The Pharma Innovation Journal 2022; 11(2): 333-337

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; 11(2): 333-337

www.thepharmajournal.com
Received: 22-11-2021
Accepted: 30-01-2022

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Behavioral responses of *Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae) to synthetic Herbivory induced plant volatile's

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Abstract
Insect pests and their natural enemies utilize the volatile semiochemicals emitted from their hosts or from food plants infested by their hosts. Volatile compounds play a major role in the plant defense mechanisms against various insect pests. This study was conducted to test the behavioral responses of egg parasitoid *Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae) to synthetic herbivory induced plant volatiles, singly, and as combinations used in a Y-tube olfactometer bioassay. This study suggests that the highest significant responses of *T. chilonis* Ishii male and female were shown to Cis-hexanol and Cis-3- hexenyl acetate at 5 ppm and 10, when tested singly, over the control. Although, methyl salicylate (MeSA) at all concentrations tested, failed to elicit significant responses over the control in both sexes of *T. chilonis* Ishii. Interestingly, among the different combinations of synthetic herbivory induced plant volatiles higher responses of *T. chilonis* Ishii male and female over the control were recorded from methyl salicylate (MeSA) + β-caryophyllene + Cis-hexanol (1:1:1) at all concentrations tested (*viz.*,1, 5, 10 and 15 ppm). Behavioral study of *T. chilonis* Ishii has manifested the differential sensitivity to synthetic herbivory induced plant volatiles and different doses of these compounds.

Keywords: Egg parasitoid, *Trichogramma chilonis* Ishii, Semiochemicals, Y-tube olfactometer

Introduction
Many *Trichogramma* specieses are generalized egg parasitoid with a broad host range including Lepidoptera, Diptera, Coleoptera, Hymenoptera, Neuroptera and Megaloptera and can destroy up to 98% of the host eggs (Mansfield and Mill, 2004) [10]. Interestingly, about 26 trichogrammatids are recorded in India, of which *Trichogramma chilonis* Ishii is of significant importance (Tyagi and Khan, 1993) [20]. Egg parasitoid *Trichogramma chilonis* Ishii play a pivotal role in agro-ecosystem to contain insect pest's problems in various agriculture crops viz. cotton, okra, brinjal, maize, tomato, sugarcane etc. Studies have shown that predators, parasitoids, and predatory mites locate their hosts by using volatile semiochemicals emitted from their hosts or from food plants infested by their hosts (Noldus, 1989; Vet and Dicke, 1992; Bernasconi *et al*., 1998; Dicke *et al*., 1998; De Moraes *et al*., 1998; Du *et al*., 1998; Ninkovic *et al*., 2001; Van den Boom *et al*., 2004; De Boer and Dicke, 2004; Ishwari *et al*., 2007; Shimoda, 2010) [14, 22, 1, 4, 3, 5, 13, 21, 2, 6, 19]. Interestingly, despite the possible importance of HIPV's for host location by egg parasitoids, few studies have directly addressed this subject (Reddy *et al*., 2002; Lou *et al*., 2005; Manrique *et al*., 2005; Moraes *et al*., 2005) [11, 8, 9, 11]. Also, little is known about the capacity of egg parasitoids to learn HIPV's for host location (Mumm *et al*., 2005; Schroder *et al*., 2008) [12, 17]. In the last few decades, there has been an increased interest in using these compounds to manipulate natural enemy behaviors to enhance biological control of insect pests in agricultural crops (James, 2003) [7]. Management of insect pests by natural enemies (predators and parasitoids) is environment friendly and economical. Hence, site specific utilization of HIPV's can play a pivotal role to direct target specific movement of natural enemies from their natural reservoirs.

Methods and Materials
Egg parasitoid *Trichogramma chilonis* Ishii culture was maintained at I.A.R.I., New Delhi under laboratory conditions (28±1 ^{0}C,60 ± 5% relative humidity, 16L:8D photoperiod,) on its factitious host (*Corcyra cephalonica*).

