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Role of rice plant volatiles on the orientation of leaf folder larval parasitoids, *Trichomma cnaphalocrocis* (Uchida) (Ichneumonidae:Hymenoptera) and *Cotesia angustibasis* (Gahan) (Braconidae: Hymenoptera)

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ABSTRACT

Orientation of the hymenopteran parasitoids to the volatiles emanating from leaf folder damaged and healthy rice plants of resistant Ptb 33, moderately resistant IR 72 and highly susceptible TN1 rice was studied under laboratory condition using multichoice glass olfactometer. Orientation of the T. cnaphalocrocis and C. angustibasis were more to the leaf folder damaged leaves than to the healthy leaves of Ptb 33, IR 72 and TN1. However, maximum number of visits was to the leaf folder damaged TN 1 leaves when compared to the resistant Ptb 33 and moderately resistant IR 72 and they differed significantly among themselves. In the GC-MS studies on volatile profile, arrays of volatile compounds have been identified. The leaf folder damaged plants of resistant and moderately resistant emitted less number of volatile compounds, while highly susceptible emitted more volatile compounds. Particularly, the volatile Docosane, was more in highly susceptible genotype TN 1 when compared to resistant Ptb 33 and moderately resistant IR 72 genotypes. It could be responsible in the attraction of T. cnaphalocrocis and C. angustibasis.

Keywords: *rice leaf folder, herbivore-induced plant volatiles, Cotesia angustibasis, Trichomma cnaphalocrocis*

Plant defense against herbivores are not limited to physical and chemical barriers that directly aim to harm their attackers, it is becoming increasingly evident that plants also imply strategies of indirect defense to attract predators and parasitoids by signaling. Many parasitoids have a keen ability to respond to a specific odour by associating the odour with hosts or host faeces (Lewis and Tumlinson, 1988; Vet and Groenewold, 1990; Turlings *et al*., 1993).

This attraction of the third trophic level is one of the presumed functions of herbivore induced plant volatiles(HIPVs), which are released more or less specifically in response to herbivore attack (Turlings and Ton, 2006). The volatile-mediated interactions between the plants, herbivores and natural enemies has received increasing attention world wide in last two decades due to improved crop protection against pests and diseases. It is an integral part of plant defense system and is present in many plant species including rice.

Rice plant volatiles can attract the natural enemies (*Anagrus nilaparvatae* Pang et Wang, *Cyrtorhinus lividipennis* Reuter) of the brown planthopper (BPH) *Nilaparvata lugens* (Stal.), which is one of the most important pests of rice world wide. This attraction effect of rice plant volatiles was significantly increased when rice stems were infested by BPH (Liu *et al*., 2006). Several authors reported on the attraction of *A. nilaparvatae* to the volatiles emitted from rice plant that were treated with jasmonic acid (Lou *et al*.,2005), *Spodoptera litura* infested rice leaves (Xu *et al.,* 2002) and induced volatile emissions among rice varieties (Lou *et a*l., 2006).

Thus, it could be proposed that herbivore-induced volatiles on natural enemy's attraction can be exploited in rice leaffolder management. Rice ecosystem maintains a pool of natural enemies. Nearly 61 parasitoids and six predators have been recorded on rice leaffolder with varying level of impact. In the present study, we investigated the volatile profile from leaffolder damaged and healthy rice plants of resistant Ptb 33, moderately resistant IR 72 and highly susceptible TN1 and their influence on the attraction of larval parasitoids, *Trichomma cnaphalocrocis* (Uchida) and *Cotesia angustibasis* (Gahan).

