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Electro-physiological and olfactometric responses of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) and *Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae) to volatiles of trap crops -*Tagetes erecta* Linnaeus and *Solanum viarum* Dunal

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ABSTRACT: Experiments were done on electro-physiological and olfactometeric responses of *Helicoverpa armigera* (Hübner) and its egg parasitoid *Trichogramma chilonis* Ishii to leaf and floral volatiles of *Tagetes erecta* Linnaeus and *Solanum viarum* Dunal. *H. armigera* female moths exhibited highest mean absolute net electrophysiological response (-0.462mv) to floral volatiles of *T. erecta* followed by volatiles from leaves (-0.395mv). In case of olfactometric responses, *T. chilonis* showed maximum net response to hexane extract of *T. erecta* flower bud (47.5%), followed by floral and leaf volatiles. However, statistically all these cues were on par. The volatile compounds identified from leaves and flowers of *T. erecta*, and leaves of *S. viarum* were 16, 17 and 21 in number, respectively. The compounds found common in both the trap crops were: 1, 2, benzenedicarboxylic acid, cis-a-bisabolene, eicosane, hexacosane, heptacosane, pentacosane, tetradecane and nonadecane. Among the floral volatiles, piperitenone was in the highest proportion (25.5%), followed by piperitone (5.39%), limonene (4.83%), trans- b-ocimene (3.35%), cis-epoxy-ocimene (4.83%), myrcene (1.13%) and BHT-aldehyde (0.34%). Studies revealed that both the trap crops -*T. erecta* and *S.viarum* are *Trichogramma* friendly.

KEY WORDS: Electrophysiological and olfactometric responses, *Helicoverpa armigera*, *Solanum viarum*, *Tagetes erecta*, trap crops, *Trichogramma chilonis*, volatile compounds

INTRODUCTION

Plants of higher palatability or ovipositional suitability lure herbivores away from other plant species of economically important crops. This behavioural trait formed the base of an attractant decoy hypothesis proposed by Atsatt and O' Dowd (1976). Trap crops are out come of this concept. Use of trap crops and natural enemies form an integral part of Biointensive Integrated Pest Management (BIPM) of *Helicoverpa armigera* (Hübner). African marigold (*Tagetes erecta* Linnaeus) has been used as trap crop for *H. armigera* in several crops including tomato and cotton (Srinivasan *et al.*, 1994; Shivayogeshwara *et al.*, 2000; Sridhar *et al.*, 2001). *H. armigera* female moths prefer to lay eggs on marigold flower buds. Patel and Yadav (1992), similarly, reported ovipositional preference of *H. armigera* females to marigold over tobacco. The authors further highlighted that open flowers were more preferred over buds, and disc florets over ray florets. Highest trapping efficiency was reported when two rows of marigold were intercropped in six rows of tobacco and appeared to increase the activity of *Trichogramma*. Meena and Lal (2002) also observed significant effect of marigold intercropped with cabbage on larval population of diamondback moth, *Plutella xylostella* (Linnaeus) compared to cabbage. *Solanum viarum* Dunal has been reported as another trap crop for *H. amigera* in tomato field in Taiwan (Talekar *et al.*, 1999). However, the information was lacking on the electrophysiological and olfactometer responses of *H. armigera* and its natural enemies to the chemical cues (volatiles) present in these trap crops. Hence, the present study was conducted to know the relative preference of *H. armigera* females and its parasitoid, *Trichogramma chilonis* Ishii to marigold and *S. viarum* and to identify the volatiles present in these trap crops.

MATERIALS AND METHODS

Insect culture and Plants

The females of *H. armigera* used for electrophysiological studies were obtained from the culture maintained on artificial diet in the Entomophagous Insect Behaviour, Laboratory of Project Directorate of Biological Control. Five-day-old mated females were used. Similarly, three-day-old adults of *T. chilonis* used for olfactometer response experiments were obtained initially from Mass Production Laboratory and further culture was maintained on *Corcyra cephalonica* (Stainton) eggs.

Tagetes erecta, and *S. viarum* plants were raised in pots from seed procured from Indian Institute of Horticultural Research, Hessaraghatta under glasshouse condition. These plants were kept free from insecticidal or fungicidal sprays.

Electrophysiological responses

Electrophysiological responses of *H. armigera* females to volatiles trapped from *T. erecta* and *S. viarum* leaves and flowers were studied using the Syntech Electroantennometer. The antenna of *H. armigera* female was dissected along with the basal segment and mounted on the indifferent electrodes containing electroconductivity gel (0.1M KCL) while the tip of the antennae was inserted carefully into the recording electrode containing the same electrolyte. The recording electrode was connected through a pre-amplifier (Syntech) to an EAG amplifier (AM 02, Syntech). The analog EAG signals were amplified 10 times and digited through an A/D interface (IADC-02, Syntech). The airflow was maintained at 0.5m/second, impulse was given for 0.5 seconds, and the response was recorded for 5 seconds. With the custom EAG programme (Ver.2.6, 1996, Syntech), the resulting electroantennograms were analysed by measuring the maximum millivolt amplitude of depolarization resulting due to a particular stimulus.

