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## GCMS analysis of volatile organic compounds in cotton genotypes against whitefly, *Bemisia tabaci* (Gunnadius)

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### Abstract

Cotton (*Gossypium hirsutum* L.) production is seriously hampered by the infestation of the whitefly, *Bemisia tabaci*. The infestation behavior of the whiteflies could be affected by the quantity of plant released volatile organic compounds. In this study, we determined the presence of volatile organic compounds in different cotton genotypes which shows varying levels of resistance to *B. tabaci* using GC-MS analysis. Totally seventeen volatile compounds were identified in the cotton plants namely 3-octanol,  $\alpha$ -pinene, caryophyllene, camphene, humulene,  $\beta$ -pinene, nonadecane,  $\beta$ -asarone,  $\alpha$ -farnesene,  $\beta$ -bisabolene, 3-Cyclohexen-1-ol, 9-octadecanone, myrcene, methoxyacetic acid, eicosane, 9, 12, 15-octadecatrienoic acid and dibutyl phthalate. However,  $\alpha$ -farnesene,  $\beta$ -bisabolene and methoxyacetic acid were present only in resistant (LRA-5166, LHDP-1) and moderately resistant genotypes (GJHV-517), respectively. Therefore these compounds might be involved in repellent activity against whitefly. This finding can be used in future to identify *B. tabaci* repellents and attractants that could be used as tools of IPM of *B. tabaci* and other whitefly pests.

**Keywords:** cotton, *Bemisia tabaci*, GC-MS, resistant, susceptible

### Introduction

Whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), is a highly polyphagous pest that causes substantial losses to the global agricultural economy and has devastating effects on more than 700 agricultural crops and ornamental plant species<sup>[1, 2]</sup>. Whitefly contains species complexes of 37 distinct populations distributed worldwide<sup>[3, 4]</sup> with 24 different biotype populations<sup>[5, 6]</sup>. The high reproductive potential, small size, and high dispersal ability of this species are responsible for its pest status and it causes severe damage to plants both directly and indirectly. Direct damage occurs due to feeding of phloem sap by the nymphs and adults which intern affects plant growth and development. Indirect damage is due to 'excretion of honeydew' (sugar-rich sticky liquid) during the feeding process of whitefly which serves as a suitable environment for the growth of sooty mould fungi (*Capnodium* sp.; Cnapiodiaceae)<sup>[7]</sup>. This sooty mould forms a dark black layer over the leaves and inhibits sunlight penetration, affecting photosynthesis which leads to leaf senescence and premature leaf drop<sup>[5, 8]</sup>. Furthermore, it is an efficient vector of >100 plant viruses (Geminiviruses) for many economically important crops and is the sole vector of begomoviruses<sup>[9, 10]</sup>.

India is the second largest producer of cotton (*Gossypium hirsutum* L.) in the world after China<sup>[11]</sup>. The whitefly attack created crises in cotton production; an estimate of 75% of the crop was destroyed in the Northern zone of India<sup>[12, 13]</sup>. The management of *B. tabaci* in cotton is predominantly dependent on massive spraying of synthetic insecticides and it becomes difficult to control with insecticides because whitefly lives on the underside of the leaves and its shorter developmental period which creates an increased level of resistance to a wide range of insecticides (Organophosphate, Carbamate and Neonicotinoids) and resistant strains have become more and more abundant<sup>[14-17]</sup>. According to the Arthropod Pesticide Resistance Database, *B. tabaci* has evolved resistance to 64 compounds worldwide<sup>[18]</sup>. Moreover, chemical control affects non-target organisms and the entire ecosystem<sup>[19, 20]</sup>.

One of the pest management practices in the IPM principles is the use of resistant varieties, as this method can be combined with other environmentally friendly management techniques such as the use of biological pesticides or biological agents<sup>[21, 22]</sup>. Plants are naturally having the resistance mechanisms viz., antixenosis, antibiosis and tolerance against the insect attack<sup>[23]</sup>.

