Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2018; 6(2): 1264-1270

Received: 07-01-2018
Accepted: 08-02-2018

Movva Vijaya
a) Biology and Biotechnology Division, CSIR-Indian Institute of Chemical Technology, Taranaka, Hyderabad, India
b) Academy of Scientific and Innovative Research, CSIR-Indian Institute of Chemical Technology, Taranaka, Hyderabad, India

Pathipati Usha Rani
Biology and Biotechnology Division, CSIR-Indian Institute of Chemical Technology, Taranaka, Hyderabad, India

S Rajna
Academy of Scientific and Innovative Research, CSIR-Indian Institute of Chemical Technology, Taranaka, Hyderabad, India

Correspondence
Movva Vijaya
a) Biology and Biotechnology Division, CSIR-Indian Institute of Chemical Technology, Taranaka, Hyderabad, India
b) Academy of Scientific and Innovative Research, CSIR-Indian Institute of Chemical Technology, Taranaka, Hyderabad, India

Induced indirect defense in chilli plant, *Capsicum annuum* L. due to feeding stress caused by herbivore, *Spodoptera litura* F.

Movva Vijaya, Pathipati Usha Rani and S Rajna

Abstract
Chilli, *Capsicum annuum* L. plants release volatile chemicals in response to the feeding stress caused by the generalist herbivore, *Spodoptera litura*. These chemicals serves as volatile cues that signal and recruit the generalist egg parasitoid, *Trichogramma chilonis* Ishii to successfully locate its host. The volatile chemicals released after the herbivory were extracted and subjected to column chromatography and the different solvent fractions were assayed in behavioral bioassays. Twelve percent Ethyl acetate fraction which was found to attract the wasps more intensively was regarded as active fraction and was analyzed by GC-MASS. Urs 12 en- 24- oic acid 3-oxo methyl ester, found only in the infested plants at high percentage (11.214%) along with several long chain hydro carbon compounds, identified as Tetradecane, Dodecanoic acid, Pentacosane, Hexacosane, Heptacosane, Triacontane which all together might be playing an important role in providing chemical cues for the parasitoid, *T. chilonis.*

Keywords: herbivore induced plant volatiles (HIPVs), GC-MASS, *Spodoptera litura, Trichogramma chilonis, Capsicum annuum* (chilli)

Introduction
Apart from green leaf volatiles, plants also possess different chemicals that are expressed after an insect or microbe attack. Herbivory and pathogen attack causes the change in the several biosynthetic pathways in plants which in turn leads to the production of herbivore induced plant volatiles (HIPV's) [1]. These HIPV's are used as cues by the parasitoid which in turn controls the attacking pest by parasitization thus play a crucial role in tritrophic interactions as well as in defense response against herbivore attack [2]. These can either be compounds that a plant does not biosynthesize unless it is damaged or compounds that are also synthesized by undamaged plants but in elevated amounts by damaged plants. The type of volatile blend emitted from the plant also depends upon the species of plant, age of the plant, insect attacked and even the age of the attacking insect [3]. In some cases, the HIPV blend produced also differs depending on the species of herbivore feeding on the plant [4]. These may be emitted from the site of damage and sometimes also from systemically undamaged parts of the plant [5]. The odors released by attacked plants serve as important cues for parasitoids and predators to locate their host/prey [6]. There are reports of about 50 different species of plants producing volatiles, which help in attracting the herbivore natural enemies drawn from five insect orders (McCormick, 2012). The main functions of HIPVs are the priming and activation of defenses in neighboring plants and the attraction of natural enemies of the attacker. Apart from the herbivore feeding, root feeding [7] also affects the behavior of predators and parasitoids and often the host eggs deposited on the plant surfaces also induce defense responses in the form of chemical volatiles [8].
The common cut worm, *S. litura* (F) (Lepidoptera: Noctuidae) and chilli, *C. annuum* (L) are selected as model pest and plant of our study. *S. litura* is widely distributed, polyphagous pest devastating with large host range, and it causes heavy damage to the chilli crop [9]. *T. chilonis* is a Hymenopteran egg parasitoid responsible for mortality of most destructive pests such as *Hellicoverpa armigera* and *S. litura* in their egg stage itself [10]. The behavior of Trichogramma is usually mediated by chemical means [11] from the attacked plants or from the attacking herbivore. Studies on rice plants infested with its stem borer pest, *Scirpophaga incertulas* Walker (YSB) revealed that the plants emit volatiles through the surface of their infested stems which induce attractant activity and stimulates the oviposition of its egg

parasitoid, *Trichogramma japonicum* Ashmead [12]. The difference in the level of parasitism on different plants may be partly caused by plant volatiles [13]. The chemical information through HIPVs attracts herbivore natural enemies and, thus benefit plants indirectly [14, 15].

Since *T. chilonis* is known for its parasitization efficacy of a wide range of lepidopteran pests, it will be interesting to study the behavior of T. chilonis to the HIPVs from chilli plants during *S. litura* feeding. In this study we focused on elucidating the chemical profile of plant volatiles emitted from both infested and uninfested chilli plants and the response of *T. chilonis* to these volatiles.