MATERIALS AND METHODS

Five centimeter long leaves were cut from the top third leaf of 50 day old plant of Ptb 33, IR 72 and TN 1. One set of leaves from each test genotype was maintained healthy and in another set leaffolder larvae instar confined for 6h. These leaves were arranged randomly in a multi choice glass olfactometer. Three cavities had seven healthy leaves alone of test genotypes and another three cavities had damaged leaves along with leaffolder larvae. After 2 hr, fifty newly emerged *Trichoma cnaphalocrocis* adults were released at the centre of the olfactometer. The number of visits made by the adult parasitoids was recorded for 2 hr and 6 hr after release to each cavity. Orientation of larval parasitoids was measured in the terms of the number of visits made by the adult parasitoids. Volatiles that emanated from leaffolder damaged and healthy plants of rice genotypes *viz*., Ptb 33, IR 72 and TN 1 were collected and identified using GC-MS as described by Xu *et al.,* (2005).

Ten rice plants of test genotype were placed in a 2000 ml long necked flat bottom flask and was closed by a double holed rubber cork. An L-shaped glass tube packed with activated charcoal was inserted in each hole and served as inlet and outlet. Air stream generated by an aerator was passed through inlet, circulated in the flat bottom flask and passed out through the outlet for 6 hr. The volatiles emanated were collected in the activated charcoal placed at the outlet. The activated charcoal placed at the outlet was washed with n-hexane, filtered with 0.25µm membrane filter and 1µl was injected into the GC-MS instrument (Perkin Elmer, Clarus 500). For collecting volatiles from the leaf folder damaged plant, six starved larvae were confined to ten plants for 2hr after which the volatiles were collected for 6 hr. Three replications were maintained.

The GC-MS system was equipped by Perkin Elmer, Clarus 500. The conditions were set as follows: Elect 1 column; temperature programmed from 60°C for 0.00 min; flow rate 1ml min^{-1} of helium. The MS method was EI+ ionization mode with 70eV Voltage; scan range 50-300 Mass (m/z), scan time 0.2 sec, and inter-scan delay 0.1 sec, Quaterapole mass analyzer. The individual compounds were identified by comparison of their mass spectra with those from NIST and the Wiley library of Mass spectral data base. The data on natural enemies were transformed into " X as per the method developed by Poisson for analysis. The data collected from the experiments were subjected to three factor FRBD analysis. The mean values of treatments were then separated by Duncan's multiple range test (DMRT).

RESULTS AND DISCUSSION

After two hours of release of *T. cnaphalocrocis*, the number of visits was minimum to the Ptb 33 healthy leaves (4.00) followed by IR 72 healthy leaves (5.33) and Ptb 33 infested leaves (5.33). Maximum number of visits was recorded to the TN 1 infested leaves (8.67) followed by TN 1 healthy leaves (7.66) (Table 1).

Table 1. Orientation of *Trichomma cnaphalocrocis* to the healthy and leaf folder damaged leaves of test genotypes

Genotypes	Number of visits made treatment^{-1}			
	2 hr		6 hr	
	Healthy leaves	Leaf folder damaged leaves	Healthy leaves	Leaf folder damaged leaves
Ptb 33	4.00 (2.00)	5.33 (2.30)	5.00 (2.23)	6.67 (2.51)
IR 72	5.33 (2.30)	6.67 (2.51)	7.33 (2.70)	8.33 (2.88)
TN 1	7.66 (2.70)	8.67 (2.58)	8.67 (2.88)	12.66 (3.55)

Mean of six replications
Figures in parentheses are square root transformation

	SED	CD(0.05)
Genotypes	0.04	0.08**
Leaves	0.03	0.06**
Time	0.03	0.06**
Genotypes x leaves	0.05	0.11 NS
Leaves x time	0.04	0.09**
Genotypes x time	0.05	0.11**
Genotypes x leaves x time	0.08	0.16**

DMRT analysis

Genotypes	Means
G1	5.16^{c}
G2	6.83^{b}
G3	8.75^{a}

Similarly after six hours of release, more number of visits was to the leaf folder infested leaves than to the healthy leaves. The number of visits was maximum to TN1 infested leaves (12.66) and minimum to Ptb 33 healthy leaves (5.00). In general the number of visits made by the adult parasitoid was more towards the infested leaves than to healthy leaves irrespective of the levels of resistance in genotypes. The number of visits was maximum to TN 1 infested leaves and was nearly 2.5 times greater than the Ptb 33 infested leaves.