Y-tube olfactometer made up of transparent Plexiglas (3 cm ID; stem 10 cm, arms 8 cm; stem-arms angle 130º) with each arm connected to a glass tube holding the odor source was used to record the behavioural responses of *Trichogramma chilonis* Ishii. During the experimentation filtered air (30 ml/min) was drawn through the olfactometer by a pump. Synthetic HIPV's were selected on the basis of literature accounts suggesting that each might have some attractive properties to some natural enemies in different agro-ecosystems to find out their possible effects on *Trichogramma chilonis* Ishii. Test compounds were obtained from commercial sources (Table 1). Selected synthetic compounds and their different combinations in 1:1 ratio were dissolved in a solvent (hexane) at different concentrations viz. 1 ppm, 5ppm, 10 ppm and 15 ppm. Whatman filter paper (2 × 2 cm) impregnated with 10 µl of test solution, left to dry for 10s, and introduced into one of the glass tube was used as the odor source. On the other arm of olfactometer, glass tube held untreated filter paper (control) of the same size. The olfactometer was disconnected from the glass tube and thoroughly washed with water, rinsed in 70% ethanol, and dried in an oven at 120ºC after every three runs. The apparatus was rotated 180˚ after five runs to exclude directional bias. Time to respond for *Trichogramma chilonis* Ishii was standardized to record maximum activity for the test compound. Male and Female of *Trichogramma chilonis* Ishii were allowed for 10 minutes to respond to the test compound and each insect was used only once. Behavioral responses of both sexes were considered positive when insects traveled at least 4 cm along arm connected to test compounds. All experiments were conducted in day light at28±2ºC and 60% relative humidity.

Table 1: Details of synthetic HIPV's used in the present study

S.N.	Compound	Purity	Supplier
1	Methyl salicylate	> 99%	Sigma- Aldrich
2	Cis-hexanol	> 98 %	Sigma- Aldrich
3	β- caryophyllene	> 80%	Sigma- Aldrich
4	D-Limonene	97%	Sigma- Aldrich
5	Cis-3-Hexenyl acetate	≥98%	Sigma- Aldrich

