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Volatiles from host plant brinjal attract the brinjal Fruit and Shoot Borer -*Leucinodes orbonalis* Guenee

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ABSTRACT

Brinjal Fruit and Shoot Borer- *Leucinodes orbonalis* Guenee is a major insect pest on brinjal- *Solanum melongena* worldwide. An effective strategy used in developing pest controlling agents is the synergism between insect pheromones and host plant volatiles, which can increase the attraction of insect pest. The present study was aimed at investigating the chemical constituents and attractant effects of the volatiles extracted from different parts of the host plant brinjal on the behavior of adult *L. orbonalis*. Bioassay using Y-shaped olfactometer revealed that the one-day old virgin female, gravid female and male insects respond positively to the host plant volatiles extracted from fruits, leaves and shoots but not to that of flowers. It was shown that the gravid females were significantly attracted to all three volatiles ($p < 0.05$). Bioassay using X-shaped olfactometer identified that all three types of insects highly preferred the volatiles from fruits ($p < 0.05$). Gas chromatography-mass spectrometry analysis of volatiles indicated that brinjal plant produces volatile secondary metabolites, which include 2,2'-(Ethane-1,2-diylbis(oxy))bis(ethane-2,1-diyl) dibenzoate (12.11%), 3,7-dimethylocta-1,6-dien-3-ol (22.38%), Benzyl alcohol (22.9%) and Benzyl alcohol (27.06%) as major constituents from fruits, shoots, leaves and flowers respectively. Responses of insects to the volatiles from host plant in the absence of visual cues direct us to focus on the importance of host plant volatiles to locate the plant. Results of this study emphasize the major role that host plant volatiles play in the attraction of insect pests towards the plant.

Introduction

Brinjal plant is an economically important plant grown in Sri Lanka and other Asian countries, especially in Bangladesh, China, India, Pakistan, Philippines and Thailand (Gunawardena et al., 1989; Kumar et al., 2006). China leads world's brinjal production followed by India (Patel et al., 2015). In Bangladesh, brinjal is the second most produced vegetable after potato while in Sri Lanka, it covers the second large extent after ash plantain (Ahmad et al., 2009; Performance Report, 2016). Brinjal is available throughout most of the year (Cork et al., 2001). Further, brinjal is a well-known vegetable rich in fiber, low in calories and provides a wide range of nutrients, minerals and multivitamins (Raigon et al., 2008; Plazas et al., 2014).

In recent years, production of brinjal is under imminent threat due to the increased management cost of insect pest Brinjal Fruit and Shoot Borer (BFSB), which is the key insect pest that attacks brinjal plant

(Mainali, 2014). Larvae of this pest cause the damage, which at initial stages adversely affect the shoot growth, and in later stages diminish fruit quality thus making it unfit for consumption (Alam and Sana, 1962). A study conducted in 12 districts of Sri Lanka by Sandanayake has shown that this insect pest causes a mean damage level of 52.5% for brinjal fruits (Gunawardena et al., 1989). At present, farmers are completely dependent on chemical insecticides to control this pest (Kumar et al., 2006). Insects like BFSB possessing extremely diverse adaptations such as hidden, protected lifestyles in adult stage and concealed habits in the larval stage cannot be easily controlled with cover sprays of insecticides (Nusra et al., 2020). Indiscriminate use of synthetic chemicals causes many unwarranted problems (Patel et al., 2015; Ahmad et al., 2009).

There are evidence to suggest that ecological interaction, especially odours between insect pests and their host, aids in the development of the most effective insect pest control strategies (Chidawanyika et al.,

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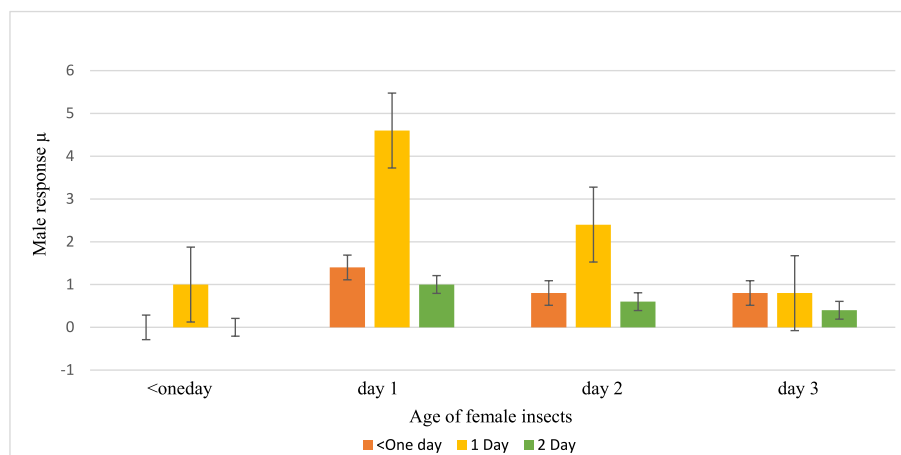


Fig. 3.2. Response profile of male BFSB to age of virgin female BFSB. (Each bar represents the mean number of male insects of five replicates. The vertical lines represent the standard error of means).

Table 3.3.1

Response of BFSB adults to brinjal fruit volatile treated and diethyl ether treated bottles during olfactometer bioassays.

Type of insect used	Dose (μl)	% Response ± SE §		χ ²	p Value *
		Plant volatile treated	Diethyl ether treated		
Gravid females	0.02	17 ± 0	3.3 ± 3.3	4**	<0.05
	0.2	26.7 ± 4.1	6.7 ± 4.1	5.33**	<0.05
	2	30 ± 3.3	6.7 ± 4.1	6.33**	<0.05
	4	46.7 ± 3.3	6.7 ± 4.1	10.33**	<0.05
	8	80 ± 3.3	0	24**	<0.05
	16	33.3 ± 5.3	10 ± 4.1	4.67**	<0.05
Virgin females	0.02	0	0	0	>0.05
	0.2	10 ± 4.1	0	3	>0.05
	2	10 ± 4.1	6.7 ± 4.1	3	>0.05
	4	30 ± 3.3	13.3 ± 3.3	2.33	>0.05
	8	43.3 ± 4.1	6.7 ± 4.1	9.33**	<0.05
	16	20 ± 3.3	6.7 ± 4.1	4**	<0.05
Males	0.02	0	0	0	>0.05
	0.2	0	0	0	>0.05
	2	6.7 ± 4.1	3.3 ± 3.3	1	>0.05
	4	20 ± 3.3	10 ± 4.1	2.33	>0.05
	8	30 ± 3.3	10 ± 4.1	4.67**	<0.05
	16	13.3 ± 3.3	6.7 ± 4.1	2	>0.05

p < 0.05, 3.84 Significant at 5% (Chi square test).

