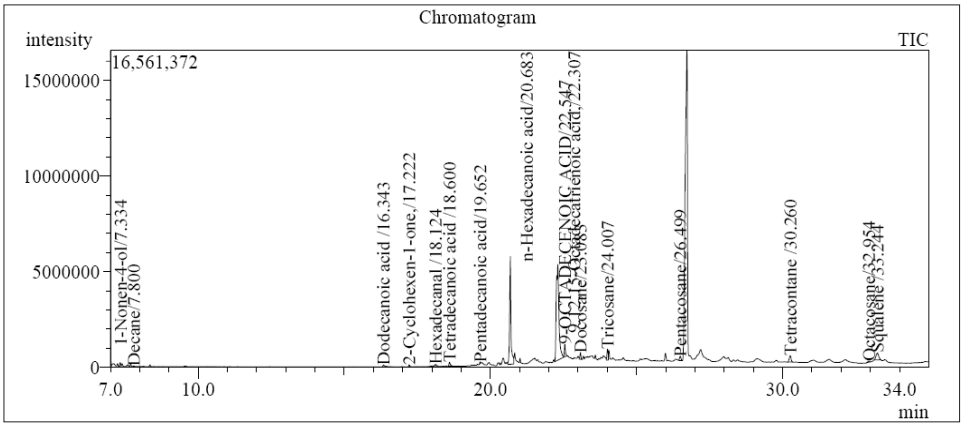
S.No	TN1 YSB damaged plants			TN1 healthy plants		
	Retention time	Area	Compounds	Retention time	Area	Compounds
1	7.334	94786	1-Nonen-4-ol	7.579	239302	1-Nonen-4-ol
2	-	-	-	7.804	104670	Decane
3	12.876	273826	Nonane	-	-	-
4	-	-	-	16.362	273146	Dodecanoic acid
5	16.364	428678	Hexadecane	-	-	-
6	17.003	315835	Pentadecane	-	-	-
7	18.122	1843974	Pentadecanal	-	-	-
8	18.601	656544	Tetradecanoic acid	-	-	-
9	19.222	716311	Heneicosane	-	-	-
10	19.654	1103691	Pentadecanoic acid	-	-	-
11	19.957	1442852	9 17-Octadecadienal	-	-	-
12	20.459	3307181	cis-9-Hexadecenoic acid	-	-	-
13	20.702	22723239	n-Hexadecanoic acid	20.678	9242243	n-Hexadecanoic acid
14	21.019	4736568	Hexadecanoic acid	-	-	-
15	-	-	-	22.317	34632582	9,12,15-Octadecatrienoic acid
16	22.331	50179705	9,12-Octadecadienoic acid	-	-	-
17	-	-	-	22.553	2922979	9-octadecenoic acid

18	-	-	-	23.089	1041062	Docosane
19	23.091	1368369	Phytol, acetate	-	-	-
20	24.010	660778	Tricosane	24.058	358856	Tricosane
21	-	-	-	25.132	1591662	2-methylhexacosane
22	26.492	1080210	Pentacosane	26.494	1392615	Pentacosane
23	28.155	1276128	Eicosane	-	-	-
24	30.264	2643176	Tetracontane	30.270	3234769	Tetracontane
25	32.942	939822	Octacosane	32.951	1739969	Octacosane
	Mean	95791673		**Mean**	58275782	