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Many studies have concentrated on the occurrence of different mechanisms of resistance including, antixenosis [24], antibiosis [25] and tolerance [26]. Our previous study confirmed the presence of different resistance mechanism in cotton genotypes [27]. Very few studies were concentrated on the volatile profile of cotton genotypes against whitefly. In this study, we have identified the volatile profile of susceptible and resistant cotton genotypes which sown varying levels of resistance to whitefly.

## Materials and Methods

### Cotton genotypes

The experiments were carried out under glasshouse conditions at the Insectary (11°00'58.1"N 76°55'45.5"E), Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore. Sixty-three cotton genotypes belonging to *Gossypium hirsutum* L. were initially screened under field conditions. From that the best performing 19 genotypes were selected for further screening in the glasshouse. Based on the damage symptoms [28] and whitefly count, six (Bunny, CCH-4474, GJHV-517, LHDP-1, LRA-5166, and TCH-1819) genotypes with different level of resistance were selected for evaluating the mechanisms of resistance. The seeds were procured from the Department of Cotton, Tamil Nadu Agricultural University, Coimbatore.

### GC-MS analysis of the compounds on leaf surface of cotton

#### Extraction of volatiles from leaf samples of cotton genotypes

Twenty gram of cotton leaf samples were immersed overnight in 20 ml of distilled hexane in separate conical flasks. The hexane was then filtered through Whatman No.1 filter paper. Anhydrous sodium sulphate @ 1 g / 10 g samples was added to the filtrate for dehydration for 2 h and passed through the silica gel (60-120 mesh) column. The hexane extract of host plant samples eluted through the column was then distilled at 60-70 °C. The left over residue was collected by rinsing the container with little quantity of HPLC grade hexane (Merck) and stored in separate vials in a deep freezer for GCMS analysis.

#### Analysis of volatile compounds using GC-MS

Chromatographic separation was carried out with GCMS-QP (Shimadzu, 2010) plus equipped with a capillary column RXI-IMS (30 m x 0.25 mm, 0.25 mm ID). Helium (99.99% pure) was used as a carrier gas with a flow rate of 0.98 ml / min. The oven temperature was programmed from 110 °C (isothermal for 2 min), with an increase of 10 to 200 °C / min, then 5 to 280 °C / min, ending with a 9 min isothermal at 280 °C. Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 40 to 550 Da.

1	Mode	:	Split (10:1)
2	Injector Temperature	:	250 <sup>o</sup>
3	Ion source Temperature	:	280 <sup>o</sup>
4	Interface Temperature	:	250 <sup>o</sup>
5	Solvent out time	:	4 min
6	Mass range	:	30-500 m / z

#### Identification of compounds

Injection was performed in split less mode (10:1) and the volume was 1 µl. The mass spectra of compounds in samples were obtained by ionization voltage at 70 eV and the detector was operated in scan mode from 500 amu. The chemical

constituents were identified by matching mass spectra of reference compounds in mass library of National Institute of Standards and Technology (NIST) version 2.0. The relative amounts of individual components were expressed as per cent peak areas relative to the total peak area.

## Results

### GC-MS profiles of volatile organic compounds in different cotton genotypes

The leaf extract of cotton plants were subjected to GC-MS analysis. GC-MS profiles of the cotton leaf surface chemicals revealed considerable differences among the cotton genotypes tested. About sixteen peaks were obtained with retention times viz., 4.490, 4.880, 7.555, 7.982, 9.520, 9.576, 9.813, 10.305, 10.425, 10.480, 10.599, 10.848, 11.807, 11.809, 13.215 and 14.316 in the GC analysis. Totally seventeen volatile compounds were identified in the cotton plants namely 3-octanol,  $\alpha$ -pinene, caryophyllene, camphene, humulene,  $\beta$ -pinene, nonadecane,  $\beta$ -asarone,  $\alpha$ -farnesene,  $\beta$ -bisabolene, 3-Cyclohexen-1-ol, 9-octadecanone, myrcene, methoxyacetic acid, eicosane, 9, 12, 15-octadecatrienoic acid and dibutyl phthalate (Table 1; Figure 1-6).