§ Six insects were used in each replicate and mean of 5 replicates.

2012). Plants synthesize and emit a wide range of volatile organic compounds which act as chemical signals inducing a variety of behavioral responses in insects (Dethier et al., 1960). Also, some studies reported that the behaviour of the larvae or adult or both stages of the insects can be influenced by plant volatiles. The highly volatile organic compounds commonly act within a long distance from the emission source while less volatile organic compounds act in a close range (Nordlund and Lewis, 1976). Further, many attractive volatile organic compounds released by plants, make navigation towards their host plants a challenge for the herbivorous insects. So, the relationship between insect pests and volatile semiochemicals of the host plants has been studied well and is recognized as a communication system within insect species, which can modify the insects' behavior (Plazas et al., 2013). Further, in some plants, these volatile organic compounds are the key compounds that are involved in the attraction of insect pests (Nusra

Table 3.3.2

Response of BFSB adults to brinjal leaf volatile treated and diethyl ether treated bottles during olfactometer bioassays.

Type of insect used	Dose (μl)	% Response ± SE §		χ ²	p Value*
		Plant volatile treated	Diethyl ether treated		
Gravid females	0.2	10 ± 4	3.3 ± 3.3	4**	<0.05
	2	30 ± 3.3	6.7 ± 4.1	5.67**	<0.05
	4	40 ± 4.1	13.3 ± 6.2	5.53**	<0.05
	8	56.7 ± 4.1	3.3 ± 3.3	15**	<0.05
	12	83 ± 5.3	0	25**	<0.05
	16	53.3 ± 3.3	6.7 ± 4.1	11.8**	<0.05
Virgin females	0.02	0	0	0	>0.05
	0.2	0	0	0	>0.05
	2	10 ± 4.1	3.3 ± 3.3	2	>0.05
	4	23.3 ± 4.1	10 ± 4.1	3.33	>0.05
	8	30 ± 3.3	3.3 ± 3.3	7.33**	<0.05
	16	23.3 ± 4.1	13.3 ± 3.3	1.67	>0.05
Males	0.02	0	0	0	>0.05
	0.2	0	0	0	>0.05
	2	0	0	0	>0.05
	4	0	0	0	>0.05
	8	10 ± 4.1	6.7 ± 4.1	1	>0.05
	16	10 ± 4.1	6.7 ± 4.1	5**	<0.05

p < 0.05, 3.84 Significant at 5% (Chi square test).

§ Six insects were used in each replicate and mean of 5 replicates.

et al., 2020).

Volatile mediated foraging behavior is important in insect pests when they target host plants. The use of host plant volatiles with the combination of insect pheromones in traps have been developed successfully as an eco-friendly insect pest management strategy to control the insect pest population in field conditions. It has been reported that red weevil (*Rynchophorus ferrugineus*) is attracted by volatiles released from the host plant coconut and that this strategy is now developed with combination of aggregation pheromone produced by red weevil as an environmental-friendly trapping system to combat the red weevil population of coconut plantations in Sri Lanka (Gunawardana and Swarnakanthi, 1995).

There is an urgent need to develop an environmental friendly method that can be used to control the BFSB in brinjal plant. Previous studies carried out on the behavior of BFSB have shown that BFSB

Table 3.3.3

Response of BFSB adults to brinjal shoot volatile treated and diethyl ether treated bottles during olfactometer bioassays.

Type of insect used	Dose (µl)	% Response ± SE §		χ ²	p Value*
		Plant volatile treated	Diethyl ether treated		
Gravid females	0.02	20 ± 3.3	3.3 ± 3.3	5**	<0.05
	0.2	23.3 ± 4.1	3.3 ± 3.3	6**	<0.05
	2	33.3 ± 0	13.3 ± 3.3	3.3	>0.05
	4	40 ± 4.1	6.7 ± 4.1	8.3**	<0.05
	8	80 ± 3.3	0	24**	<0.05
	16	50 ± 5.3	10 ± 4.1	8.8**	<0.05
Virgin females	0.02	0	0	0	
	0.2	6.7 ± 4.1	3.3 ± 3.3	1	>0.05
	2	10 ± 4.1	3.3 ± 3.3	4**	<0.05
	4	20 ± 3.3	10 ± 6.7	4.3**	<0.05
	8	30 ± 3.3	6.7 ± 4.1	5.67**	<0.05
	16	16.7 ± 5.3	6.7 ± 4.1	5**	<0.05
Males	0.02	0	0	0	
	0.2	0	0	0	
	2	3.3 ± 3.3	0	1	>0.05
	4	10 ± 4.1	6.7 ± 4.1	3	>0.05
	8	16.7 ± 0	6.7 ± 4.1	3	>0.05
	16	10 ± 4.1	3.3 ± 3.3	2	>0.05
20	3.3 ± 3.3	0	1	>0.05	

p < 0.05, 3.84 Significant at 5% (Chi square test).

§ Six insects were used in each replicate and mean of 5 replicates.

female produced sex pheromone which attracted the BFSB male insects and reported that the traps baited with sex pheromone achieved a reduction in insect pest population in the field conditions (Attygalle et al., 1988; Gunawardena et al., 1989; Ranjithkumar et al., 2013; Cork et al., 2003). There are no studies describing the behavioral effects of BFSB to the host plant volatiles. Hence, the present study was aimed at evaluating the effects of host plant volatiles obtained from different parts of brinjal plant on the behavioral responses: especially host-finding behavior of adult BFSB in the absence of visual cues. Subsequently, the volatile chemical constituents present in different parts of the host plant brinjal were analyzed.

Material and methods

Insect material

Infested fruits from the market were spread out in plastic containers with a layer of sawdust and covered with net. These were kept undisturbed for 6–9 days. Numerous pupae were collected. One kilogram of infested brinjal offered an average of 20 pupae which were formed preferably on sawdust. The groups of pupae were transferred into transparent plastic jars. The mouth of the jars was covered with pieces of mosquito net. After adult emergence, the male and female adults were separated from the jars. Pairs of adults were transferred into separate jars containing a few pieces of cotton wool soaked with 5% sucrose solution and covered carefully with small porous net in order to get gravid females for the behavioural bioassays. The insects were maintained at room temperature (25 ± 2 °C) at humidity conditions (80%) under 12:12 L: D photoperiod in order to be collected for the bioassays (Gunawardena et al., 1989).