Results

Preliminary Y-tube olfactometer studies of *Trichogramma chilonis* Ishii indicated that hexane did not elicit any response from *Trichogramma chilonis* Ishii. The responses of *T. chilonis* Ishii to synthetic HIPV's are presented in Table 2. Males of *T. chilonis* Ishii preferred cis- Hexenol (5ppm, 10 ppm, and 15 ppm), Cis-3-hexenyl acetate (5ppm, 10 ppm) and D-Limonene (5 ppm) and gave significant choices over the control, while no significant preference was shown for methyl salicylate (MeSA) and β-caryophyllene at any of concentration (viz. 1ppm, 5 ppm, 10ppm and 15 ppm) under investigation. Highest significant responses of *T. chilonis* Ishii male were shown to Cis-hexanol at 5ppm (N = 25, P= 0.001) and 10 ppm (N= 25, P= 0.001), followed by Cis-3- hexenyl acetate at 5 ppm (N = 25, P= 0.001) over the control, respectively (Table 2, fig. 1). Similarly, significantly higher preference of *T. chilonis* Ishii female was shown to Cis-hexanol at 5 ppm (N= 25, P= <0.0001) and 10 ppm (N= 25, P= 0.006), whereas non significant responses were recorded from 1 ppm (N= 25, P= 0.07) and 15 ppm (N= 25, P= 0.07) concentration of Cis-hexanol over the control, respectively. Interestingly, *T. chilonis* Ishii female preference to all concentrations of Cis-3-hexenyl acetate viz. 1 ppm (N= 25, P=0.024), 5ppm (N= 25, P= 0.001), 10 ppm (N= 25, P= 0.0001) and 15 ppm (N= 25, P= 0.024) respectively, was significantly higher over the control. Although very fewer responses were recorded from D-limonene (5 ppm) and β-caryophyllene (1ppm) over the control. Methyl salicylate (MeSA) at all concentrations tested, failed to elicit significant responses over the control in both of sexes *T. chilonis* Ishii. Similarly, D-limonene (at 1, 10 and 15 ppm), Cis-hexanol (1ppm) and β-caryophyllene (5, 10 and 15 ppm) also failed to elicit significant responses in both sexes of *T. chilonis* Ishii. While, Cis-3- hexenyl acetate (1 and 15 ppm) and β-caryophyllene (1ppm) failed to elicit significant responses in *T. chilonis* Ishii male but significant responses over control, were recorded in *T. chilonis* female. The responses of *T. chilonis* Ishii male and female to different combinations of synthetic HIPVs are presented in Table 3. Significantly higher responses of *T. chilonis* male were recorded from methyl salicylate (MeSA) + β-caryophyllene + Cis-hexanol (1:1:1) at 1 ppm (N = 25, P = 0.02), 5 ppm (N = 25, P = 0.02), 10 ppm (N = 25, P = 0.001) 15 ppm (N = 25, P = 0.001), respectively over the control followed by the combination of β-caryophyllene + Cis-hexanol (1:1), 5 ppm (N = 25, P = 0.024), 10 ppm (N= 25, P = 0.006) and 15 ppm (N =25, P = 0.006), respectively. Whereas, preference of *T. chilonis* male to the combination of methyl salicylate (MeSA) + β-caryophyllene (1:1) and methyl salicylate + Cis-hexanol (1:1) at all concentrations tested was not significantly different from the control (Table: 3, fig. 2). Similarly, *T. chilonis* Ishii female preference was significantly higher to the combination of methyl salicylate (MeSA) + β-caryophyllene + Cis-hexanol (1:1:1) at 5 ppm (N = 25, P = 0.001), 10 ppm (N = 25, P = 0.001) 15 ppm (N = 25, P = 0.001) respectively, followed by the combination of β-caryophyllene + Cis-hexanol (1:1), 5 ppm (N = 25, P = 0.006), 10 ppm (N= 25, P = 0.001) and 15 ppm (N =25, P = 0.0001), respectively. Conversely, the combination of methyl salicylate (MeSA) + β-caryophyllene (1:1) and methyl salicylate + Cis-hexanol (1:1) at all concentrations tested not attracted significantly higher *T. chilonis* Ishii female over control (Table: 3, fig. 2). Similarly, β-caryophyllene + Cis-hexanol (1:1) at 1 ppm also failed to elicit significant responses over the control in both sexes of *T. chilonis* Ishii. Present findings conform to those made by Reddy *et al*. (2002) who found egg parasitoid *T. chilonis* Ishii was highly attracted by hexenyl-acetate. Likewise, Vijaya *et al.* (2018) reported Ethyl acetate released from *Spodoptera litura* damaged Chilli, *Capsicum annuum* L. plants attracted higher numbers of egg parasitoid, *Trichogramma chilonis* Ishii in behavioral bioassays. Our findings are in close agreement with Peñaflor *et al*. (2011) who reported that the generalist egg parasitoid *T. pretiosum* was attracted by (Z)-3-hexenal, (E)-2-hexenal, (E)-3-hexen-1-ol, and (Z)-3 hexenyl acetate and a blend of aromatic compounds, monoterpenes and homoterpenes (3E)-4,8-dimetil-1,3,7- nonatriene (DMNT), respectively. Likewise, Sen *et al.* (2005) reported significantly higher EAG responses in *T. chilonis* Ishii females with citronellal, phytol, caryophyllene, R-(+)-limonene, linalool, carvacrol and citronellol while *T. chilonis* Ishii males responded to citronellol, caryophyllene, linalool, R-(+)-limonene and amyl acetate. In summary, our results show that the highest significant responses of *T. chilonis* Ishii male were shown to Cis-hexanol and Cis-3- hexenyl acetate at 5 ppm and 10 ppm over the control, respectively. Whereas, *T.*

chilonis Ishii female showed higher preference to Cis-hexanol at 5 ppm and 10 ppm and Cis-3- hexenyl acetate at 1ppm, 5 ppm 10 ppm and 15 ppm. Although, methyl salicylate (MeSA) at all concentrations tested, failed to elicit significant responses over the control in both of sexes *T. chilonis* Ishii.