Absolute net EAG responses (-mv) to the test cues (trap crop volatiles) were calculated by subtracting the mean absolute EAG response of control stimulations immediately preceding ($control_x$) and following ($control_{x+1}$) the presentation of the absolute EAG response of the test cue.

Absolute net EAG_x (-mv) = EAG_x - [($control_x$ + $control_{x+1}$)/2].

Bioassay

Y-Tube olfactometer assay earlier described by Ngi-Sang *et al.* (1996) was used to test attraction in 3-day-old female *T. chilonis*. The parasitoids were introduced singly in the stem of the glass olfactometer and observed for their choice behaviour. Parasitoids reaching the cue source within 10 minutes and remaining arrested for 15 seconds were recorded as having made choice. Individual parasitoids were given a choice between air permeated volatiles/ water extracts of *T. erecta* and *S. viarum* and clean air control. All the test cues were replicated four times with ten parasitoids per replicate and the experiment was conducted at ambient laboratory temperature (25±2°C). The data were subjected to analysis of variance.

Collection of volatiles

For volatiles collection healthy potted plants of *T. erecta* and *S. viarum* with leaves or flowers were introduced into a collection chamber and clean filtered airstreams was drawn over leaves or flowers and volatiles were trapped in the activated charcoal kept in corning glass-tube for one hour. The volatiles were eluted from charcoal traps with HPLC-grade hexane and concentrated under vacuum concentrator.

Analysis of volatiles

Concentrated samples were injected into the column and analyzed using GC-MS. Analyses was performed on a Hewlett-Packard 6890 GC coupled with a 5973 Mass Spectral Detector using HP-5MS (Hewlett-Packard, Avondale, Pennysylvania) cross linked 5 per cent phenyl methyl siloxane column (30m x 0.25mm ID x 0.25mm film thickness). The mass spectra of unknown compounds were compared with those in the Wiley spectral database. Standards of compounds were obtained from Sigma Aldrich® and the retention time and mass spectra were compared with the peaks for confirmation.

RESULTS AND DISCUSSION

Bioassay of trap crop volatiles and extracts

In dual-choice Y-tube glass olfactometric bioassay, highest mean net response (47.50%) of *T. chilonis* females to marigold flower bud extracts was recorded (Table 1). However, statistically this was on par with net response of *T. chilonis* exhibited towards volatiles from marigold flowers (45.00%) and leaves (42.50%). Volatiles from *S. viarum* leaves (37.50%) were on par with *S. viarum* flowers (35.00%), *T. erecta* leaves (42.50%) and *T. erecta* flower bud extract at half concentration (37.50%). This reveals that volatiles from marigold and *S. viarum* attract *T. chilonis.* Manjunath *et al.* (1970) reported 5-85 per cent parasitization of *H. armigera* eggs on marigold by *Trichogramma* spp. (mostly *T. chilonis*) around Bangalore and concluded that ovipositing *Trichogramma* females were attracted to bright colour of marigold. Patel and Yadav (1992) reported that intercropping marigold in tobacco did not affect the activity of the predatory mirid, *Cyrtopeltis tenuis* Reuter and appeared to increase egg parasitization by *Trichogramma*. Subsequently, Bruce and Cork (2001) reported attraction of *H. armigera* females to marigold (*T. erecta*) headspace sample equivalent to 0.4 flowers per hour emission from live flower through wind-tunnel bioassay. Further, the authors reported attraction of *H. armigera* females to the mixture of benzaldehyde, (S)-(-)-limonene, (R, S)-(±)- linaloql, (E)- myroxide, (Z) – beta- ocimene, phenylacetaldehyde and (R)-(-)–piperitone (1:1.6:0.7: 1.4:0.4: 5.0:2.7) which was found in the marigold. Cunningham *et al.* (2004) evaluated the response of *H. armigera* males in dual- choice wind tunnel studies to phenylacetaldehyde and α-pinene and found phenylacetaldehyde more attractive than α-pinene.