Plant material

Samples of brinjal plant (“Lena iri” variety) mature leaves, shoots, mature fruits and full-bloom flowers were collected separately from the unsprayed brinjal field at Agriculture Research Station, Kandakuliya,

Table 3.4

Response of BFSB adults to multiple-choice olfactometer bioassays.

Type of insect used	Dose (µl)	% Response ± SE §		χ ²	p Value*
		Plant volatile treated	Diethyl ether treated or plant volatile treated		
Gravid females	Fruit volatile:	37.3 ± 1.6	10.7 ± 1.6	11.52**	<0.05
	Diethyl ether				
	Leaf volatile:	29.3 ± 1.6	10.7 ± 1.6	7.33**	<0.05
	Diethyl ether				
	Shoot volatile:	17.3 ± 1.6	10.7 ± 1.6	1.73	>0.05
	Diethyl ether				
	Fruit volatile: Leaf	37.3 ± 1.6	29.3 ± 1.6	1	>0.05
	volatile: Leaf				
	Fruit volatile:	37.3 ± 1.6	17.3 ± 1.6	5.78**	<0.05
	volatile:				
	Shoot volatile:	29.3 ± 1.6	17.3 ± 1.6	2.73	>0.05
	Leaf volatile: Shoot				
volatile:					
Virgin females	Fruit volatile:	25.3 ± 1.3	12.0 ± 1.3	4**	<0.05
	Diethyl ether				
	Leaf volatile:	18.7 ± 1.3	12.0 ± 1.3	1.6	>0.05
	Diethyl ether				
	Shoot volatile:	8.0 ± 1.3	12.0 ± 1.3	1	>0.05
	Diethyl ether				
	Fruit volatile: Leaf	25.3 ± 1.3	18.7 ± 1.3	0.77	>0.05
	volatile: Leaf				
	Fruit volatile:	25.3 ± 1.3	8.0 ± 1.3	7.06**	<0.05
	volatile:				
	Shoot volatile:	18.7 ± 1.3	8.0 ± 1.3	3.53	>0.05
	Leaf volatile: Shoot				
volatile:					
Males	Fruit volatile:	17.0 ± 1.6	14.0 ± 1.3	0.4	>0.05
	Diethyl ether				
	Leaf volatile:	14.7 ± 1.3	14.0 ± 1.3	0.4	>0.05
	Diethyl ether				
	Shoot volatile:	5.3 ± 1.3	14.0 ± 1.3	4**	<0.05
	Diethyl ether				
	Fruit volatile: Leaf	17.0 ± 1.6	14.7 ± 1.3	0.8	>0.05
	volatile: Leaf				
	Fruit volatile:	17.0 ± 1.6	5.3 ± 1.3	5.67**	<0.05
	volatile:				
	Shoot volatile:	14.7 ± 1.3	5.3 ± 1.3	4**	<0.05
	Leaf volatile: Shoot				
volatile:					

p < 0.05, 3.84 Significant at 5% (Chi square test).

§ Six insects were used in each replicate and mean of 5 replicates.

Kalpitiya in October 2017. All parts of the collected plant samples were washed thoroughly with running water followed by distilled water and air dried until it acquired a constant weight. Samples were stored at 0(-5) °C using airtight polythene bags. Air dried samples were cut into small pieces before steam distillation (Senanayake et al., 2016).

Host plant volatile collection

Steam distillation method was used for the extraction of volatile constituents from different parts of the brinjal plant. The air-dried small

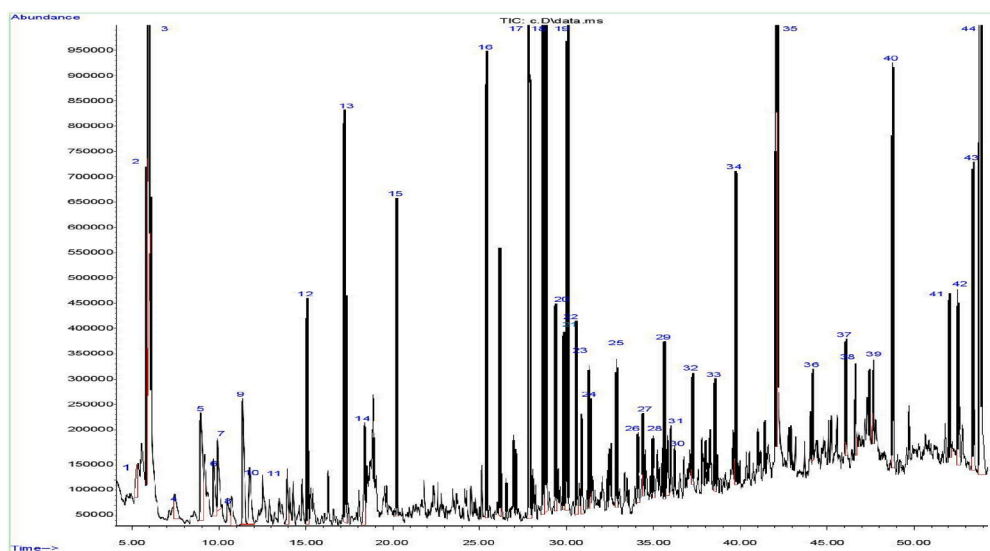


Fig. A1. GC–MS chromatogram of the steam distilled extract of brinjal plant fruit volatiles.

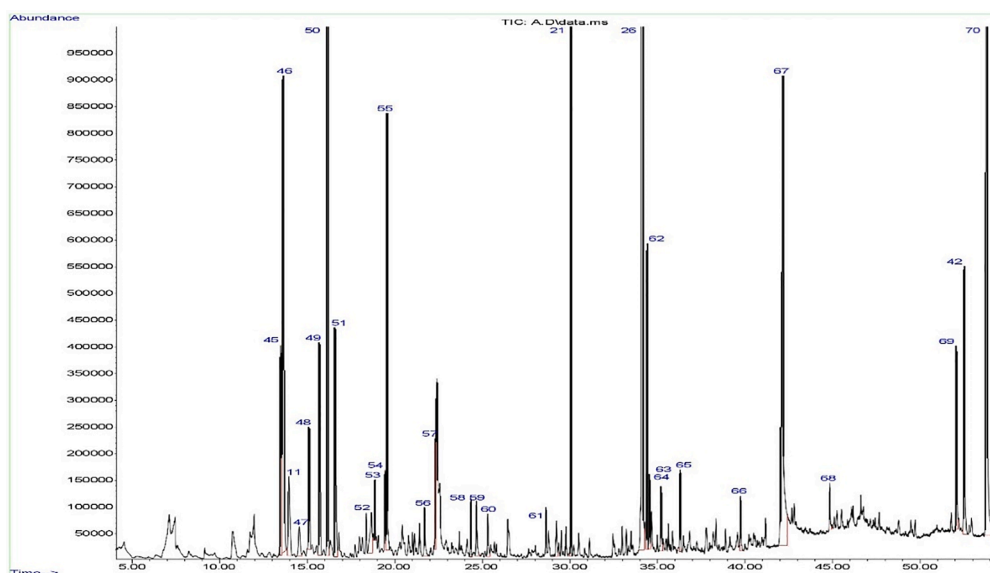


Fig. A2. GC–MS chromatogram of the steam distilled extract of brinjal plant shoot volatiles.

