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Plant volatile diversity in different tomato genotypes and its influence on parasitization efficiency of *Trichogramma chilonis* Ishii on *Helicoverpa armigera* (Hübner)

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ABSTRACT: Evaluation of 15 tomato genotypes (Varieties/ hybrids) for their influence on the parasitization efficiency of *Trichogramma chilonis* Ishii on *Helicoverpa armigera* (Hübner) eggs under screen house condition revealed significant differences in terms of per cent parasitization. Least parasitization was recorded on Arka Abha (20%) and highest on Arka Ahuti (50%), followed by Anand-1 (46.66%). Olfactory response of *T. chilonis* to tomato fruit volatiles did not show significant differences. However, leaf volatiles showed significant differences in their attraction to *T. chilonis*. The fruit volatiles identified from different genotypes comprised 16 compounds, which include alkane hydrocarbons, monoterpenes, sesquiterpenes, diterpenes and other metabolites. It revealed great fruit volatile diversity in terms of number of compounds (qualitative) and their proportions (quantitative). Maximum compounds were identified from variety Ramya and Pusa Ruby (12 each). The major compound present in the fruit volatiles was Linalool-L and its proportionate concentration varied from 1.79 (Pusa Ruby) to 91.12 per cent (Arka Abha). This was followed by heptadecane (present in all the genotypes) and the proportionate concentration varied from 2.89 (A. Saurabh) to 62.9 per cent varalakshmi. The leaf volatile profiles of the genotypes evaluated include 19 compounds. Except genotype varalakshmi in which α-pinene constituted 69.69 per cent of total volatiles, in all other genotypes heptadecane was the main compound and the concentration varied from 21.72 (in Ramya) to 60.75 per cent (in A. Abha). Some of the compounds identified α-phellandrene, α-pinene, trans-caryophyllene, (Z)-α-farnesene, trans-α-ocimene and selinene are known for their synomonal activity.

KEY WORDS: Genotypes, *Helicoverpa armigera*, parasitizing efficiency, tomato, *Trichogramma chilonis*, volatile diversity

INTRODUCTION

Plant allelochemicals mediate mutually beneficial interactions, and play an important role as chemical signals in the host location behaviour of natural enemies. Plants can be attractive towards natural enemies of insects that attack them. Natural enemies use herbivore-induced synomones to locate their hosts. This common feature, termed indirect resistance, can be exploited to increase the efficiency of natural enemies in biological control of insect pests. Earlier studies have indicated the influence of different crops on the relative dominance of parasitoids of *Heliothis* /

Helicoverpa species or their success in enhancing parasitism by several species of parasitoids (Bhatnagar *et al.*, 1982; Murray and Rynne, 1994; Murray *et al.*, 1995; Ballal and Singh, 2003; Tandon and Bakthavatsalam, 2005). Further, it was reported that there is a great amount of diversity in plant volatile profile among different varieties/ hybrids of chickpea (Tandon and Bakthavatsalam, 2001), pigeon pea (Tandon and Bakthavatsalam, 2003) and sunflower (Tandon and Bakthavatsalam, 2004), which influences the success or failure of natural enemies. Bottrell *et al.* (1998) reviewed critically and discussed the selection of plant varieties and modification as a realistic strategy to manipulate natural enemies for successful biological control of crop pests. The present studies were conducted to know the influence of different genotypes (varieties/ hybrids) of tomato on the parasitization efficiency of the egg parasitoid *Trichogramma chilonis* Ishii on *Helicoverpa armigera* (Hübner) and the profile of tomato leaf and fruit volatiles. The ultimate aim is to identify *T. chilonis* friendly tomato genotypes.

MATERIALS AND METHODS

The experiments were conducted during 2003-04 in the Entomophagous Insect Behaviour Laboratory at Project Directorate of Biological Control (PDBC), Bangalore.

Insects cultures

The cultures of host insect, *H. armigera* and its egg parasitoid, *T. chilonis* were initially obtained from Mass Production Laboratory and thereafter multiplied in the Entomophagous Insect Behaviour Laboratory of PDBC.

Tomato genotypes

Fifteen genotypes comprising of varieties and hybrids were grown in earthen pots (30cm diam) in a screen house. The genotypes evaluated were: Anjali, Anand-1, Arka Abha, Arka Ahuti, Arka Alok, Arka Ashish, Arka Meghali, Arka Saurabh, Arka Vikas, Challenger-1, Junagarh Ruby, Ramya, Pusa Ruby, Varalakshmi and 101-Super. Most of them were obtained from Indian Institute of Horticultural Research, Hessaraghatta, Bangalore. Two plants of each variety/ hybrid (per pot) constituted a single replication. Each genotype was replicated five times.