Table 1. Mean net response of *Trichogramma chilonis* to volatiles

Sl. no.	Trap crop	Mean net response (%)
1.	*Solanum viarum* leaves	37.50
2.	*S. viarum* flowers	35.00
3.	*Tagetes erecta* leaves	42.50
4.	*T. erecta* flowers	45.00
5.	*T. erecta* flower bud extracts (100%)	47.50
6.	*T. erecta* flower bud extracts (50%)	37.50
	SEM±	2.29
	CD (P=0.05)	6.92
	CV (%)	11.25

Electrophysiological responses

The data on absolute net EAG response of *H. armigera* female are presented in Table 2. Highest absolute net EAG response was given to volatile mixture released by marigold flower (0.462 –mv) which was statistically on par with marigold leaves (0.395-mv). Least response was shown to volatiles from *S. viarum* leaves. This indicates that *H. armigera* female moths are more receptive to volatile cues from marigold flowers in comparison to *S. viarum* leaves or flowers. Hence, it can prove a better trap crop for *H. armigera* over *S. viarum*. Bruce and Cork (2001) found seven compounds from *T. erecta* flowers to be electro-physiologically active (at µg dose of each compound on filter paper) to elicit EAG responses.

Table 2. Absolute net EAG response of *H. armigera* females to trap crop volatiles

Sl. no.	Volatile source	Mean absolute net EAG_x response (-mv)*
1.	*Tagetes erecta* leaves	0.395
2.	*T. erecta* flowers	0.462
3.	*Solanum viarum* leaves	0.304
4.	*S. viarum* flowers	0.340
	SEM ±	0.036
	CD (P=0.05)	0.110
	CV (%)	3.940

Analysis of volatiles

The chemical profile of marigold (leaves and flowers) and *S. viarum* (leaves) was obtained by GC-MS analysis. The list of 24 compounds identified from the leaves of both the trap crops is presented in Table 3. These volatiles pertain to organic groups like alkanes, alkyls, monoterpenes, sesquiterpenes and alcohols. The major compounds identified from *T. erecta* and *S. viarum* are: 1, 2, benzenedicarboxylic acid, diet (3.21 %, 0.00%); 1,2, benzenedicarboxylic acid, dibu (2.32%, 1.94%); pentadecane (1.07%, 1.62%); octadecane (0.70%, 0.36%); tetradecane (0.64% 0.14%); cis-alpha-bisabolene (0.61%, 0.16%); heptadecane (0.69%, 0.25%); hexadecane (0.55%, 0.92%) and eicosane (0.55%, 0.30%), respectively.

Seventeen compounds were identified from volatiles trapped from *T. erecta* flowers using GCMS (Table 4). Based on percentage of total volatiles, highest quantity of piperitenone (25.05%) was found in the volatiles followed by dodecane (13. 86%), piperitone (5.32%), limonene (4.83%), trans-α-ocimene (3.35%) and hexadecanoic acid, methyle ester (2.41%). Bruce and Cork (2001) identified seven volatiles, namely, benzaldehyde, (S)-)(-)- limonene, (R, S)-(±)-linalool, (E)- myroxide, (Z) – beta-ocimene, phenylacetaldehyde and (R)-(-)–piperitone from marigold (*T. erecta*) flowers and all were found electrophysiologically active against *H. armigera*. Burguiere *et al.* (2001) also reported that ocimene, β-phellandrene (both terpenoid), benzaldehyde and phenylacetaldehyde (both aromatic compounds) elicited highest electrophysiological response in female *H. armigera*. Bruce and Cork (2001) also identified compounds like benzaldehyde, (s)-(-)-limonene, (R, S)-(±)-linalool, (E)-myroxide, (Z)-β-ocimene, phenylacetaldehyde and (R)-(-) piperitone.

From the electrophysiological studies it can be concluded that *H. armigera* female moths do respond to volatiles released by *T. erecta* and *S. viarum* leaves and flowers, and the most preferred cues were volatiles from *T. erecta* flowers. Further, olfactometric responses of *T. chilonis* to the cues from these two trap crops further revealed that beside attracting *H. armigera* they also attract *T. chilonis*. Thus these two trap crops have proved as *Trichogramma* friendly. *H. armigera* eggs laid on trap crops (*T. erecta or S. viarum*) in an organic crop production system can be managed locally by releasing *T. chilonis*.