Appendix I

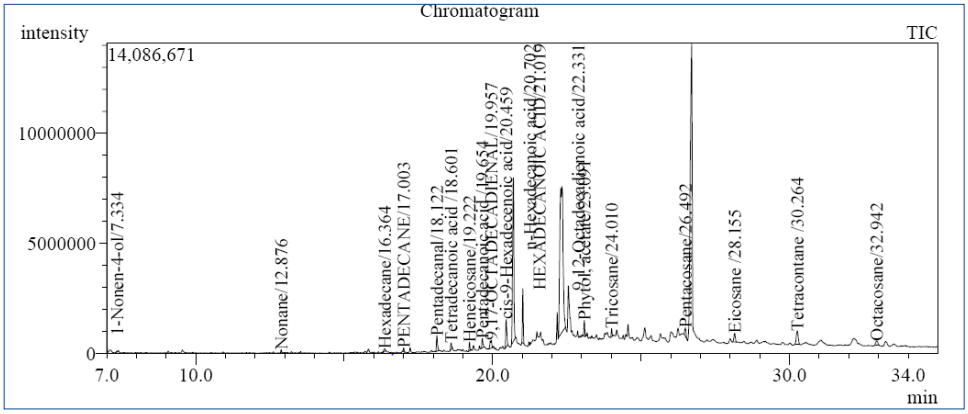


Chromatogram of the synomones extracted from YSB-damaged TKM 6 plants

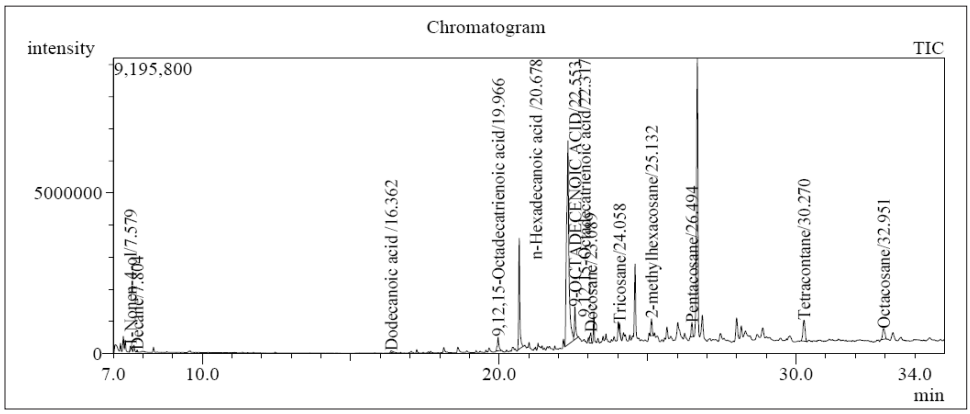


Chromatogram of the synomones extracted from healthy TKM 6 plants

Appendix Ii



Chromatogram of the synomones extracted from YSB-damaged TN 1plants



Chromatogram of the synomones extracted from healthy TN 1plants

4. Discussion

Attraction of the adult egg parasitoid to the synomones extracted from the YSB-damaged TKM 6 and YSB-damaged TN 1 was compared with the healthy TKM 6 and healthy TN 1. The adult egg parasitoid attracted was maximum to the synomone extract in the order of YSB-damaged TN 1, YSB-damaged TKM 6, healthy TN 1 and healthy TKM 6. The adult egg parasitoid attracted was 27, 22, 18 and 15 numbers at four hours after release to YSB-damaged TN 1, YSB-damaged TKM 6, healthy TN 1 and healthy TKM 6 respectively. YSB-damaged TKM 6 had 1.40 times more attraction of the adult egg parasitoid compared to the healthy TKM 6. Similarly, YSB-damaged TN 1 had 1.48 times more attraction of the adult egg parasitoid compared to the healthy TN 1.

The attraction of egg parasitoid females to synomones induced by the feeding activity of immatures and adults has been explored in a few cases, involving Mymaridae and Scelionidae. Manrique*et al*., (2005) reported that volatiles released by several herbaceous plants infested by adults of *Lygus hesperus* Knight attracted the egg parasitoid, *Anaphesiole* Girault. Moraes *et al*., (2005) and Manrique*et al.,* (2005) allowed the herbivore females to feed and oviposit on the host plants, therefore synomone induction also as a consequence of oviposition cannot be excluded.

Identification of the hydrocarbon profile in synomones through GC-MS revealed that eighteen hydrocarbon compounds have been identified in YSB-damaged TKM 6 of which two compounds namely n-Hexadecanoic acid and 9,12-Octa decadienoic acid had more peak area. 2-Methoxy-4-vinylphenol, Tetradecanal, Hexadecanoic Acid, 9, 12-Octa decadienoic acid and Octadecanoic acid were presentonly in the YSB-damaged TKM 6 and not in healthy TKM 6.In healthy TKM 6, 16hydrocarbon compounds were identified of which 9,12,15-Octa decatrienoic acid and 9-Octadecenoic acid had more peak area. Three compounds Decane, 9,12,15-Octa decatrienoic acid and 9-Octadecenoic acid were detected only in healthy TKM 6 and not in YSB-damaged TKM 6.

Twenty three hydrocarbon compounds were detected in the YSB-damaged TN 1. Two compounds n-Hexadecanoic acid and 9, 12-Octadecadienoic acid had more peak area. Nonane,Hexadecane, Pentadecanal, Tetradecanoic acid, Heneicosane, Pentadecanoicacid, 9,17-Octadecadienal, cis-9-Hexadecenoic acid, 9,12-Octadecadienoic acid, Phytol acetate and Eicosane were present only in the YSB-damaged TN 1 and not in the healthy TN 1. Synomone extracted from healthy TN 1 had totally 13 hydrocarbon compounds. Seven compounds *viz*., Decane, Dodecanoic acid, 9,12,15-Octa decatrienoic acid, 9-octadecenoic acid, Docosane and 2 methyl hexacosane were present in healthy TN 1 and not in YSB-damaged TN 1.

Most oviposition-induced synomones known so far are perceived by the parasitoid as olfactory stimuli (volatile synomones) (Meiners and Hilker 1997, 2000; Hilker*et al*., 2002, 2005; Colazza*et al*., 2004), although there are few exceptions of chemical cues which act after parasitoid's landing on the plant substrate, thus apparently being perceived through contact chemoreception (contact or short range synomones) (Fatouros*et al*., 2005, 2007, Conti *et al*., 2006).

To conclude, two compounds *viz*., n-Hexadecanoic acid and 9, 12-Octadecadienoic acid had more peak area in YSB-damaged TKM 6 and TN 1. These two compounds have to be further explored for the attraction of the egg parasitoid.

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