pieces of plant material from each part (500 g) were steam distilled separately for 4 h at a distillation rate of 50 ml/hour. The condensed solution (250 ml) was saturated with NaCl and extracted with diethyl ether (50 ml × 2). Anhydrous Na₂SO₄ was added to the organic layer to remove water and concentrated under reduced pressure using rotary evaporator at 35 °C. Remaining solvent was evaporated under slow stream of nitrogen and carefully stored in sealed dark glass vials at 0–(-5) °C until later analysis. Volatiles were collected separately from fruits, leaves, shoots and flowers. All glass apparatus used in the collection of volatiles were cleaned thoroughly using chromic acid followed by soap water, acetone and finally distilled water. They were oven dried subsequently. Different dilutions of the host plant volatiles were prepared using diethyl ether in all experiments (Senanayake et al., 2016).

Behavioral bioassay

Two-choice Y-shaped olfactometer bioassay-finding the age of the adults for the bioassays

The olfactometer described by Hershberger and Smith in 1967 was

modified to suit our purpose. A “Y” shaped olfactometer with 3 connected glass tubes (30 cm long, 8 cm diameter, 120° angles between arms) with an opening at the intersection of the 3 arms for the vacuum pump was used as the olfactometer. The opening on the intersection of the arms facilitated the air circulation in the olfactometer. The ends of the two tubes of the olfactometer were connected to perforated, plastic, transparent, wide mouthed bottles (250 ml) through the lids while the third end of the tube was used to introduce insects. Two bottles were used, one with 3 virgin females and the other without insects. They were connected to both arms of the olfactometer. After switching on the vacuum pump, a transparent plastic bottle (500 ml) containing batches of six male insects previously kept in a separate dark room was connected to the third arm of the olfactometer and released individually. The number of insects that moved into the arm with virgin females and the arm without insects were recorded. It was considered a choice when the insect moved a distance that exceeded half of that one side of the arm within 5 min. Three different age groups of virgin females (<oneday, day 1 and day 2) were tested separately with four different age groups of male insects (<oneday, day 1, day 2 and day 3). The bioassay was

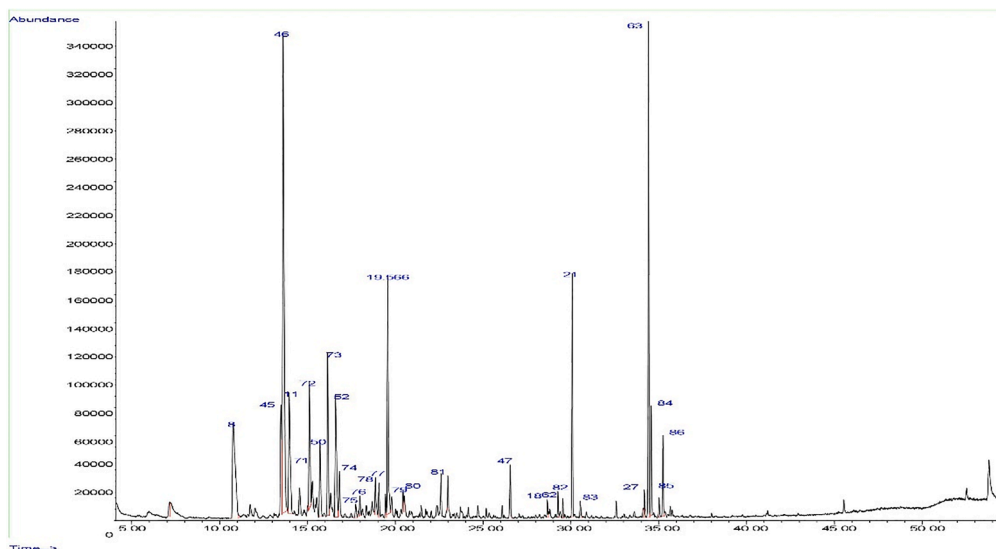


Fig. A3. GC–MS chromatogram of the steam distilled extract of brinjal plant leaf volatiles.

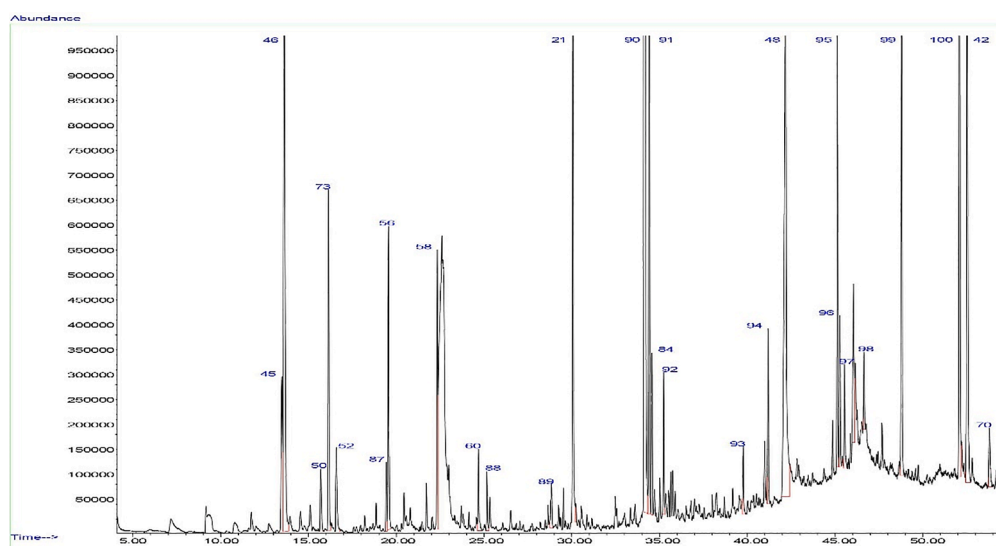


Fig. A4. GC–MS chromatogram of the steam distilled extract of brinjal plant flower volatiles.

replicated 5 times (Gunawardena et al., 1989).