Parasitization efficiency of *T. chilonis*

Tomato plants of different varieties/ hybrids were grown in clay pots (30 cm diam) and finally two seedlings were retained in each pot. Potted tomato plants at flower initiation stage were artificially infested with one-day-old *H. armigera* eggs at the rate of 5 eggs per plant placed on topmost leaves close to flower buds (one egg / leaf). The number of adult egg parasitoids (*T. chilonis*) released was estimated based on total host eggs placed on all plants, sex ratio of the parasitoid (1: 1), and parasitoid: host ratio of 1: 20. Two-day-old mated *T. chilonis* were released after 3 hours of infestation of tomato plants of 15 varieties kept under screen house condition with *H. armigera* eggs. After two days, all the eggs were collected in Petri-plates for recording observations on parasitization. Per cent parasitization was recorded initially based on blackening of eggs and later on confirmed by adult emergence. Two years, pooled data were converted into arcsine values and then subjected to analysis of variance.

Y-tube olfactometer responses of *T. chilonis*

Y-tube olfactometer bioassay earlier described by Ngi-Sang *et al.* (1996) was used to test attraction in 3-day-old females of *T. chilonis*. The parasitoids were introduced singly in the stem of Y-tube glass olfactometer and observed for their choice behaviour. Parasitoids reaching the cue source within ten minutes and remaining arrested for 15 seconds were recorded as having made choice. Individual parasitoids were given a choice between air-permeated volatiles from tomato leaves/ fruits and clean air from control. 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.

EAG responses of *H. armigera* to green leaf volatiles of different tomato varieties / hybrids

Electrophysiological responses of *H. armigera* females to green leaf volatiles released by 15 different genotypes of tomato (Table 3) were studied using the Syntech electroantennometer. The antenna of *H. armigera* females was dissected along with the basal segment and mounted on the different electrode containing electroconductivity gel (0.1M KCL) while tip of the recording electrode containing the same electrode was connected through a pre-amplifier (Syntech) to an EAG amplifier (AM 02 Syntech). The analog EAG signals were amplified 10 times and digitized 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 EAGs were analyzed by measuring the maximum millivolt amplitude of depolarization resulting due to a particular stimulus. Absolute Net EAG responses (-mv) to the cues were calculated by using the following formula: Absolute Net EAG responses (-mv) = EAG_x - [($Control_{x+}$ $Control_{x+1)}$ / 2]

Collection of volatiles

For collection of volatiles from healthy potted plants of ten varieties of tomato (Table 2), leaves/fruits were introduced into the collection chamber and clean filtered air stream was drawn over, and volatiles were trapped in activated charcoal kept in Corning glass-tube traps for two hours. The volatiles were eluted from charcoal traps with HPLC-grade hexane and concentrated under vacuum concentrator.

Analysis of volatiles from leaves and fruits

Freshly trapped and concentrated volatile samples from leaves and fruits of different genotypes of tomato were injected individually into the column and analysed using GCMS. Analysis was performed on a Hewlett-Packard 6890 GC coupled with a 5973 Mass Spectral Detector using HP-5MS (Hewlett-Packard, Avondale, Pennsylvania) 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. Volatile compounds listed in Table 4 and 5 have 90–99 per cent matching quality.

RESULTS AND DISCUSSION

Influence of tomato genotypes on parasitization efficiency of *T. chilonis*

There was significant variability in the influence of 15 tomato genotypes on parasitization efficiency of the parasitoid, *Trichogramma chilonis* of *Helicoverpa armigera* eggs (Table 1). Highest parasitization of *H. armigera* eggs was recorded on variety Arka Ahuti (50.00%), followed by Anand-1 and both were statistically on par. Least parasitization was recorded on Arka Abha (20.00%), followed by Ramya (21.66%) and Anjali (23.33%) and all were on par. Arka Abha is a bacterial wilt resistant variety developed by Indian Institute of Horticultural Research. Majority of the genotypes evaluated were found to be Trichogramma friendly. Nordlund *et al.* (1985) reported that volatile synomones from tomato plants stimulated search behaviour in *T. pretiosum*, resulting in increased rates of parasitism of eggs of *Heliothis zea* in both laboratory and field.