After two hours of adults braconids release, the number of visits was minimum to the Ptb 33 healthy leaves (4.67) followed by Ptb 33 infested leaves (5.67). Maximum number of visits was recorded to the TN 1 infested leaves (9.33) followed by TN 1 healthy leaves (8.67) (Table 2). At six hours after release, the number of visits was maximum to TN1 infested leaves (19.66) and minimum to Ptb 33 healthy leaves (9.00). In general the number of visits made by the adult parasitoid was more to the infested leaves than to healthy leaves. The number of visits was maximum to TN 1 infested leaves and was nearly two times greater than the Ptb 33 infested leaves.

Table 2. Orientation of *Cotesia angustibasis* to the healthy and leaf folder damaged leaves of test genotypes.

Genotypes	Number of visits made treatment^{-1}			
	2 hr		6 hr	
	Healthy leaves	Leaf folder damaged leaves	Healthy leaves	Leaf folder damaged leaves
Ptb 33	4.67 (2.15)	5.67 (2.37)	9.00 (3.46)	11.00 (4.28)
IR 72	6.33 (2.99)	8.67 (3.31)	12.00 (2.94)	18.34 (3.05)
TN 1	8.67 (2.51)	9.33 (2.94)	16.00 (4.00)	19.66 (4.43)

Mean of six replications
Figures in parentheses are square root transformation

	SED	CD(0.05)
Genotypes	0.04	0.09**
Leaves	0.03	0.07**
Time	0.03	0.07**
Genotypes x leaves	0.06	0.13**
Leaves x time	0.05	0.10**
Genotypes x time	0.06	0.13**
Genotypes x leaves x time	0.09	0.18 NS

DMRT analysis

Genotypes	Means
G1	7.58^{c}
G2	11.33^{b}
G3	13.41^{a}

Five compounds were identified in healthy Ptb 33 namely 5-methyl heptadelane, 1-Tetradecanol, Decane, Octadecane and Docosane. In leaffolder damaged Ptb 33 four compounds were identified *viz.,* Decane, Trans-Asarone, Octadecane and Docosane. Decane, Octadecane and Docosane compounds were found both in healthy and leaf folder damaged Ptb 33 plants. However, Trans-asarone was found in leaf folder damaged plants (Table 3).

Healthy plants had three volatile compounds *viz*., Octadecane, Docosane and Tetrapentacosane and leaf folder damaged plants three volatile compounds *viz*., Decane, Octadecane, and Docosane (Table 4). Octadecane and Docasane compound was found in healthy and leaf folder damaged plants. Tetrapentacosane was found only in healthy plants. Decane was found only in leaf folder damaged plants (Table 4).

Healthy TN 1 plants had eight volatile compounds *viz*., Cyclopentane, 1-Hexadecane, pentadecane, Trans-asarone, 3-methyl pentacosane, Octadecane, 4-phenylisocoumarin and Diisooctyl-phthalate were recorded. In leaf folder damaged TN 1 had three volatile compounds *viz.,* Decane, Docosane and Octadecane were recorded.

Octadecane compound was found in healthy and leaf folder damaged TN 1 plants. Whereas, Pentadecane was found only in healthy plants. While Docasane and Decane were found only in leaf folder damaged plants. Cyclopentane, 1-Hexadecane, trans-asarone, 3-methyl pentacosane, 4-phenylisocoumarin and Diisooctyl-phthalate were found in healthy plants (Table 5).