Two-choice Y-shaped olfactometer bioassay-using plant volatiles

The olfactometer described in 2.4.1 was used to check the attraction of host plant volatiles to all three types of insects (virgin females, gravid females and males) separately. Two Whatman no. 1 filter papers (2.5 cm × 2.5 cm) were used, one treated with a known amount of host plant volatile extract dissolved in diethyl ether and the other treated with equal amount of diethyl ether. Both filter papers were air dried for one minute to evaporate the solvent. The air-dried filter papers were placed in the middle of the two bottles separately and connected to both arms of the olfactometer. After switching on the vacuum pump, a transparent, plastic bottle (500 ml) containing batches of six test insects previously kept in a separate dark room was connected to the third arm of the olfactometer and released individually. The number of insects that moved into the host plant volatile treated and diethyl ether treated arms within 5 min were recorded. Seven doses of fruit volatiles (0.02–20 mg), leaf volatiles (0.2–20 mg) and shoot volatiles (0.02–20 mg) were tested separately for virgin female, gravid female and male insects and each dose was replicated 5 times. The doses of different parts of the plant

volatiles were determined by trial and error. Placement of host plant volatile treated and diethyl ether treated filter papers were interchanged randomly in subsequent replicates. The mean number of insects that responded to the two treatments at each dose was compared by using Chi Square test (Senananyake et al., 2016).

Multiple-choice X-shaped olfactometer bioassay

The Multiple-choice bioassay was conducted to identify the most preferable/ most attractant volatile to the host. A modified X-shaped olfactometer with 4 connected glass tubes (30 cm long, 8 cm diameter, 90° angles between tubes) with an opening at the intersection of the four arms for the introduction of the test insects was used as the olfactometer. The ends of the four tubes of the olfactometer were connected with perforated, plastic, transparent, wide mouthed bottles (250 ml) with a hole (3 cm diameter) at the end, which was covered with small porous net in order to facilitate the air circulation in the olfactometer. Currents of air were passed through each of this opening by using four portable USB fans at a rate of 25 cm s⁻¹. Four Whatman no. 1 filter papers (2.5 cm × 2.5 cm) were used, three treated with peak dose identified from two-choice bioassay of each host plant volatile and the other treated

Table B1

Gas chromatography-mass spectrometry analysis of volatile organic compounds emitted by brinjal plant fruit volatiles.

Peak No	Retention time (min)	Name of the compound	Percentage
1	5.3151	(Z)-3-Octene	0.58
2	5.8559	1,1-Diethoxyethane	1.65
3	5.9895	Diethyl acetal	9.44
4	7.4273	1,1'-Oxybis-ethane	0.56
5	8.9414	3-Nitropropanoic acid	2.04
6	9.6794	3,4-dihydroxy-3,4-dimethyl- 2,5-Hexanedione	0.91
7	9.9403	2,5,8,11,14-Pentaoxahexadecan-16-ol	1.06
8	10.7292	Benzaldehyde	0.55
9	11.359	2-Methyl-1,3-dioxolane	2.08
10	11.7662	3-Methyl-2-butanol	0.99
11	13.9292	Benzeneacetaldehyde	0.63
12	15.0744	Cyclooctane	2.24
13	17.2311	n-Octyl methanoate	4.22
14	18.3826	5-(1-Ethoxy-ethoxy)-4-methyl-hex-2-enal	0.96
15	20.2467	n-Octyl acetate	1.95
16	25.4062	2,4-Dimethyl-6- <i>tert</i> -butylphenol	4.42
17	27.8492	N,N'-bis(1-methylethyl)- 1,4-Benzenediamine	9.33
18	28.6508	2,6-bis(1,1-dimethylethyl)- 2,5-Cyclohexadiene-1,4-dione	4.52
19	28.8035	2,6-di(<i>t</i> -butyl)-4-hydroxy-4-methyl-2,5-cyclohexadien-1-one	4.25
20	29.3825	2,6-di(<i>t</i> -butyl)-5,6-epoxy-4-methyl-4-hydroxy-2-cyclohexanone	2.46
21	30.0632	Butylated hydroxytoluene	3.68
22	30.5785	3-methoxy- Octane	1.40
23	31.2974	2-(1-phenylethyl)- 1,3-Dioxolane	0.99
24	31.4183	Pentamethyldisilane	1.42
25	32.8879	9-ethyl-10-methylanthracene	1.54
26	34.1157	Benzyl benzoate	0.57
27	34.4021	(-)-(9R,10S)-10-Acetyl-9,10-dimethylbicyclo[6.4.0]dodec-1(8)-ene	0.57
28	34.9746	1-(1-hydroxyethyl)-1-(diethylphosphonyl)-2-methylene-Cyclopropane	0.50
29	35.649	Ethylhexyl benzoate	0.97
30	35.7953	4,5,6,7,8,9,10,11,12,13-decahydro- 1H-Cyclododecapyrazole	0.60
31	35.9925	1-Bromoeicosane	0.52
32	37.2395	3,5-Diethoxycarbonyl-2,6-dimethylpyridine	0.78
33	38.5691	4-Phenylpyridine	0.74
34	39.7588	Phthalic acid, isobutyl nonyl ester	2.00
35	42.0682	1,2-Benzenedicarboxylic acid	4.80
36	44.1931	2-Propionylthiophene	0.57
37	46.089	(8Z)-8-Hexadecene	0.81
38	46.6425	Stearic acid	0.77
39	47.6795	1-Octadecene	0.51
40	48.7865	1-Naphthyl benzoate	3.27
41	52.0692	n-Tetracosane	1.24
42	52.5273	2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-phenol	1.92
43	53.4116	Phthalic acid, di(6-methylhept-2-yl) ester	2.88
44	53.8188	2,2'-(Ethane-1,2-diylbis(oxy))bis(ethane-2,1-diyl) dibenzoate	12.11

with equal amount of diethyl ether. They were placed separately after air-drying for one minute in the middle of the bottles and connected to the four arms of the olfactometer. Maximum mean number of insects attracted to the dose in each host plant volatile was considered as the peak dose. After switching the USB fans on, a transparent plastic bottle (500 ml) containing batches of fifteen test insects previously kept in a separate dark room was connected to the opening at the intersection of the four arms of the olfactometer and released individually. The number of insects that moved into the host plant volatile treated and diethyl ether treated arms within 5 min were recorded. Virgin female, gravid female and male insects were assayed separately and replicated 5 times. Placement of host plant volatile treated and diethyl ether treated filter

Table B2

Gas chromatography-mass spectrometry analysis of volatile organic compounds emitted by brinjal plant shoot volatiles.