The compounds viz., 3-octanol,  $\alpha$ -pinene, 9-octadecanone, 9, 12, 15-octadecatrienoic acid and dibutyl phthalate are present in all the cotton genotypes. While  $\alpha$ -farnesene,  $\beta$ -bisabolene and methoxyacetic acid were present only in resistant (LRA-5166, LHDP-1) and moderately resistant genotypes (GJHV-517), respectively. The volatile compound, caryophyllene was present exclusively in GJHV-517, LRA-5166 and TCH-1819 however, the compound, camphene was present only in susceptible genotypes, Bunny and GJHV-517 but absent in all other genotypes. The compound, humulene was present only in resistant genotypes, LRA-5166, LHDP-1 and CCH-4474 whereas,  $\beta$ -pinene and 3-cyclohexen-1-ol was present in all the genotypes except in GJHV-517.

The compound, nonadecane was present only in CCH-4474 and GJHV-517 nevertheless  $\beta$ -acarose which was present in both resistant and susceptible genotypes. The compound, myrcene was present in Bunny, CCH-4474 and LHDP-1.

## Discussion

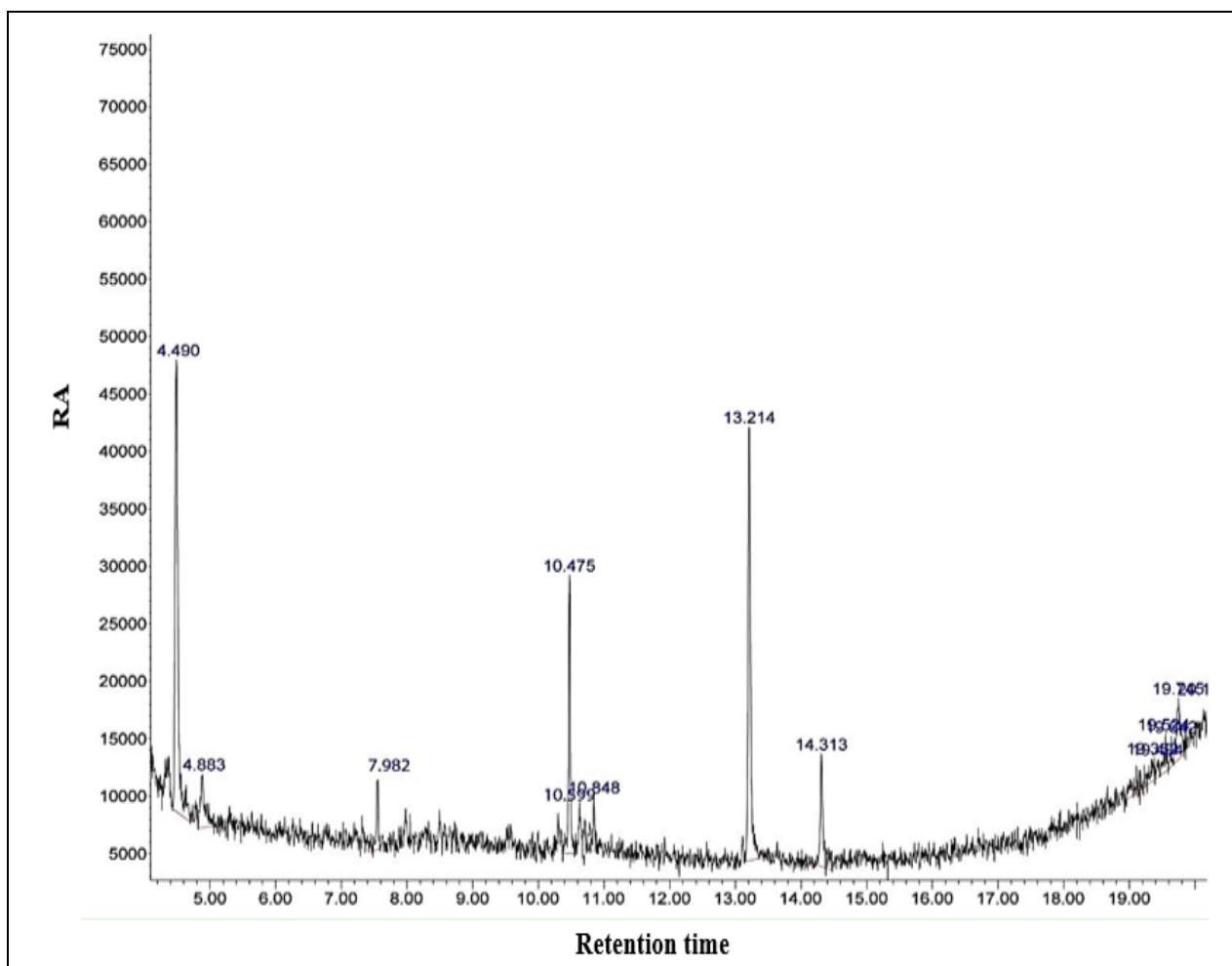
GC-MS profiles of cotton leaf surface chemicals revealed considerable differences among the cotton genotypes tested. Totally seventeen volatile compounds were identified in the cotton leaf extract using hexane. Out of seventeen compounds, five compounds viz., 3-octanol,  $\alpha$ -pinene, 9-octadecanone, 9, 12, 15-octadecatrienoic acid and dibutyl phthalate are present in all the cotton genotypes but the peak area percent was different. The peak area per cent of 3-octanol,  $\alpha$ -pinene,  $\beta$ -pinene, 9, 12, 15-octadecatrienoic acid and dibutyl phthalate were more in susceptible genotypes than resistant. A number of monoterpenes and sesquiterpenes have been reported to display repellent property to *B. tabaci*. For example, virus infected tomato plant was significantly susceptible to *B. tabaci* and that plant had a significant lower concentration of the volatiles  $\alpha$ -pinene, limonene, 4-carene, thymine,  $\beta$ -phellandrene, caryophyllene,  $\alpha$ -cedrene,  $\beta$ -cedrene, and  $\alpha$ -humulene than the healthy plant [29]. Similarly, significantly less amount of monoterpenes (e.g., p-cymene, 1, 8-cineole) and sesquiterpenes (e.g.,  $\alpha$ -copaene,  $\beta$ -cedrene) emitting healthy plants were more preferred by *B. tabaci* females than infested plants [30]. In the present study,  $\alpha$ -farnesene,  $\beta$ -bisabolene and methoxyacetic acid were present only in resistant genotypes as compared to susceptible which