The glandular trichomes (Type-vi), which contributed to resistance factor of PI 134417 variety of tomato against several insect pests also adversely affected an array of egg and larval parasitoids (Kauffman and Kennedy, 1989). Significant portion of the reduction of egg parasitism in *H. zea* and *Manduca sexta* is attributed to the effects of 2-tridecanone and/or 2-undecanone present in the tips of glandular trichomes on the foliage of PI 134417 variety besides adverse effect of trichomes on wasps (Kashyap *et al.*, 1991). Four years field study on resistance of a wild tomato variety to *H. zea* and its effect on egg parasitoids, i.e., the *T. pretiosum* and *T. exiguum*, revealed highest parasitism (43%) on *Lycopersicon esculentum*, followed by F1 hybrid (14%), back

Table 1. Influence of tomato genotypes on the parasitization efficiency of *T. chilonis* on *H. armigera* eggs

Variety/ hybrid	Mean per cent parasitization
Anjali	23.33 (28.85)*
Anand-1	46.66 (43.09)
Arka Abha	20.00 (26.57)
Arka Ahuti	50.00 (45.01)
Arka Alok	31.66 (34.23)
Arka Ashish	33.33 (35.25)
Arka Meghali	36.66 (37.26)
Arka Saurabh	36.66 (37.26)
Arka Vikas	36.66 (37.26)
Challenger –1	43.33 (41.15)
Junagarh Ruby	30.00 (33.16)
Ramya	21.66 (27.71)
Pusa Ruby	26.66 (31.09)
Varalakshmi	40.00 (39.29)
101-Super	26.66 (31.09)
SEM±	1.24
CD (P=0.05)	3.60
CV (%)	6.12

*Figures in parentheses are arcsine-transformed values.

cross (2%) and PI 134417 (2%). The low parasitism on the latter two lines was attributed to high trichome density and high concentration of methyl ketone levels. Similar behaviour of *Trichogramma* sp. and *Telenomus sphingidis* was observed by Farrar *et al.* (1994) on parasitization of *M. sexta* eggs on these tomato lines. Kennedy (2003) stated that trichome mediated defenses are significant in *L. hirsutum* f. *glabratum* and have implications in negative tritrophic effects mediated by direct contact of parasitoid and predators with trichomes.

Earlier, Tandon and Bakthavatsalam (2001, 2003) reported the influence of chickpea and pigeon pea genotypes on the parasitization efficiency of *T. chilonis* on *H. armigera* eggs. The authors related it to morphological characters like glandular trichomes and malic acid produced by them. Basit *et al.* (2001) recorded great variation in parasitism by *T. chilonis* on *Corcyra cephalonica* eggs placed on leaves of different varieties of rice under laboratory condition. Tandon and Bakthavatsalam (2004) studied the influence of sunflower genotypes on parasitism of *H. armigera* eggs by *T.chilonis*. The present studies are in conformity with previous reports and have demonstrated the role of tomato varieties/ hybrids as a factor that helps *T.chilonis* in host searching and thereby enhancing the extent of parasitism.

Table 2. Olfactory response of *T. chilonis* to fruits and leaves of tomato genotypes

Variety/ hybrid	Net response to fruits(%)	Net response to leaves(%)
Arka Abha	36.66 (37.22)	26.60 (31.00)*
Arka Ahuti	33.33 (35.22)	30.00 (33.21)
Arka Alok	36.66 (37.22)	40.00 (39.15)
Arka Ashish	33.33 (35.22)	30.00 (33.21)
Arka Meghali	33.33 (37.22)	23.33 (28.78)
Arka Saurabh	30.00 (33.32)	30.00 (33.21)
Arka Vikas	36.66 (37.26)	36.66(37.22)
Ramya	30.00 (33.22)	36.66 (37.22)
Pusa Ruby	33.33 (35.22)	33.33(35.22)
Varalakshmi	36.66 (37.22)	36.66(37.22)
SEM±	1.78	2.09
CD (P=0.05)	NS	6.23
CV (%)		10.73

*Figures in parentheses are arcsine-transformed values.

Olfactory responses of *T. chilonis* to fruit and leaf volatiles

In dual choice Y-tube glass olfactometer bioassay, highest mean net response (40.00%) of *T. chilonis* females to volatiles from leaves of Arka Alok variety was recorded (Table 2), which was on par with net response of Arka Vikas, Ramya, Varalakshmi and Pusa Ruby. Least net response was shown towards Arka Meghali (23.33%), followed by Arka Abha. There was no significant difference in the response of *T. chilonis* adults to volatiles released by fruits of different tomato genotypes, which varied from 30.00 to 36.66 per cent. Nordlund *et al.* (1985) reported attraction of females of *T. pretiosum* towards tomato volatiles in the Y-tube olfactometer. Guerrieri *et al.* (2003) reported the attractiveness of two tomato ecotypes, AN5 and AN7 towards *Aphidius ervi*, the most effective parasitoid *of Macrosiphum euphorbiae*, and the key pest of tomato grown in Southern Italy. It was revealed that a high level of attractiveness could be either constitutive (AN5) or induced (ecotype AN7).