Interestingly, among the different combinations of HIPV's higher responses of *T. chilonis* Ishii male and female over the control were recorded from methyl salicylate (MeSA) + β-caryophyllene + Cis-hexanol (1:1:1) at all concentrations tested (*viz.*,1, 5, 10 and 15 ppm).

Table 2: Behavioral responses of *Trichogramma chilonis* Ishii in Y-tube olfactometer to synthetic HIPVs.

Synthetic HIPVs	Concentration	Male response (mean ±SE)				Female response (mean ±SE)			
		N	**Source**	**Control**	**P value**	***N***	**Source**	**Control**	**P value**
Methyl salicylate	1ppm	25	11±0.10^{a}	14±0.10^{a}	0.559	25	9±0.09^{a}	16±0.09^{a}	0.165
	5ppm	25	12±0.11^{a}	13±0.11^{a}	0.846	25	13±0.1^{a}	12±0.1^{a}	0.846
	10ppm	25	15±0.11^{a}	10±0.11^{a}	0.327	25	14±0.10^{a}	11±0.10^{a}	0.559
	15ppm	25	10±0.10^{a}	15±0.10^{a}	0.327	25	10±0.1^{a}	15±0.1^{a}	0.327
Limonene	1ppm	25	13±0.10^{a}	12±0.10^{a}	0.846	25	13±0.10^{a}	12±0.10^{a}	0.07
	5ppm	25	18±0.09^{a}	07±0.09^{b}	0.024	25	18±0.09^{a}	7±0.09^{b}	0.024
	10ppm	25	15±0.1^{a}	10±0.1^{a}	0.327	25	17±0.1^{a}	8±0.1^{a}	0.07
	15ppm	25	13±0.1^{a}	12±0.1^{a}	0.85	25	17±0.1^{a}	8±0.1^{a}	0.07
Cis-3-Hexenyl acetate	1ppm	25	17±0.1^{a}	08±0.1^{a}	0.07	25	18±0.09^{a}	7±0.09^{b}	0.024
	5ppm	25	18±0.09^{a}	07±0.09^{b}	0.024	25	20±0.08^{a}	5±0.08^{b}	0.001
	10ppm	25	20±0.08^{a}	05±0.08^{b}	0.001	25	21±0.07^{a}	4±0.07^{b}	0.0001
	15ppm	25	17±0.09^{a}	08±0.09^{a}	0.07	25	18±0.09^{a}	7±0.09^{b}	0.024
Cis-hexanol	1ppm	25	17±0.1^{a}	8±0.1^{a}	0.071	25	17±0.1^{a}	8±0.1^{a}	0.07
	5ppm	25	21±0.7^{a}	4±0.7^{b}	0.001	25	22±0.07^{a}	3±0.07^{b}	<0.0001
	10ppm	25	21±0.7^{a}	4±0.7^{b}	0.001	25	19±0.09^{a}	6±0.09^{b}	0.006
	15ppm	25	18±0.7^{a}	7±0.7^{b}	0.02	25	17±0.1^{a}	8±0.1^{a}	0.07
β- caryophyllene	1ppm	25	16±0.1^{a}	9±0.1^{a}	0.165	25	19±0.09^{a}	6±0.09^{b}	0.006
	5ppm	25	17±0.7^{a}	8±0.7^{a}	0.07	25	17±0.1^{a}	8±0.1^{a}	0.07
	10ppm	25	12±0.1^{a}	13±0.1^{a}	0.846	25	14±0.10^{a}	11±0.10^{a}	0.559
	15ppm	25	11±0.1^{a}	14±0.1^{a}	0.559	25	12±0.10^{a}	13±0.10^{a}	0.846

* For each odour source, different letters indicate significant differences from control (Paired t test).