2. Materials and Methods

2.1 Plant maintenance

Chili plants, *Capsicum annuum* ('Garima' variety), obtained from the nursery of vegetable section, Acharya N. G. Ranga Agricultural University (ANGRAU), Rajendra nagar, Hyderabad (AP) were grown in clay pots filled with red soil (20 cm height × 24 cm diameter) in laboratory glass house under standard, pest-free conditions (30 ± 5°C, 60 ± 5% RH and 16-h light: 8-h dark photoperiod).

2.2 Insect maintenance

2.2.1 *Spodoptera litura* F. (Lepidoptera: Noctuidae)

The initial *S. litura* (NBAII-MP-NOC-02) egg masses were procured from ICAR-NBAIR, Bangalore. Neonate larvae of *S. litura* were kept in plastic tubs (25 cm diameter) and were provided with fresh castor leaves, in our laboratory under controlled room conditions [28 ± 2°C, RH 65± 5% and photoperiod of 16:8 (light: dark)]. Healthy fourth instar larvae of *S. litura* were used for the experiments. The insects used in the experiments are from a single egg mass which emerged on the same day to eliminate the errors caused due to their age differences.

2.2.2 *Trichogramma chilonis* I. (Hymenoptera: Trichogrammatidae)

The initial cultures of *T. chilonis* (NBAII-MP-TRI-13) were brought from Project Directorate of Biological Control, Bangalore and cultured in our laboratory under controlled conditions at 28±2 ºC and at 65± 5 % relative humidity (RH) using the eggs of *S. litura* for its parasitization. The parasitoids were fed with diluted honey soon after their eclosion and prior to the experiments [16].

2.3 Plant treatment and extraction

Healthy 4th instar *S. litura* larvae were released on to the chilli plant at the rate of 3 insects per plant and were allowed to feed till half of the leaf is consumed. The leaves remained after the feeding as well as the same amount of normal pest unfed leaves were excised from the plant 24hrs after infestation and dipped in DCM solvent for 20-25 seconds with continuous shaking at the rate of 22 gm/10 ml at room temperature [16]. The extracts obtained were filtered through Whatmann no. 1 filter paper and air dried. This resulting material is termed as 'crude extract'.

2.4 Sample separation

The crude extracts which showed positive activity in behavioral assays were further separated and purified by column chromatography.

2.5 Semi purified column fractions

Semi purified fractions were obtained from the crude leaf extracts by eluting with different solvents in silica gel columns. The extracts were subjected to column chromatography using hexane and ethyl acetate solvent systems. Silica gel (mesh size 60-120) is used as the stationary phase and hexane and ethyl acetate solvent system for eluting the compounds. The column was allowed to saturate with hexane and The compound (crude leaf surface extract) was eluted with different ratios of Hexane: Ethyl acetate, 2, 4, 8, 12, 20, 50, and 100 percent Ethyl acetate (EA) and pure Hexane (H), The fractions obtained after elution are considered as the semi purified fractions and were used for all the bioassays along with the crude extracts.

2.6 Behavioral bioassays

The test materials were subjected to behavioral bioassays using *T. chilonis* parasitoids. In these experiments, the parasitoids responses towards each test sample are measured in 3 different types of assays i.e., orientation assays in culture tubes, parasitoids responses in Y- Olfactometer, ovipositional assay (Petri plate method). In all the experiments, female parasitoids were mated upon emergence and did not have any experience with the host herbivore or the host plant–associated cues prior to bioassays.

2.6.1 Orientation assays in culture tubes

This assay was first described by [12], in which behavior in the form of recognition of the test sample and responding by moving towards the sample were recorded. In these assays, flat bottomed culture tubes (9cm long, 2.5 cm diameter) were used as the arenas for testing parasitoids behavior towards the volatile chemicals. On a piece of absorbent paper the test material (20µl) was applied as a localized spot and the solvent was allowed to evaporate for 2 minutes. Then the paper was hung from the inner side of the tube. One gravid female was released at the bottom of the tube and the lid was closed and observed visually for 15 minutes for wasp's behavior. The behaviors were classified on 0-4 scale. The scale is as follows: 0. No reaction and no movement; 1. Upward movement towards the paper strip; 2. Circular movements around the paper strip; 3. Landing on the paper strip; 4. Parasitoid antennation and probing on the paper. The behavior of 15 females was measured for each test material and the tests were repeated three times.

2.6.2 Parasitoids responses in Y- Olfactometer

Method reported by [16] was followed to test the orientation behavior of the parasitoids, in which a glass Y- tube with two arms is used. It can also be considered as a dual choice test for the parasitoid between samples from both pest fed and unfed plants. Fixed quantity of test material (20µl) was applied on to a small piece of absorbant cotton and after allowing the solvent to evaporate for 2 minutes, they were placed at the end of one of the two arms of the Y tube. One gravid female of *T. chilonis* was released into the basal arm of the tube and observed for 15 minutes for the orientation towards her preferred arm.