Bjorksten and Hoffman (1998) investigated the influence of tomato plant cues on searching behaviour and parasitism in the egg parasitoid, *Trichogramma* nr. *brassicae* on *Sitotroga cerealella* allowed to emerge on tomato plants. Females searched significantly longer on tomato seedlings and the effect lasted for 1-2 days and parasitized significantly more hosts on tomato than females emerging in tubes or lettuce leaves. Thaler *et al.* (2002) reported that jasmonate-deficient tomato plants were less attractive to predatory mites. Damaged wild tomato plants (rich in jasmonate) induced a greater production of volatile compounds (i.e. β-caryophyllene, α-pinene, β-pinene and α-phillandrene) compared with jasmonate deficient plants.

EAG responses of *H. armigera* to green leaf volatiles of different tomato varieties/hybrids

The data generated on EAG responses of *H. armigera* female antenna exhibited the largest mean absolute response (-1.429mv) as indicated by the peak amplitude to honey used as standard cue.

Table 3. EAG response of *H. armigera* to leaf volatiles of different tomato varieties/hybrids

Variety hybrid	Mean Absolute Net EAG response (-mv)
Anjali	0.221 (-0.657)*
Anand-1	0.668 (-0.175)
Arka Abha	0.796 (-0.106)
Arka Ahuti	0.798 (-0.124)
Arka Alok	1.166 (0.015)
Arka Ashish	0.500 (-0.305)
Arka Meghali	0.728 (-0.154)
Arka Saurabh	0.960 (-0.040)
Arka Vikas	1.234 (0.069)
Challenger 1	0.468 (-0.807)
Junagarh Ruby	0.323 (-0.491)
Ramya	0.690 (-0.170)
Pusa Ruby	0.932 (-0.032)
Varalakshmi	0.569 (-0.097)
101-Super	0.251(-0.600)
Honey (Standard)	1.429 (0.148)
SEM±	0.067
CD (P=0.05)	0.197

Figures in parentheses are log transformed values.

Among the tomato genotypes, highest response (1.234-mv) was shown by *H. armigera* towards volatiles produced by crushed leaves of Arka Vikas, followed by Arka Alok, which were on par statistically (p=0.05). In the next group come Arka Suarabh, Pusa Ruby, Arka Ahuti, and Arka Abha – all being on par with each other. Anjali was least perceptive, followed by 101-Super and Junagarh Ruby. This gives us a clear indication about the differential response of female antenna of *H. armigera* towards green leaf volatiles released by the different tomato genotypes. Tandon and Bakthavatsalam (2005) studied the EAG response of *H. armigera* females to leaf and flower volatiles of *Tagetes erecta* and *Solanum viarum* - the two effective trap crops for *H. armigera*. *T. erecta* flower buds were found most attractive, followed by the leaves. Bakthavatsalam *et al.* (2001) made similar observations on the influence of different cultivars of sunflower on the electroantennogram response of *Chrysoperla carnea*. Electrophysiological techniques can determine which volatiles present in a particular crop or variety are active. Once these volatiles/ varieties are identified, their function in modifying insect behaviour can be explored.

Analysis of volatiles from leaves and fruits

Recent critical review on plant volatiles by Natalia *et al.* (2006) reveals that about 1700 volatile compounds from leaves, flowers and fruits as well as roots have been isolated and identified from more than 90 plant families. These compounds defend plants from arthropod herbivores and pathogens causing diseases, and provide productive

Table 4. Profile of volatiles released by fruits of different varieties and hybrids of tomato

Volatile components	Varieties /hybrids									
	V.Lakshmi	A. Meghali	A. Ashish	A. Abha	A. Alok	A. Ahuti	A. Vikas	A. Saurabh	Ramya	P. Ruby
(Z)- β-Farnesene	-	-	-	-	-	-	-	-	+	-
Cembrene	+	+	+	-	-	+	-	+	+	+
Cis-Linalool Oxide	-	-	+	+	+	+	-	+	+	+
Eicosane	-	-	-	-	+	+	-	+	-	-
Heptadecane	+	+	+	+	+	+	+	+	+	+
Hexadecane	-	+	+	-	+	+	-	-	+	+
Kaur-16-ene	-	-	+	-	-	-	-	-	+	+
Linalool-L	-	+	+	+	+	+	-	+	+	+
6-methyl-5-hepten-2-one	-	-	+	-	-	-	-	-	-	-
N-nonadecane	+	+	+	-	+	+	+	-	+	+
Octadecane	+	-	+	+	+	+	+	+	+	+
Pentadecane	+	+	+	+	+	+	+	+	+	+
Phenol, 2, 4-bis (1-dimethyl ethyl	-	+	-	-	+	-	-	+	-	-
N-Tetradecane	+	+	+	-	+	+	-	-	+	+
Trans-linalool Oxide	-	-	-	-	-	-	-	-	+	+
(E, E)-7,11,15-Trimethyl-3-methylen	-	-	-	-	-	-	-	-	-	-

Matching quality of all the compounds is above 90 %.