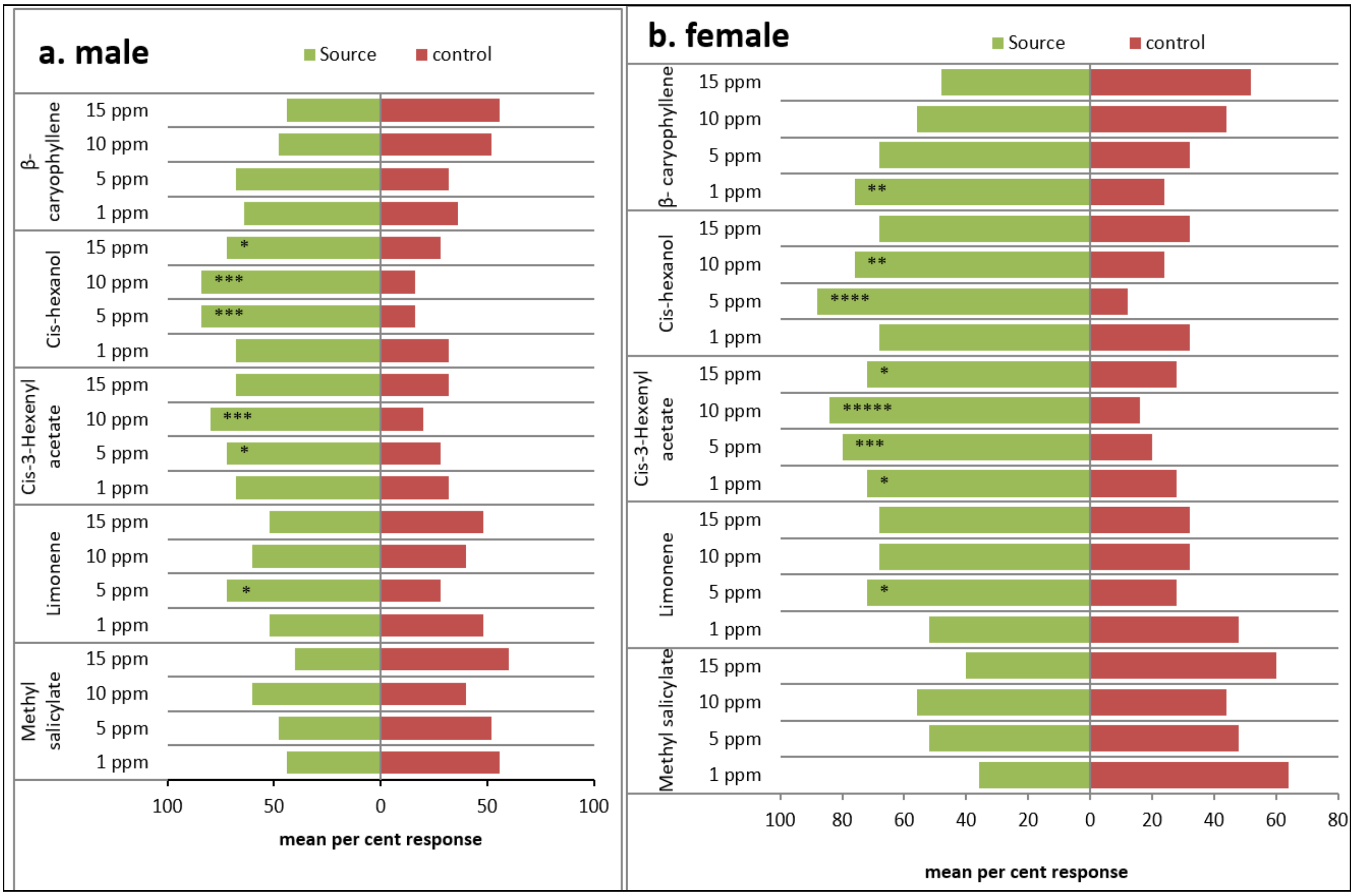

Fig 1: Behavioral responses (mean per cent response) of *Trichogramma chilonis* Ishii (a) male and (b) female to synthetic HIPVs in Y-tube olfactometer. Asterisks indicate significant differences over the control (Paired t test: N = 25, *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$, ****$P \leq 0.0001$).