2.6.3 Ovipositional assay (Petri plate method)

This was performed to test the efficacy of the test material which is indicated by increased oviposition rate. The rate of *T. chilonis* parasitization on *S. litura* infested leaf crude extracts, and their coloumn fractions treated host eggs were measured and compared to that of normal, healthy *C. annuum* plant leaf extracts treated host eggs through ovipositional assays performed in the laboratory [16]. The large 150mm diameter

plastic petri dishes formed experimental arenas where the parasitoids were offered treated and untreated host eggs. One day old S. litura eggs were glued on to white card paper in such a way that each card paper (1cm×1cm) should contain about 50 host eggs. The surfaces of the host eggs were uniformly treated with the test sample at the rate of 20µl per 50 eggs and the solvent was evaporated and tested for oviposition efficacy. Briefly, the cards containing treated eggs are pasted on to a filter paper disc that lined the petri dish. Each petri dish contained three treated and three control egg cards pasted alternately. A freshly emerged, honey fed and mated female parasitoid, A gravid female parasitoid, *T. chilonis* was released at the centre of the petri dish and the lid closed. In dual choice experiments, each petri dish contained three sample extracts and three control (control vs infested plant extract; control vs uninfested plant extract; infested vs uninfested plant extracts) treated egg cards. In multiple-choice tests, all three samples (control, healthy plant extract, infested plant extract) treated eggs were offered simultaneously, which were then placed diagonally opposite to each other in alternating sectors. Ovipositional assays were conducted using dual choice and multiple-choice experiments. The experiments were performed in the laboratory at 28±2°C, with RH of 65±5% and under a fluorescent light bank of 1200 Lux.

2.7 Gas chromatograph – mass spectrometer (GC – MS)

The chromatographic fractions that evoked positive anemotactic responses or stimulated the enhanced parasitization in *T. chilonis* in behavioral bioassays were further analyzed in gas chromatography coupled with mass spectroscopy (GC-MS) to identify the active chemical(s). The concentrates of the hexane: ethyl acetate elute of the chilli plant extracts were injected into the GC (Agilent 6890 GC) equipped with 5973 N model mass selective detector. An HP–5 MS (Hewlett Packard, Palo Alto, CA) capillary column was used. The column oven was programmed after an initial delay of 2 min from 50 to 300º C at 10º C/min. Helium was used as carrier gas, with a flow rate of 1.2 ml/min. The sample (1 µl) was introduced in a split ratio 10: 1 at an injection temperature of 150°C compounds were identified by comparison of the mass spectra with those in the Wiley library.

2.8 Statistical analysis

All statistical analyses were performed using biostat 2008. Statistical differences between two groups in dual choice tests were evaluated with the paired t-test while multiple-choice test results were analysed by one way ANOVA to identify significant differences in the various behaviors among the three or more treatment groups (Biostat 2008).

3. Results

3.1 Behavioral assays

3.1.1 Orientation response of T. chilonis in culture tube assay

The experimental studies on *T. chilonis* using crude extractions of different test concentrations i.e., 10, 20, 30 and 40 µg/ml indicated that the egg parasitoids are attracted towards almost all the samples confirming the presence of volatile compounds. The wasps showed highest attraction towards the leaf surface extracts of *S. litura* fed leaves which is confirmed by the utmost antennal probing, circular movements and landing on the sample patch in search of its host. *T. chilonis* also moved towards the normal plant extract treated patch but with least excitement and failed to land on the sample compared to the infested leaf extract treated patch. Maximum response of the parasitoid was observed at 20 µg/ml concentration of crude extract of both infested and uninfested plants. Solvent control is also used in the assay to rule out the errors. The responses of *T. chilonis* to different test concentrations of the crude extracts were listed in Table 1.

Table 1: Parasitization by *T. chilonis* on host eggs treated with pest infested and uninfested leaf crude extracts.

Sample	Concentrations tested			
	10µg/ml (T): (S)	**20µg/ml (T): (S)**	**30µg/ml (T): (S)**	**40µg/ml (T): (S)**
Solvent control	10±0.7:1.8±0.13	7±0.8:2±0.1	6.2±1.0:2.2±0.1	7±0.8:2.8±0.2
Uninfested sample	7.5±0.8:2.4±0.2	7±0.8:2.8±0.2	6±0.6:2.8±0.1	8±0.8:3±0.2
Infested sample	9±1.2:3.3±0.2	9±0.6:3±0.2	12±0.8:3.4±0.1	8±0.8:3.4±0.1

Values are means of time (T) ±SE: maximum score (S) ±SE (n=100).

Culture tube assays conducted for the semi purified fractions to test the presence of active compound containing fraction confirmed that 8 and 12 percent ethyl acetate fractions are the most preferred fractions of *T. chilonis.* Among these two fractions highest antennal probing and wing vibration was observed towards the 12 percent ethyl acetate fraction of the infested plant. The behavioral responses of the parasitoid, *T. chilonis* to different semi purified fractions of both infested and normal plant samples were listed in Table 2. From the culture tube bioassays we conclude that the 12 percent ethyl acetate fraction of infested plant extract was found to possess an active compound which is induced by the plant in response to insect attack.

Table 2: Parasitization by *T. chilonis* on host eggs treated with fractions obtained from pest infested and uninfested plant crude extracts through coloumn chromatography.