might be involved in repellent activity against whitefly. These findings of the present study are in line with the results reported by Parimala *et al.* [31] and Khan *et al.* [32] that the compounds,  $\alpha$ -caryophyllene,  $\beta$ -myrcene,  $\beta$ -carene, eicosane and diethyl phthalate were present in cuticular wax of cotton and may involve in resistance against whitefly. Similarly,

Bleeker *et al.* [33] reported that *B. tabaci* prefer cultivated tomato plants to wild tomato plants, and their work showed that wild tomato plants released higher levels of terpenes, such as p-cymene,  $\alpha$ -terpinene,  $\gamma$ -terpinene and phellandrene, which act as a repellent to *B. tabaci*.

**Table 1:** Comparative volatile profile of different cotton genotypes

S.No.	Compound	RT	Peak area %					
			Bunny	CCH-4474	GJHV-517	LHDP-1	LRA-5166	TCH-1819
1.	3-Octanol	4.490	26.94	12.69	23.56	6.35	8.93	16.75
2.	$\alpha$ -Pinene	4.880	5.95	3.56	4.89	1.75	2.02	2.90
3.	Caryophyllene	7.555	-	-	7.71	-	4.02	3.82
4.	Camphene	7.982	3.30	-	3.30	-	-	-
5.	Humulene	7.982	-	0.31	-	1.34	2.17	-
6.	$\beta$ -Pinene	9.520	1.66	2.64	-	3.62	4.63	1.40
7.	Nonadecane	9.576	-	1.36	3.29	-	-	-
8.	$\beta$ -Acarose	9.813	-	-	-	11.65	23.05	22.60
9.	$\alpha$ -Farnesene	10.305	-	-	-	-	2.68	-
10.	$\beta$ -Bisabolene	10.425	-	-	-	6.61	-	-
11.	3-Cyclohexen-1-ol	10.480	11.02	8.63	-	13.63	20.92	10.43
12.	9-Octadecanone	10.599	2.53	2.71	2.22	2.86	3.12	4.84
13.	Myrcene	10.848	1.30	2.63	-	1.66	-	-
14.	Methoxyacetic acid	11.807	-	-	2.33	-	-	-
15.	Eicosane	11.809	-	4.82	-	3.60	1.18	4.63
16.	9, 12, 15-Octadecatrienoic acid	13.215	21.95	17.52	3.62	4.74	2.69	3.98
17.	Dibutyl phthalate	14.316	8.19	7.66	10.42	2.21	3.55	5.00