Table 3: Behavioral responses of *Trichogramma chilonis* Ishii in Y-tube olfactometer to different combinations of synthetic HIPVs.

Synthetic HIPVs	Concentration	Male response (mean ±SE)				Female response (mean ±SE)			
		N	Source	Control	P value	*N*	Source	Control	P value
β- caryophyllene + Cis-hexanol (1:1)	1ppm	25	14 ± 0.10^{a}	11 ± 0.10^{a}	0.559	25	16 ± 0.1^{a}	9 ± 0.1^{a}	0.165
	5ppm	25	18 ± 0.09^{a}	07 ± 0.09^{b}	0.024	25	19 ± 0.09^{a}	6 ± 0.09^{b}	0.006
	10ppm	25	19 ± 0.09^{a}	6 ± 0.09^{b}	0.006	25	20 ± 0.08^{a}	5 ± 0.08^{b}	0.001
	15ppm	25	19 ± 0.09^{a}	6 ± 0.09^{b}	0.006	25	21 ± 0.07^{a}	4 ± 0.07^{b}	0.0001
Methyl salicylate+ Cis-hexanol (1:1)	1ppm	25	16 ± 0.1^{a}	9 ± 0.1^{a}	0.165	25	17 ± 0.09^{a}	08 ± 0.09^{a}	0.07
	5ppm	25	15 ± 0.1^{a}	10 ± 0.1^{a}	0.327	25	15 ± 0.1^{a}	10 ± 0.1^{a}	0.327
	10ppm	25	16 ± 0.1^{a}	9 ± 0.1^{a}	0.165	25	16 ± 0.1^{a}	9 ± 0.1^{a}	0.165
	15ppm	25	17 ± 0.1^{a}	08 ± 0.1^{a}	0.07	25	16 ± 0.1^{a}	9 ± 0.1^{a}	0.165
Methyl salicylate+ β- caryophyllene (1:1)	1ppm	25	11 ± 0.1^{a}	14 ± 0.1^{a}	0.559	25	12 ± 0.10^{a}	13 ± 0.10^{a}	0.846
	5ppm	25	10 ± 0.1^{a}	15 ± 0.1^{a}	0.327	25	13 ± 0.10^{a}	12 ± 0.10^{a}	0.846
	10ppm	25	13 ± 0.10^{a}	12 ± 0.10^{a}	0.846	25	15 ± 0.1^{a}	10 ± 0.1^{a}	0.327
	15ppm	25	14 ± 0.10^{a}	11 ± 0.10^{a}	0.559	25	16 ± 0.1^{a}	9 ± 0.1^{a}	0.165
β- caryophyllene + Cis-hexanol + Methyl salicylate (1:1:1)	1ppm	25	18 ± 0.7^{a}	7 ± 0.7^{b}	0.02	25	17 ± 0.1^{a}	8 ± 0.1^{a}	0.07
	5ppm	25	18 ± 0.7^{a}	7 ± 0.7^{b}	0.02	25	20 ± 0.08^{a}	05 ± 0.08^{b}	0.001
	10ppm	25	21 ± 0.7^{a}	4 ± 0.7^{b}	0.001	25	21 ± 0.7^{a}	4 ± 0.7^{b}	0.001
	15ppm	25	20 ± 0.08^{a}	05 ± 0.08^{b}	0.001	25	21 ± 0.7^{a}	4 ± 0.7^{b}	0.001

* For each odour source, different letters indicate significant differences from control (Paired t test).