Coloumn fractions	Chilli plants	
	Fed by *S. litura*	**Normal plants**
100% Hexane	4.8±0.9:2.4±0.2	3.4±0.2:2.2±0.1
2% Ethyl acetate: Hexane	4.6±0.2:2±0	3.8±0.3: 2±0
4% Ethyl acetate: Hexane	5.8±1.2:2±0	2±0:3±0
8% Ethyl acetate: Hexane	4.2±0.3:3±0.2	7±0.8:2.4±0.1
12% Ethyl acetate: Hexane	3.8±0.3:3.4±0.2	6±0.6:2.2±0.1
20% Ethyl acetate: Hexane	5.4±0.8:2.9±0.2	6.8±1.6:2±0.4
50%Ethyl acetate: Hexane	6±0.6:2.2±0.1	3.8±0.3:2±0
100% Ethyl acetate	5.2±0.8:2±0	3.8±0.3:2±0

Values are means of time (T) ±SE: maximum score (S) ±SE (n=100).

3.1.2 Orientation behavior of T. chilonis in Y Olfactometer

Laboratory behavioral assays performed in Y- Olfactometer with *T. chilonis* revealed the parasitoids choice towards the volatile chemicals from the *S. litura* infested and uninfested leaf extracts and their chromatographic fractions of *C. annuum* plants. The volatile chemicals from the test samples directly affected the olfactory signal system of the *T. chilonis* in host location. The crude samples were tested in Y tube in which infested and uninfested samples were provided in both arms concurrently at concentrations ranging from 10-40 µg/ml. Y tube experiments revealed that the wasp is able to differentiate the signals from infested and uninfested arms. Highest scores were recorded towards the infested arm in which high antennal probing and continuous movement of the wasp was observed around the sample and was found maximum at 20µg/ml concentration of the crude extract as presented in Fig. 1.

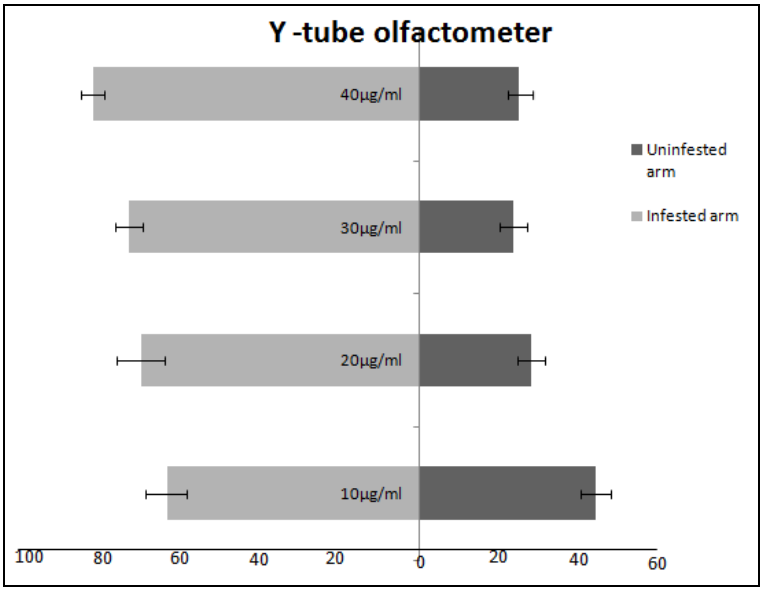

Fig 1: Orientation preference of *T. chilonis* in Y tube olfactometer towards crude extracts of both infested and uninfested chilli plants at different test concentrations. Values are mean ± SE (n=100)

In Y- Olfactometer, parasitoids preference for 12 percent EA: H fraction of the infested leaf extract was more (86±1.8) than any other fractions tested. Parasitoids responses towards different fractions were listed in Table 3.

Table 3: Orientation preference of T.chilonis towards pest fed and normal chilli plants and their chromatographic fractions in Y- tube olfactometer.

Chromatographic Fractions	Infested plant extract	Normal plant extract
100% Hexane	51±2.2	52.8±2.4
2% Ethyl acetate: Hexane	4±1.02	5.5±1.3
4% Ethyl acetate: Hexane	49.6±2.2	54.8±4.8
8% Ethyl acetate: Hexane	59.5±4.7	36.5±6.5
12% Ethyl acetate: Hexane	86±1.8	13.4±1.7
20% Ethyl acetate: Hexane	45±3.9	58.6±4.1
50%Ethyl acetate: Hexane	47.2±4.3	58.6±4.1
100% Ethyl acetate	32.2±3.7	32.8±3.1

Values are mean ± SE (n=100).

3.1.3 Ovipositional preferences of T. chilonis

Laboratory ovipositional bioassays were conducted in petri dishes using surface treated host eggs with different concentrations in dual choice. Almost always *T. chilonis* preferred to oviposit on *S. litura* infested leaf extract treated host eggs. When the solvent as well as the normal leaf extracts treated host eggs were given as an option for oviposition in dual choice assays, the wasps preferred to oviposit on the normal plant extract treated eggs compared to the solvent treated. However, when *S. litura* fed and normal plant extracts were offered for oviposition, highest rate was observed on the host eggs treated with pest infested plant extracts, indicating the presence of oviposition stimulants as presented in Fig. 2a. In multiple choice tests, highest parasitization rate was observed in the infested plant extract treated eggs as presented in Fig. 2c. It is interesting to note that the extracts from the normal, healthy chilli plants that are not being exposed to any pest feeding has also stimulated oviposition up to some extent. The lowest dose applied, that is 10µg/ml crude leaf extract per 50 eggs caused a low incidence of parasitazation, while 20µg/ml triggered a maximum parasitization.