**Fig 1:** Volatile compound identified from Bunny

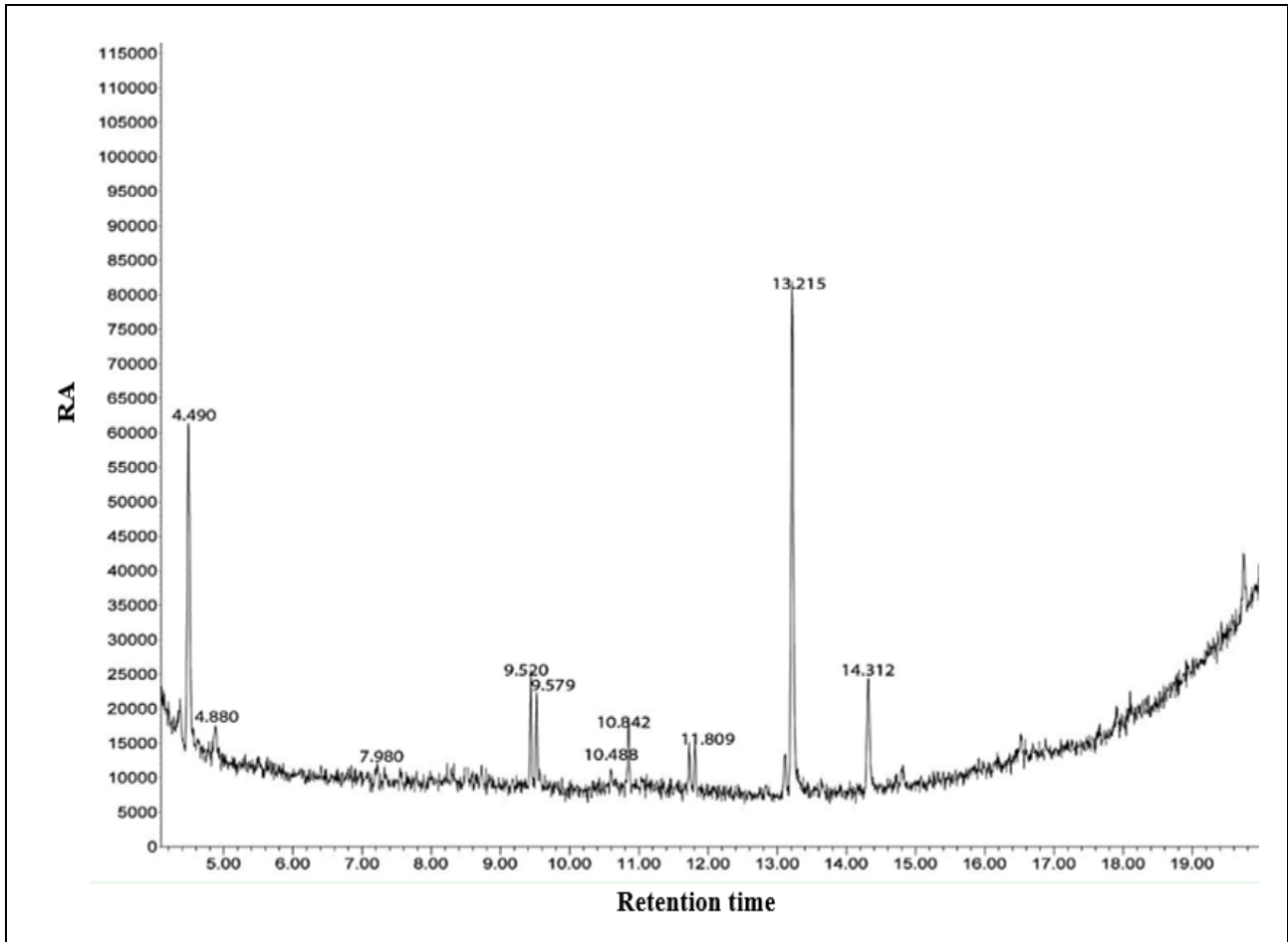