Table 3. Green leaf volatiles of *Tagetes erecta* and *Solanum viarum*

Sl. no.	Compound	Per cent of total	
		T. erecta	*S. viarum*
1.	Allyl anisole	0.00	0.05
2.	**1, 2, Benzenedicarboxylic acid, dibu**	**2.32**	**1.94**
3.	1, 2, Benzenedicarboxylic acid, diet	3.21	0.00
4.	**Cis –alpha- bisabolene**	**0.61**	**0.16**
5.	Camphene	0.00	0.02
6.	Decane	0.13	0.09
7.	Dodecane	0.18	0.09
8.	Diethyle phatalate	0.00	3.60
9.	**Eicosane**	**0.55**	**0.30**
10.	**Heptadecane**	**0.69**	**0.25**
11.	**Hexadecane**	**0.55**	**0.92**
12.	3-Hexanol	0.00	0.69
13.	Limonene	0.00	0.05
14.	Methyl chavicol	0.07	0.00
15.	**Nonadecane**	**0.55**	**0.30**
16.	Nonane	0.00	0.05
17.	**Octadecane**	**0.70**	**0.36**
18.	**Pentadecane**	**1.07**	**1.62**
19.	2-Pentanol, 2-methyl	0.00	0.99
20.	Phenol, 2, 4-bis	0.07	0.00
21.	α- pinene	0.00	0.06
22.	**Tetradecane**	**0.64**	**0.14**
23.	Tridecane	0.25	0.09
24.	Undecane	0.14	0.11

Table 4. Volatiles from flowers of *Tagetes erecta*

Sl. no.	Compound	Per cent of total *T. erecta*
1.	1, 2, Benzenedicarboxylic acid, dime	1.19
2.	BHT-aldehyde	0.34
3.	**Dodecane**	**13.86**
4	**Heptadecane**	**2.20**
5.	Hexadecane	1.20
6.	**Hexadecanoic acid, methyl ester**	**2.41**
7.	**Limonene**	**4.83**
8.	Myrcene	1.13
9.	Nonane	0.39
10.	Octadecane	1.14
11.	Cis-epoxy Ocimene	1.22
12.	**Trans-Beta-Ocimene**	**3.35**
13.	Pentadecane	1.00
14.	**Piperitone**	**5.39**
15.	**Piperitenone**	**25.05**
16.	α- terpinolene	1.56
17.	Sabinene	0.54

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REFERENCES

Atsatt, R. R. and O' Dowd, D. J. 1976. Plant defence guilds. *Science*, **193**: 24-29.

Bruce, T. J. and Cork, A. 2001. Electrophysiological and behavioral responses of female *Helicoverpa armigera* to compounds identified in flowers of African marigold, *Tagetes erecta*. *Journal of Chemical Ecology*, **27**: 1119-1131.

Burguiere, L., Marion-Poll, F. and Cork, A. 2001. Electrophysiological responses of female *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) to synthetic host odours. *Journaal of Insect Physiology*, **47**: 509-514.

Cunningham, J. P., Moore, C. J., Zalucki, M. P. and West, S. A. 2004. Learning, odour preference and flower foraging in moths. *Journal of Experimental Biology*, **207**: 87-94.

Manjunath, T. M., Phalak, V. R. and Subramanian, S. 1970. First record of egg parasites of *H. armigera*

(Hübner) (Lepidoptera: Noctuidae) in India. *Technical Bulletin, Commonwealth Institute of Biological Control*, **13**:111-115.

Meena, R. K. and Lal, O. P. 2002. Effect of Intercropping on the incidence of diamondback moth, *Plutella xylostella* on cabbage. *Journal of Entomological Research*, **26**: 141-144.

Ngi-Sang, A. J., Overholt, W. A., Njagi, P. G.N., Dicke, M., Ayertey, J. N. and Lwande, W. 1996. Volatile infochemicals in host and host plant habitat location by *Cotesia flavipes* Cameron and *C. sesamiae* (Cameron) (Hymenoptera: Braconidae), two larval parasitoids of graminaceous stem borers. *Journal of Chemical Ecology*, **22**: 307-323.

Patel, R. K. and Yadav, D. N. 1992. Impact of Intercropping marigold on *Heliothis armigera* (Hübner) and its natural enemies in seed crop of tobacco. *Tobacco Research*, **18**: 65-72.

Shivayogeshwara, B., Gouda, B. L. V., Shankar, S. and Patil, N. M. 2000. Marigold in the Management of *Helicoverpa armigera* (Hübner) in tobacco crop. *Insect Environment*, **6**: 4.

Sridhar, V., Pandey, V. and Rao, E. S. 2001. Suitability of African marigold (*Tagetes erecta* Linn.) as a trap crop to *Helicoverpa armigera* (Hübner): influence of type and colour of flowers. *Orissa Journal of Horticulture*, **29**: 84-86.

Srinivasan, K., Moorthy, P. N. K. and Raviprasad, T. N.1994. African marigold as a trap crop for the management of the fruit borer, *Helicoverpa armigera* on tomato. *International Journal of Pest Management*, **40**: 56-63.

Talekar, N. S., Hau, T. B. H. and Chang, W. C. 1999. *Solanum viarum*, a trap crop for *Helicoverpa armigera*. *Insect Environment*, **5**: 142.