Peak No	Retention time (min)	Name of the compound	Percentage
45	13.4773	2-Ethyl-1-hexanol	3.20
46	13.6109	Benzyl alcohol	7.61
11	13.9481	Benzeneacetaldehyde	1.49
47	14.5334	Tetradecane	0.57
48	15.0869	2,6-Dimethyl-7-octen-2-ol	1.62
49	15.6849	Ethyl 2-(5-methyl-5-vinyltetrahydrofuran-2-yl)propan-2-yl carbonate	2.98
50	16.143	3,7-dimethylocta-1,6-dien-3-ol	22.38
51	16.5756	Benzeneethanol	3.15
52	18.6623	2-Methyl-1-hexen-3-yne	0.97
53	18.8532	Epoxylinolol	0.52
54	19.4321	Beta. fenchyl alcohol	0.76
55	19.553	Methyl salicylate	4.63
56	21.7161	3,7-dimethyl-2,6-Octadien-1-ol	0.56
57	22.3332	Hydroquinone	0.81
58	24.3499	Dodecamethylcyclohexasiloxane	0.51
59	24.6744	5-pentyl-2(5H)-furanone	0.62
60	25.317	(2E,6E)-4-Methyl-2,6-octadiene-4,5-diol	0.53
61	29.2423	4-(2,6,6-Trimethyl-1-cyclohexen-1-yl)- 3-buten-2-one	0.48
21	30.063	Butylated hydroxytoluene	5.12
27	34.1283	(-)-(9R,10S)-10-Acetyl-9,10-dimethylbicyclo[6.4.0]dodec-1(8)-ene	10.48
62	34.4082	1-Methyl-5,8-dimethoxy-1,2,3,4-tetrahydro-1,4-iminonaphthalene	2.87
63	34.5673	3,6-Dimethyl-2-(1-(trimethylsilyl)ethen-2-yl)pyrazine	0.73
64	35.2289	3-[(2S,4R)-3-benzoyl-2-(<i>tert</i> -butyl)-1,4-dimethyl-5-oxoimidazodin-4-yl]propionsau	0.70
65	36.3168	Zerumbone	0.80
66	39.765	Phthalic acid, isobutyl octyl ester	0.57
67	42.1698	Palmitic acid	9.77
68	44.8546	n-Nonadecanol-1	0.46
69	52.0691	Hexanedioic acid	2.03
42	52.5144	2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-Phenol	3.21
70	53.7931	Diethylene glycol dibenzoate	9.87

papers were interchanged randomly in subsequent replicates.

In both two-choice and multiple-choice bioassays, the olfactometer was placed horizontally on a black background in low-light condition. The light intensity just above the olfactometer was maintained same in every direction it faced. At each trial, the olfactometer was cleaned by washing thoroughly with a detergent and blowing air for 15 min. The olfactometer was rotated 90° at each replicate in a clockwise direction to control any directional effect. The bioassay was carried out between 20 and 24 h. Test insects and filter paper strips were not used repeatedly. A bioassay was conducted with diethyl ether treated filter paper strip vs a filter paper strip without any treatment to certify that diethyl ether has no effect on the behavior of the test insects.

Analysis of plant volatiles

The volatile extracts obtained from steam distillation of different parts of the host plant brinjal were analyzed on a GC having the following specifications: Shimadzu's new-generation GC-2025 capillary gas chromatograph with FID (Hydrogen flame ionization detector) and Rtx-wax capillary column, 30 m × 0.25 mm; 0.25 µm film thickness. The GC oven was programmed at an initial temperature of 40 °C held for 1 min, raised to 60 °C at the rate of 2 °C/min, followed by a raise to 100 °C at a rate of 5 °C/min and subsequently reaching a final temperature of 200 °C at the rate of 10 °C/min. High purity nitrogen gas was used as the carrier gas (1 ml/min). The injector and detector temperatures were 240 °C and 270 °C respectively, and 1 µl of the volatile extract in diethyl ether (2 mg/ml) was injected. Further, in this study, each volatile extract

Table B3

Gas chromatography-mass spectrometry analysis of volatile organic compounds emitted by brinjal plant leaf volatiles.

Peak No	Retention time (min)	Name of the compound	Percentage
8	10.7545	Benzaldehyde	2.12
45	13.4838	2-Ethylhexanol	4.67
46	13.611	Benzyl alcohol	22.90
11	13.9546	Benzeneacetaldehyde	5.97
71	14.5462	1-methyl-4-(1-methylethyl)- Benzene	1.20
72	15.1061	2-Methyl-6-methylene-7-octen-2-ol	4.68
50	15.7041	Ethyl 2-(5-methyl-5-vinyltetrahydrofuran-2-yl)propan-2-yl carbonate	3.18
73	16.1431	3,7-dimethyl- 1,6-Octadien-3-ol	5.46
52	16.5948	Benzeneethanol	5.78
74	16.8047	1,2,3,4-tetramethyl- Benzene	1.59
75	17.7463	2,6,6-Trimethyl-2-cyclohexene-1,4-dione	0.54
76	17.9753	2-ethyl-1,4-dimethyl- Benzene	0.54
77	18.866	linalool Z-pyranic oxide	0.92
78	19.0632	Naphthalene	1.08
79	19.4513	Camphene	0.65
56	19.5658	Methyl salicylate	7.49
80	20.4374	1-Ethenyl-4-hydroxybenzene	0.71
81	22.9886	1-Benzazole	0.90
47	26.5449	Tetradecane	1.71
18	28.6507	2,6-Bis(1,1-dimethylethyl)- 2,5-cyclohexadiene-1,4-dione,	0.52
62	29.2551	4-(2,6,6-trimethyl-1-cyclohexen-1-yl)- 3-Buten-2-one	0.64
82	29.5287	Octadecane	0.47
21	30.0694	Butylated hydroxytoluene	6.05
83	30.5275	5,6,7,7a-tetrahydro-4,4,7a-trimethyl-2(4H)-Benzofuranone	0.51
27	34.1793	(-)-(9R,10S)-10-Acetyl-9,10-dimethylbicyclo[6.4.0]dodec-1(8)-ene	0.62
63	34.4147	1-Methyl-5,8-dimethoxy-1,2,3,4-tetrahydro-1,4-iminonaphthalene	13.41
84	34.5673	2,8-Dimethoxy-5,6-quinolinedione	2.98
85	35.0127	1-(4-Isopropylphenyl)-2-methylpropyl acetate	0.54
86	35.2417	2-methyl-6-vinylheptane-2,5-dien-4-one	2.15