Fig 2: Volatile compound identified from CCH-4474

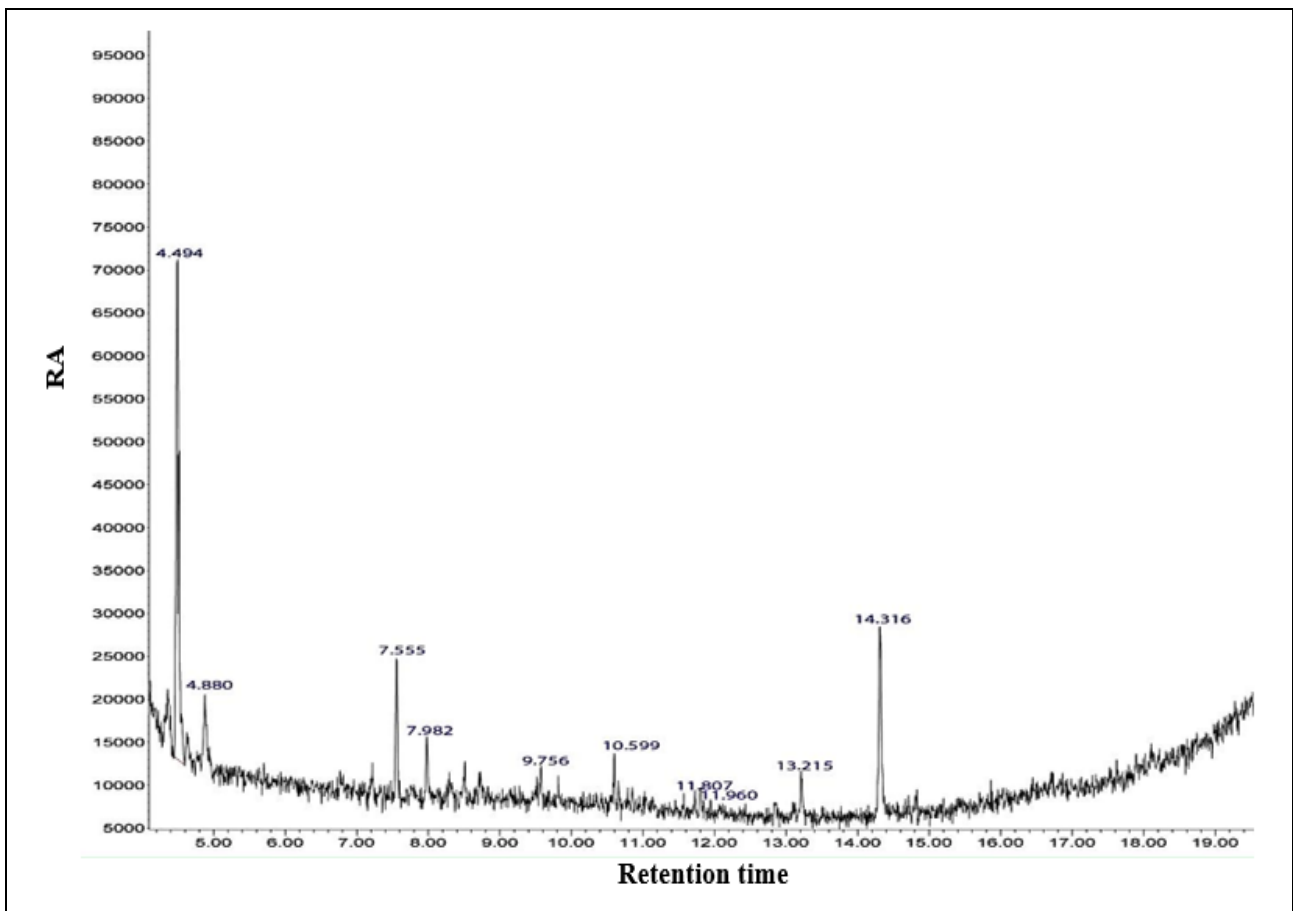


Fig 3: Volatile compound identified