Orientation of the Ichneumonids and Braconids adults towards healthy and leaf folder damaged leaves of Ptb 33, IR 72 and TN 1 revealed that visits were maximum to the leaffolder damaged leaves than to the healthy leaves. Similarly, maximum number of visits was to the healthy and leaffolder damaged TN 1 leaves

Table 3. Volatile profile of the healthy and leaf folder damaged plants of resistant Ptb 33

Sl. No.	Healthy plants			Leaf folder damaged plants		
	RT*	Area	Name	RT	Area	Name
1.	5.19	-	5- methylheptadecane	7.07	-	Decane
2.	5.50	-	1-Tetradecanol	11.90	207212	Trans-asarone
3.	7.07	-	Decane	13.86	340036	Octadecane
4.	11.4	372536	Octadecane	16.03	246103	Docosane
5.	13.85	-	Docosane	-	-	-

*RT - Retention time

when compared to the resistant Ptb 33 and moderately resistant IR 72. Lou *et al.* (2005) too demonstrated the attraction of *A. nilaparvatae to* volatiles released from *Nilaparvata lugens* infested plants, whereas there was no attraction to volatiles from undamaged plants and artificially damaged plants.

The volatile profile of healthy and herbivore damaged plants of resistant Ptb 33, moderately resistant IR 72 and susceptible TN 1 showed variation. Docosane compound was more in leaf folder damaged plants of Ptb 33 compared to healthy plants. Docosane compound was high in leaf folder damaged plants of IR 72 compared to healthy plant. In susceptible TN 1 the docosane concentration was maximum in leaf folder damaged plants and absent in healthy plants. Thus docosane from leaf folder damaged leaves irrespective of the level of resistance possibly have acted as an attractant to the parasitoids. In addition, the compound decane in higher concentration was present in leaf

Table 4. Volatile profile of the healthy and leaf folder damaged plants of moderately resistant IR 72

Sl. No.	Healthy plants			Leaf folder damaged plants		
	RT*	Area	Name	RT	Area	Name
1.	10.97	2121810	Octadecane	7.07	-	Decane
2.	13.87	1137126	Docosane	11.47	902841	Octadecane
3.	18.96	-	Tetrapentacosane	13.86	1198989	Docosane

*RT - Retention time

Xu *et al.* (2002) reported *Spodoptera litura* infested rice leaves released much higher amounts of volatiles than those in mechanical damaged and healthy plants. The contents of several green leaf volatiles (CE)-2-hexenal, (2)-3-hexen-1-01, (E)-2-hexen-1-01), methyl salicylate (MeSA) and terpenoids were increased dramatically in *S. litura* infested plants.

Table 5. Volatile profile of the healthy and leaf folder damaged plants of highly susceptible TN 1

Sl. No.	Healthy plants			Leaf folder damaged plants		
	RT*	Area	Name	RT	Area	Name
1.	5.44	-	Cyclopentane	7.55	1761346	Decane
2.	8.52	-	1-hexadecane	17.63	4977218	Docosane
3.	9.69	30944	Pentadecane	18.04	2478193	Octadecane
4.	11.89	242000	Trans-asarone	-	-	-
5.	13.19	-	3-methyl pentacosane	-	-	-
6.	13.84	77362	Octadecane	-	-	-
7.	16.82	-	4-phenylisocoumarin	-	-	-
8.	20.70	-	Diisooctyl-phthalate	-	-	-

*RT - Retention time

folder damaged TN 1 plants. Docosane as an attractant for the parasitoids have already been documented. Jones *et al.* (1973) reported docosane, tricosane, tetracosane, and pentacosane to increase parasitism by *Trichogramma* when applied to host eggs. Padmavathi and Paul (1998) found tricosane to be the best hydrocarbon for attracting *Trichogramma chilonis* followed by octacosane and docosane. Usharani *et al.* (2007) accounted yellow stem borer eggs treated with docosane, tetracosane, pentacosane, and eicosane enhanced host egg parasitization.

Thus to summarize, in the process of preliminary exploration of chemically mediated tritrophic interactions in rice under leaf folder herbivory, an array of volatile compounds have been identified. The leaffolder damaged plants of resistant and moderately resistant emitted less number of volatile compounds, while susceptible and highly susceptible emitted more volatile compounds. It was also evident that the leaffolder damaged leaves increased the parasitoid attraction *T. cnaphalocrocis* and *C. angustibasis*. Particularly, the volatile Docosane, was more in highly susceptible genotype when compared to resistant and moderately resistant genotypes. It could be responsible in the attraction of natural enemies as reported by earlier workers.

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