Among the chromatographic fractions that stimulated positive responses in *T. chilonis* during behavioral assays, 12 percent EA: H eluted fraction caused highest parasitization than the 8 percent EA: H. The results obtained with ovipositional assays on *T. chilonis* in both dual choice and multiple choice tests using column fractions are given in Fig. 2b & 2d. The enhanced rate of parasitization on infested plant extracts treated host eggs implies that herbivory induced the plants to produce considerable amounts of volatiles that provide chemical cues to the parasitoids.

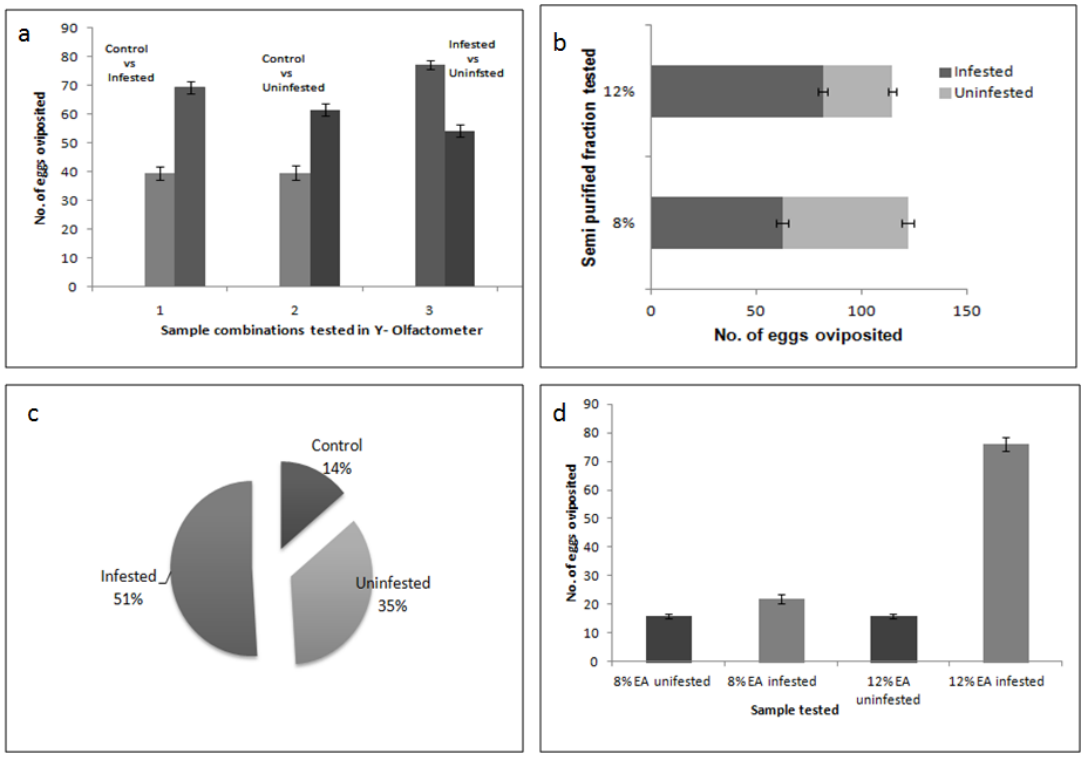

Fig 2: Parasitization of *S. litura* eggs, surface treated with the crude leaf extracts from pest fed and normal chilli plants by *T. chilonis* in a) Dual choice tests with crude samples; b) Dual choice tests with column fractions; c) Multiple choice tests with crude samples; d) Multiple choice tests with column fractions. Values are means of number of eggs parasitized (Mean±SE) (n=100)

3.2 GC-MS analysis

Among the semi purified fractions 12 percent EA: hexane fraction was regarded as bioactive fraction, since this was shown highest activity among all the chromatographic fractions tested in all the behavioral bioassays. Hence, this fraction was subjected to further analysis for chemical elucidation. The GC-Mass analysis of the infested chilli plants revealed the presence of a large number of chemicals and the chemical profile of this fraction is listed in Table 4 with their RT values and chromatograms were given in Fig. 3a and 3b. Significant amounts of Urs-12-en-24-oic acid 3-oxo- methyl ester (11.214%) was observed only in the infested chilli plants, while amounts of Pentadecane, Hexadecane, Heptadecane, Octadecane, Nonadecane, Eicosane, Docosane, Tetracosane, Octacosane, Nonacosane are decreased in the infested plants compared to uninfested chilli plants. A few hydrocarbon chemicals like Tetradecane, Dodecanoic acid, Pentacosane, Hexacosane, Heptacosane, Triacontane and fatty acid like Hexadecanoic acid were shown to be present only in the infested plant extracts. Apart from several hydrocarbon chemicals there were also few unknown compounds in all the chilli plant leaf extracts.