Table 5. Profile of volatiles released by leaves of different varieties and hybrids of tomato

Volatile components	Varieties /hybrids									
	V.Lakshmi	A. Meghali	A. Ashish	A. Abha	A. Alok	A. Ahuti	A. Vikas	A. Saurabh	Ramya	P. Ruby
α -Pinene	+	+	+	-	+	-	-	-	-	-
α-Phellandrene	+	-	-	-	-	-	-	-	-	-
β- Selinene	-	-	-	-	-	-	-	-	-	+
(Z)-β-Farnesene	+	-	-	+	+	-	-	+	-	+
Cembrene	-	+	-	-	+	+	+	+	-	-
Eicosane	-	+	-	-	+	-	-	-	+	-
n-Heptadecane	+	+	+	+	+	+	+	+	+	+
Hexacosane	-	-	-	-	-	-	-	-	+	-
Hexadecane	-	-	-	-	+	-	+	-	+	+
Hexadecanoic acid	-	-	-	-	-	-	-	-	+	-
Nonadecane	+	+	-	-	+	+	+	+	+	+
n-Octadecane	+	+	+	+	+	+	+	+	+	+
n-Pentadecane	+	+	+	+	+	+	+	+	+	+
Sabinene	-	-	-	-	+	-	-	-	+	
n-Tetradecane	+	+	+	+	+	+	+	+	+	+
Trans - Caryophyllene	-	-	-	-	-	-	-	-	+	-
Tridecane	-	-	-	-	-	-	-	-	+	-
(E, E)-7,11,15-trimethyle-3-methyl	-	+	+	-	-	-	-	-	+	-
Trans- β- Ocimene	-	-	-	+	-	-	-	-	-	-

Matching quality of all the compounds above 90 %.

advantage by attracting pollinators and seed dispersers. In the present study, identification of plant volatiles (from leaves or fruits) can provide vital clues to understanding of the tritrophic interactions between tomato genotypes, *H. armigera* and its key egg parasitoid, *T. chilonis*.

The chemical composition of fruit volatiles of different tomato genotypes revealed the presence of 16 fractions (Table 4). The matching quality of these compounds was above 90 per cent. The identified volatile fractions comprised alkane hydrocarbons (7), monoterpenes (3), sesquiterpene (1), diterpene (2) and other metabolites (3). Among the alkane hydrocarbons, two compounds, *i.e.*, pentadecane and heptadecane were most common and identified in all the genotypes studied, followed by octadecane (9), n-tetradecane (7) and hexadecane (6). Eicosane was identified from three genotypes-Arka Ashish, Arka Ahuti and Arka Saurabh, and tridecane from Ramya. The monoterpene fractions of fruit volatiles included cis-linalool oxide, linalool-L and trans-linalool oxide, which were found in 7, 8 and 5 genotypes, respectively. Cembrene (monocyclic diterpene) was isolated from 7 genotypes. (Z)-β-farnesene (sesquiterpene) and tridecane were observed only in the volatiles of genotype Ramya, and a flavouring compound 6-methyl-5-hepten-2-one from Arka Ashish and (E, E)-7,11,15-trimethyl-3-methylen in A. Ashish and Pusa Ruby. El-Sayed (2006) reported several species of natural enemies including *Aphidius ervi* (Braconidae), *Megarhyssa nortoni nortoni* and *Mastrus ridibundus* (Ichneumonidae) utilizing 6-methyl-5-hepten-2-one in their chemical communication system. However, if the volatile fractions are considered in terms of percentage of total volatiles, linalool–L present in the fruits varied from 1.79 (Pusa Ruby) to 91.12 (Arka Abha). Similarly, heptadecane, prevalent in all the genotypes, constituted from 2.89 per cent (Arka Saurabh) to 62.9 per cent (Varalakshmi). Regarding volatile diversity in these genotypes, Arka Ashish, Ramya and Pusa Ruby were very rich having 12 fractions each, while Arka Vikas was pure having only 4 fractions.

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