was subjected to GC–MS analysis (Agilent 7890B Gas Chromatograph coupled to a Agilent 5977B Series Mass Selective Detector equipped with a capillary column (30 m × 0.25 mm; 0.25 µm film thickness)) for the identification of constituents present. Helium was used as the carrier gas (1 ml/min). The GC oven was programmed at an initial oven temperature of 40 °C, increased to 50 °C at a rate of 1 °C/min, followed by an increase to 210 °C at a rate of 4 °C/min and subsequently reaching a final temperature of 230 °C at a rate of 8 °C/min. The MS data of eluted compounds were acquired and compared with the retention times to those of authentic standards and with mass spectra from NIST library.

Statistical analysis

Data obtained from different bioassays were subjected to statistical analysis (descriptive statistics, Tukey's mean comparison and chi-square test) using SPSS (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.) and the results were presented accordingly. The number of test insects which selected the baited and non-baited arms in each bioassay for different plant volatile doses were counted and the mean numbers of each bioassay was the average of all 5 replicates. Insects that did not respond to either arm were not considered in the analyses. Tukey's mean comparison test was performed to check the significant difference between the means of the three types of test insects responding to the plant volatiles during the bioassays. Means and standard errors (SE) of each data sets are presented in tables and figures. Further, the olfactory responses in all the bioassays were analyzed with a Chi-square test.

Table B4

Gas chromatography-mass spectrometry analysis of volatile organic compounds emitted by brinjal plant flower volatiles.

Peak No	Retention time (min)	Name of the compound	Percentage
45	13.4773	2-Ethylhexanol	1.46
46	13.6109	Benzyl alcohol	27.06
50	15.6913	Ethyl 2-(5-methyl-5-vinyltetrahydrofuran-2-yl)propan-2-yl carbonate	0.55
73	16.1303	3,7-dimethyl- 1,6-Octadien-3-ol	2.37
52	16.5756	Benzeneethanol	0.71
87	19.4322	Alpha. Terpeneol	0.41
56	19.553	Methyl salicylate	1.99
58	22.3396	Hydroquinone	1.92
60	24.6808	5-Pentyl-2(5H)-furanone	0.54
88	25.1643	Eugenol	0.34
89	28.8479	Cyclododecane	0.37
21	30.063	Butylated hydroxytoluene	4.25
27	34.1919	(-)-(9R,10S)-10-Acetyl-9,10-dimethylbicyclo[6.4.0]dodec-1(8)-ene	8.55
90	34.4146	5,7-Dimethoxy-1-methylindole	5.96
84	34.5673	2,8-Dimethoxy-5,6-quinolinedione	0.92
91	35.2416	4-(1-methylethyl)- Benzenemethanol	0.80
92	39.765	Phthalic acid, decyl isobutyl ester	0.43
93	41.1837	Hexadecanoic acid, methyl ester	1.02
48	42.1953	Palmitic acid	12.93
94	45.1345	Linoleic acid	5.85
95	45.2681	Triolein	0.87
96	45.5353	Oleyl alcohol, trifluoroacetate	0.68
97	46.6423	9,12-Octadecadienoic acid	0.49
98	48.7926	2-Naphthyl benzoate	3.48
99	52.0818	Bis(2-ethylhexyl)adipate	5.06
100	52.2135	Hexacosane	5.30
42	52.5335	2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl- Phenol	5.16
70	53.7868	Diethylene glycol dibenzoate	0.55

Results

Extraction of volatiles

Steam distillation of different parts from the brinjal plant gave a pale-yellow oil with pleasant odour and the weight of the volatile extracts obtained from the fruits, leaves, shoots and flowers were 20.5, 16.8, 11.3 and 12.6 mg respectively.

Two-choice olfactometer study-finding the age of the adults

The adult insects showed hardly any activity soon after emergence and during the daytime. It was also revealed that adult male and female of BFSB produce a highest response during 20.00–24.00 h when the adults were one day old. According to the above observation, one-day old adults were used throughout the experiments. Fig. 3.2 shows the response profile of male BFSB to age of virgin female BFSB. Male response $\mu = \mu_1 - \mu_2$, where μ_1 = mean number of male insects selecting arm with virgin females and μ_2 = mean number of male insects selecting arm without insects.

Two-choice olfactometer study using plant volatiles

Results revealed that all types of insects were attracted to host plant volatiles except flowers'. However, gravid females were highly attracted to all three volatiles ($p < 0.05$, Tukey's mean comparison). The attractiveness of the test insects for volatiles increased with the volatile concentration. However, after a level, the insects' response decreased when the concentration level was increased further. The maximum mean number of insects attracted to the volatile dose was considered as the peak dose. Doses were taken from the concentrated steam distilled extract of the plant. A minimum dose of 0.2 µl and maximum dose of 12

Table B5

Comparison of GC–MS analysis volatile organic compounds emitted by different parts of the brinjal plant.

Peak No	Name of the compound	Fruit volatile	Shoot volatile	Leaf volatile	Flower volatile
8	Benzaldehyde	✓		✓	
11	Benzeneacetaldehyde	✓	✓	✓	
18	2,6-bis(1,1-dimethylethyl)-2,5-cyclohexadiene-1,4-dione	✓		✓	
21	Butylated hydroxytoluene	✓	✓	✓	✓
27	(-)-(9R,10S)-10-Acetyl-9,10-dimethylbicyclo[6.4.0]dodec-1(8)-ene	✓	✓	✓	✓
42	2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methylphenol]	✓	✓		✓
45	2-Ethyl-1-hexanol		✓	✓	✓
46	Benzyl alcohol		✓	✓	✓
47	Tetradecane		✓	✓	
48	Palmitic acid		✓	✓	✓
50	Ethyl 2-(5-methyl-5-vinyltetrahydrofuran-2-yl)propan-2-yl carbonate		✓	✓	✓
52	Benzeneethanol		✓	✓	✓
56	Methyl salicylate		✓	✓	✓
58	Hydroquinone		✓		✓
60	5-pentyl-2(5H)-furanone		✓		✓
62	4-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-3-buten-2-one		✓	✓	
63	1-Methyl-5,8-dimethoxy-1,2,3,4-tetrahydro-1,4-iminonaphthalene		✓	✓	
70	Diethylene glycol dibenzoate		✓		✓
73	3,7-dimethyl-1,6-Octadien-3-ol			✓	✓
84	2,8-Dimethoxy-5,6-quinolinedione			✓	✓