Table 4: List of chemical compounds detected by GC-MASS analysis of the 12percent Ethyl acetate (active fraction) of the both infested and uninfested plant extracts. NA- not assigned.

Retention time	Compound name	Area (%)	
		Uninfested plant	Infested plant
10.930	Tetradecane	NA	0.261
12.191	Pentadecane	1.008	0.443
12.973	Dodecanoic acid	NA	1.472
13.389	Hexadecane	1.360	0.773
14.524	Heptadecane	1.780	0.565
15.596	Octadecane	4.854	1.380
16.617	Nonadecane	0.874	0.348
17.248	Hexadecanoic acid	NA	1.508
17.589	Eicosane	8.388	2.237
19.430	Docosane	9.669	2.879
21.120	Tetracosane	9.205	3.536
21.914	Pentacosane	NA	1.746
22.684	Hexacosane	NA	3.670
23.415	Heptacosane	NA	1.627
24.134	Octocosane	5.457	2.775
25.521	Triacontane	4.101	2.000
27.362	Nonacosane	2.161	1.050
31.108	Urs-12-ene-24-oic acid,3-oxo-, methyl ester	NA	11.214

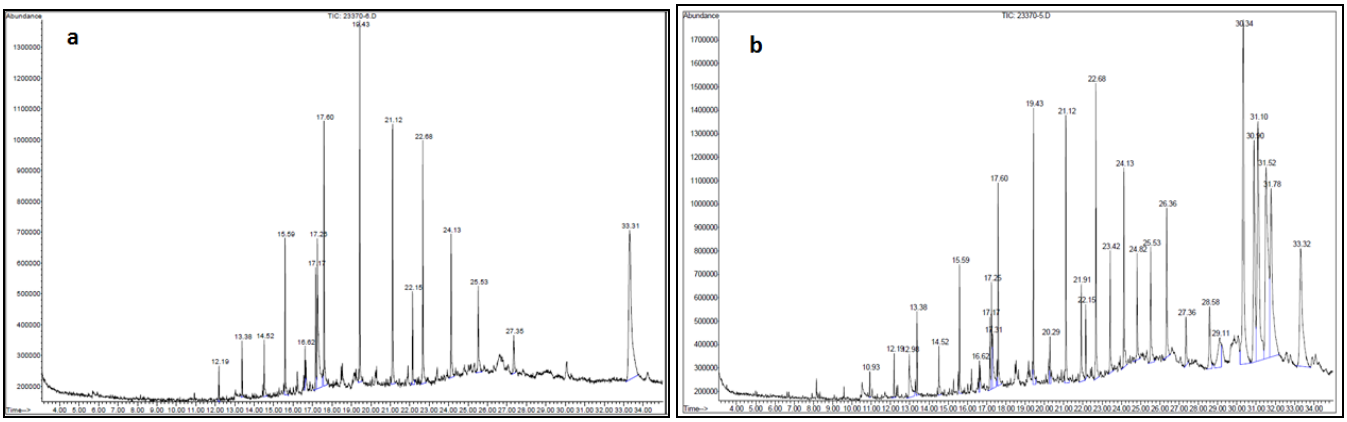

Fig 3: Representative total ion current chromatograms of GC–MS analysis of the fraction eluted with 12% ethyl acetate: hexane (a) Uninfested plant extract (b) Infested plant extract. The number on the bars indicates retention times

4. Discussion

Plants generally emit volatiles and the quantity increases when they are under herbivore attack [17]. These volatiles can act as cues for indirect defense for the plants by attracting the natural enemies, predators and parasitoids. Among the chromatographic fractions too, parasitoids response towards 8 percent and 12 percent ethyl acetate: hexane fraction is higher as is the case of the other behavioral assays i:e orientation in culture tube and in Y- olfactometer. These above two fractions caused increased parasitization in *T. chilonis* in ovipositional bioassays. These results suggest that the active chemicals that are induced due to the feeding of *S. litura* in chilli plants can be extracted with 8 percent and 12 percent ethyl acetate: hexane. Interestingly, percentage oviposition on host eggs treated with 8 percent ethyl acetate: hexane of pest infested chilli leaf as well as normal, healthy chilli leaf extract did not differ much. This suggests that certain chemicals that stimulate oviposition occur in chilli plants (whether infested with pest or not) that can be eluted with 8 percent ethyl acetate: hexane combination. These might be common chemicals and may not be the herbivore induced. Whereas, 12 percent ethyl acetate: hexane could elute HIPVs that are highly attractant to parasitoids in orientation assays and also could induce parasitization extensively, on treated host eggs. We suspect that, 12 percent ethyl acetate: hexane fraction of infested plant extracts consist chemicals that are bioactive and needed further identification of individual components. Contrary to our results, [18] studies on the maize plants showed the inability of undamaged plant chemicals in attracting a generalist egg parasitoid, *Trichogramma pretiosum*, which can respond innately to herbivore induced plant volatiles. *Plutella xylostella* feeding on the cruciferous plants were reported to attract the female solitary parasitoid wasps with more intensive antennal searching and ovipositor probing behaviors (Kugimiya *et al*. 2010). Though HIPVs do not directly indicate the occurrence of eggs, their exploitation could be of use if the host has overlapping generations [19]. Furthermore, HIPVs may help phoretic egg parasitoids to locate adults to find out a new oviposition sites with eggs [20]. The plasticity in behavior may be advantageous to the egg parasitoid, as HIPVs may be associated only with eggs under certain environmental situations: In times of high herbivore density, it may be practically common to find egg masses and larvae occurring simultaneously on the same plants, while in times of low occurrence, this would be less likely. [21] [22] showed that *Telenomus podisi* (Ashmead) (Hymenoptera: Scelionidae), a generalist egg parasitoid of pentatomids, is innately attracted by HIPVs released by soybean and chickpea under *Euchistus heros* attack.