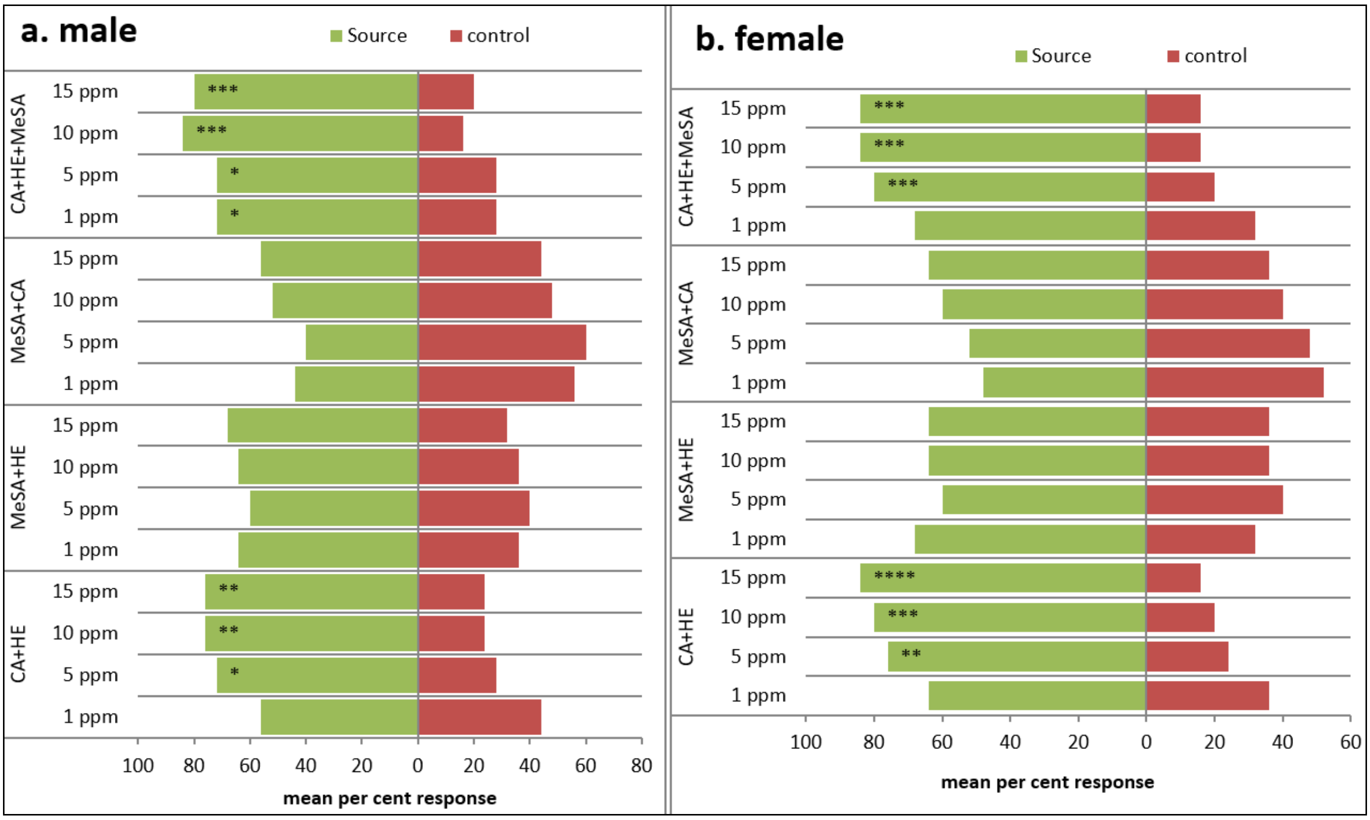

Fig 2: Behavioral responses (mean per cent response) of *Trichogramma chilonis* Ishii (a) male and (b) female to different combinations of synthetic HIPVs in Y-tube olfactometer. Asterisks indicate significant differences over the control (Paired t test: N = 25, **P*≤ 0.05, ***P*≤ 0.01, ****P*≤ 0.001, *****P*≤0.0001). Code for treatments: CA+HE, β- caryophyllene + Cis-hexanol (1:1); MeSA+HE, Methyl salicylate+ Cis-hexanol (1:1); MeSA+CA, Methyl salicylate+ β- caryophyllene (1:1); CA+HE +MeSA, β- caryophyllene + Cis-hexanol + Methyl salicylate (1:1:1).

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