μl of leaf volatiles attracted 10% (SE of ± 4) and 83% (SE of ± 5.3) of gravid female insects respectively. That of shoot volatiles attracted 20% (SE of ± 3.3) and 80% (SE of ± 3.3) of gravid female insects to a minimum dose of 0.02 μl and a maximum dose of 8 μl respectively. Fruit volatiles attracted 17% (SE of ± 0) and 80% (SE of ± 3.3) of gravid female insects to a minimum dose of 0.02 μl and a maximum dose of 8 μl respectively. Tables 3.3.1, 3.3.2 and 3.3.3 were indicate the chi-square test results during olfactometer studies.

Multiple-choice olfactometer study

Results revealed that all three categories of insects positively responded (were attracted) to the volatiles. Among them, gravid females were highly attracted to all three volatiles. All three types of insects highly preferred the volatiles from fruits ($p < 0.05$, Tukey's mean comparison). Volatile preference can be shown as: shoot volatiles < leaf volatiles < fruit volatiles. Table 3.4 shows the chi-square test results for each combination of plant volatiles and control during the multiple-choice olfactometer studies.

Volatile analysis

The volatile compounds emitted from different parts from the brinjal plant were identified using GC–MS. At least 100 compounds were detected from the plant and the highest number of 44 chemical constituents of volatiles were present in fruits. Results showed that a wide range of chemical compounds belonged to different classes of organic compounds: hydrocarbons, green leaf volatiles (GLVs), alcohols, fatty acids and other volatiles. Brinjal plant produces volatile secondary metabolites, which include 2,2'-(Ethane-1,2-diylbis(oxy))bis(ethane-

2,1-diyl) dibenzoate (12.11%), 3,7-dimethylocta-1,6-dien-3-ol (22.38%), Benzyl alcohol (22.9%) and Benzyl alcohol (27.06%) as major constituents from fruits, shoots, leaves and flowers respectively. GC–MS data comparison between the recorded and library mass spectra with similarity index higher than 90% and relative retention times were used for the identification of compounds present in each part of the brinjal plant. Same number is used to identify the same chemical constituents in each chromatogram as well as in the tables. Figs. A.1, A.2, A.3 and A.4 show the GC–MS chromatogram of steam distilled extract of brinjal plant fruit, shoot, leave and flower volatiles respectively. Tables B.1, B.2, B.3 and B.4 show the entire volatiles profile of brinjal fruits, shoots, leaves and flowers respectively. Table B.5 indicates the comparison of all four types of volatiles present in different parts of the brinjal plant.

Discussion

Plant volatiles are known to produce a wide range of behavioral responses in insects (Reddy and Angel, 2004). Insects have a highly sensitive olfactory system that can detect and discriminate relevant volatile organic compounds with high degree of selectivity and specificity (Chung et al., 2002; Tamiru et al., 2015). Unlike chemical insecticides, volatiles are difficult for insect to develop resistance against and work by a non-toxic mode of action through modifying the behavior of the pest (Bruce, 2010). Particularly important ones are the effects of host plants volatiles on pheromone behavior, which appears to be part of male strategies (to maximize encounters with females) as well as female strategies (to gain access to new feeding and oviposition sites) (Reddy and Angel, 2004).

The enhancement of sex attraction which could be induced by host plant volatile compounds suggests that more effective traps can be devised for the management of insect pests. Traps based solely on pheromones are unlikely to be fully competitive with signals emanating from food or plants. Moreover, it has been suggested that mating disruption dispensers could be developed for moth species using small amounts of expensive active pheromonal ingredients by adding small amounts of selected inexpensive host plant volatiles to the pheromone blend (Ochieng et al., 2002). Existing pheromone traps of BFSB, trap only the male insects by disturbing the mating behavior; while these volatile compounds from the host plant may enhance the sex attraction and disrupt the host-finding behavior; this may trap both male and female BFSB (Gunawardena et al., 1989; Gunawardana and Swarnakanthi, 1995).

In order to understand the chemical ecology, especially the communication systems between the insects, in a better way and to maximize their potential utility for pest management, the volatile semiochemicals emitted by insects and their host plants must be analyzed both qualitatively and quantitatively (Heath and Manukian, 1992). Further in this study volatile constituents extracted from flowers did not show any response during the olfactometer studies. The reason might be, BFSB feed primarily and oviposit solely on fruits, shoots and leaves. It is possible that the attractive compounds present in the leaves, fruits and shoots are absent from flowers; alternatively, the amount of volatiles being released from the flowers may have been below the behavioral threshold for response.

The collection of host plant volatiles on to a Super Q or Porapak Q absorbent could have led to a cleaner, a less crowded sample of host attractants, but this approach was found not practical. In this study the chemical composition of volatile fractions was not identified. In the present study, the behavioral responses of BFSB for the host plant volatiles revealed under controlled laboratory conditions. Study of insects' behavior under field conditions are more complicated due to the interference of various abiotic factors and their influence on the behaviour of the insects. Although this is a preliminary study, our results support the idea that interaction between host plant volatiles and insect pest should receive more attention. Results of this study will be helpful in designing

research studies to develop the integrated pest management due to the impact of these host plant volatiles on insect pests. Additionally, further studies need to be conducted to elucidate the attractant effects of host plant volatiles in natural field conditions.

Conclusion

This study reports for the first time the volatile profile of different parts of the brinjal plant. The two-choice olfactometer bioassay results revealed that the insects were attracted to the volatiles of host plant. The results from the multiple-choice olfactometer bioassay indicated that the attractant effect was higher to fruits' volatiles. In conclusion there is an attractant effect for the host plant volatiles towards the insect pest. The present study suggests that gas Chromatography coupled with electroantennogram (GC-EAG) studies can be carried out to isolate and identify the exact attractant chemical compositions in the host plant volatiles blend. Successful identification of the active compounds can lead to develop an eco-friendly attractant-based trap which can be used to control this key pest on brinjal plant.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A: Figures of volatile analysis

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Appendix B: Tables of volatile analysis

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