Several hydrocarbon compounds like tetradecane, pentadecane, hexadecane, heptadecane, octadecane, nonadecane, eicosane, docosane, tetracosane, pentacosane, hexacosane, heptacosane, octacosane, triacontane and nonacosane evoked from chilli plants after *S. litura* damage. Plants use these hydrocarbon compounds as defensive elements towards the attacking pests. The background studies reveal that behavioural changes in Trichogramma spp. can be brought about mainly by hydrocarbons [23] [24] or by terpenes [25]. A study on *T. japonicum* in rice yellow stem borer infestation suggests that quantity of long chain hydrocarbons and organic acids is important for the assessment and oviposition stimulation of *T. japonicum* [12]. The volatile chemicals docosane, tetracosane, pentacosane, and eicosane which are observed in our study are reported to cause enhanced host egg parasitization by *T. japonicum* when their synthetic forms are applied on to the host eggs [26]. This gives us clear evidence that these chemicals are released from plants as an indirect defense response to provide cues to the parasitoids. Plants not only alter volatile compound emission during the pest attack but also change in chemical composition is observed at its different growth stages during its vegetation. Daniel Forero *et al*., (2009) reported the change in volatile compounds of chile pepper (Capsicum annuum L. var. glabriusculum) at two ripening stages. The blend of volatiles differs according to the plant species, herbivore species and even the mode of feeding of the attacking herbivore. not only the infested parts of the plant emit volatiles to call upon the parasitoid (Usha Rani and Sandhyarani, 2011) but also systemically undamaged parts of the plants also plays their role in providing the chemical cues to the parasitoid (Rodriguez-Saona *et al*., 2010).

A triterpeniod compound, Urs 12-en, 24-oic acid 3-oxo methyl ester is recorded only in the infested plants in higher amounts. Presence of large quantities of triterpenes in latex and resins of some plants is generally believed to be a chemical defense against pathogens and herbivores [27]. Apart from their role in defense against herbivore triterpenes are also reported to have antimicrobial property (Abbas *et al*., 2014). Triterpenes are a class of terpenes which are regarded as the plant secondary 354 meatbolites acting as toxins and feeding deterants to many herbivorous insects and sometimes to mammals and thus possess important defensive roles in plants. Presence of triterpenoid compounds like Urs 12-en, 24-oic acid 3-oxo methyl ester in chilli plants is perhaps to discourage further feeding by the pest insect. Hexadecanoic acid which is found only in the infested plants is a saturated fatty acid which is also known to have nematicide, pesticide activity [28].

5. Conclusion

The current study demonstrated that female adults of *T. chilonis* were significantly attracted to the odors of the *S. litura* infested plant surface chemicals. From our experimental studies we conclude that a blend of chemicals were released in *S. litura* infested chilli plants in addition to the common volatile chemicals present in uninfested plants which are serving as cues for its natural parasitoid as a mechanism of induced indirect defense. The results of this research could help in enhancing the efficacy of the use of Trichogramma wasps as biocontrol agents against varied lepidopteran pests.

6. Acknowledgment

Authors are grateful to Department of Science and Technology, INSPIRE, New Delhi for research grant. One of the authors MV is thankful to DST- INSPIRE fellowship and AcSIR for Ph.D registration. One of the authors SR is thankful to ICAR-Indian Institute of Natural Resins and Gums. We thank Dr. U. V. R. Vijay Saradhi of the Indian Institute of Chemical Technology, Hyderabad, India, for analytical help and Director, CSIR-Indian Institute of Chemical Technology for the facilities.

7. References

1. De Moraes CM, Mescher MC, Tumlinson JH. Caterpillar- induced nocturnal plant volatiles repel nonspecific females. Nature, 2001; 410:577-580.
2. Pare PW, Tumlinson JH. Plant volatiles as a defense against insect herbivores. Plant Physiol. 1999; 121:325-331.
3. McCormick AC, Unsicker SB, Gershenzon J. The specificity of herbivore-induced plant volatiles in attracting herbivore enemies. Trends. Plant Sci. 2012; 17(5):303-310.
4. De Moraes CM, Lewis WJ, Paré PW, Alborn HT, Tumlinson JH. Herbivore-infested plants selectively attract parasitoids. Nature, 1998; 393:570-3.
5. Rodriguez-Saona CR, Frost CJ. New evidence for a multi-functional role of herbivore-induced plant volatiles in defense against herbivores. Plant. Signal. Behav. 2010; 5(1):1-3.
6. Penaflor MFGV, Bento JMS. Herbivore-induced plant volatiles to enhance biological control in agriculture. Neotrop. Entomol. 2013; 42(4):331-343.
7. Griffiths BS, Ritz K, Wheatley RE. Nematodes as indicators of enhanced microbiological activity in a Scottish organic farming system. Soil. Use. Manage. 1994; 10:20-24.
8. Hilker M, Meiners T. Early herbivore alert: Insect eggs induce plant defense. J Chem. Ecol. 2006; 32:1379-1397.
9. Tatagar MH, Mohankumar HD, Shivaprasad M, Mesta RK. Bio-efficacy of flubendiamide 20 WG against chilli fruit borers, *Helicoverpa armigera* (Hub.) and *Spodoptera litura* (Fb.). Karnataka Journal Agricultural Sciences, 2009; 22(3-Spl. Issue):579-581.
10. Puneeth P, Vijayan VA. Biocontrol efficacy and viability of *Trichogramma chilonis* on *Corcyra cephalonica* and *Spodoptera litura* under laboratory conditions. Int. J Biol. Sci. 2013; 3(1):76-79.
11. Noldus LPJJ. Semiochemicals, foraging behaviour and quality of entomophagous insects for biological control. J Appl. Entomol. 1989; 108:425-451.
12. Usha Rani P, Sandhyarani K. Specificity of systemically released rice stem volatiles on egg parasitoid, Trichogramma japonicum Ashmead behavior. J Appl. Entomol. 2011; 136:749-760.
13. Romies J, Shanowar TG, Zebitz CPW. Response of Trichogramma egg parasitoids to coloured sticky traps. Biocontrol. 1998; 43:17-27.
14. Arimura G, Kopke S, Kunert M, Volpe V, David A, Brand P, *et al*. Effects of Feeding *Spodoptera littoralis* on Lima Bean leaves: IV. Diurnal and nocturnal damage differentially initiate plant volatile emission. Plant. Physiol. 2008; 146:965-973.
15. Kugimiya S, Shimoda T, Weinberg E, Uefune M, Takabayashi J. Host-searching responses to herbivory-associated chemical information and patch use depend on mating status of female solitary parasitoid wasps. Ecol. Entomol. 2010; 35(3):279-286.
16. Usha Rani P, Lakshminarayana M. Defense mechanisms in plants– Their use in biotechnological approach for the management of insect pests. Pesticide Res. J. 2008; 20(A):33-38.
17. Kessler A, Baldwin IT. Defensive function of herbivore induced plant volatiles in nature. Science. 2001;' 291(5511):2141-2144.
18. Penaflor MFGV, Erb M, Miranda LA, Wereneburg AG, Bento JMS. Herbivore-induced plant volatiles can serve as host location cues for a generalist and a specialist egg Parasitoid. J Chem. Biol. 2011; 37:1304-1313.
19. Vinson SB. Parasitoid-host relationship. Chemical Ecology of Insects, 1984, 205-233.
20. Fatouros NE, Dicke M, Mumm R, Meiners T, Hilker M. Foraging behavior of egg parasitoids exploiting chemical information. Behavioral Ecology, 2008; 19:677-689.
21. Moraes MCB, Laumann R, Sujii ER, Pires C, Borges M. Induced volatiles in soybean and pigeon pea plants artificially infested with the neotropical brown stink bug, *Euschistus heros*, and their effect on the egg parasitoid, *Telenomus podisi*. Entomologia Experimentalis et Applicata., 2005; 115:227-237.
22. Moraes MCB, Laumann RA, Pareja M, Sereno FTPS, Michereff MFF, Birkett MA, *et al*. Attraction of the stink bug egg parasitoid *Telenomus podisi* to defence signals from soybean activated by treatment with cis-jasmone. Entomologia Experimentalis et Applicata, 2009; 131:178-188.
23. Ananthakrishnan TN, Senarayan R, Murugesan S, Annadurai RS. Kairomones of *Heliothis armigera* and *Corcyra cephalonica* and their influence on the parasitic potential of *Trichogramma chilonis* (Trichogrammatidae: Hymenoptera). J Biol. Sci. 1991; 16:111-119.
24. Padmavathi CH, Paul AVN. Saturated hydrocarbons as kairomonal source for the egg parasitoid, *Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae). J Appl. Entomol. 1998; 122:29-32.
25. Sen A, Raina R, Joseph M, Tungikar VB. Response of Trichogramma chilonis to infochemicals: an SEM and electrophysiological investigation. Biocontrol. 2005; 50:429-447.
26. Usha Rani P, Kumari SI, Sriramakrishna T, Sudhakar TR. Kairomones extracted from Rice Yellow Stem Borer and their Influence on egg parasitization by *Trichogramma japonicum* Ashmead. J Chem. Ecol. 2007; 33:59-73.
27. Mahato SB, Sen S. Advances in triterpenoid research, 1990-1994. Phytochem. 1997; 44:1185-1236.
28. Abbas K, Uzma Niaz, Talib Hussain, Asif Saeed M, Zeeshan Javaid, Arfat Idrees, Shahid Rasool. Antimicrobial activity of fruits of Solanum nigrum and Solanum xanthocarpum. Acta. Pol. Pharm. Drug Research. 2014; 71:415-421.