from GJHV-517

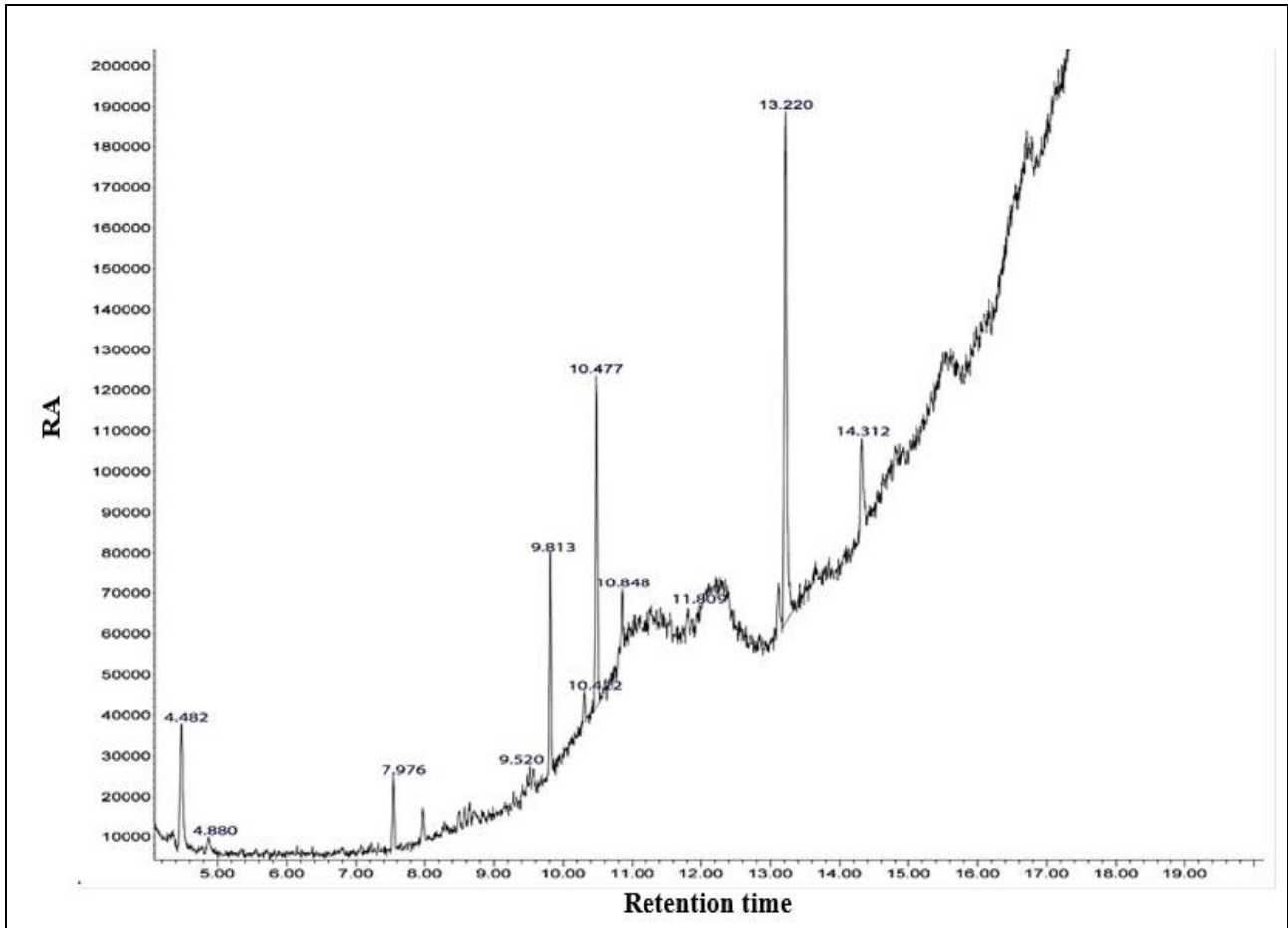


Fig 4: Volatile compound identified from LHD-1

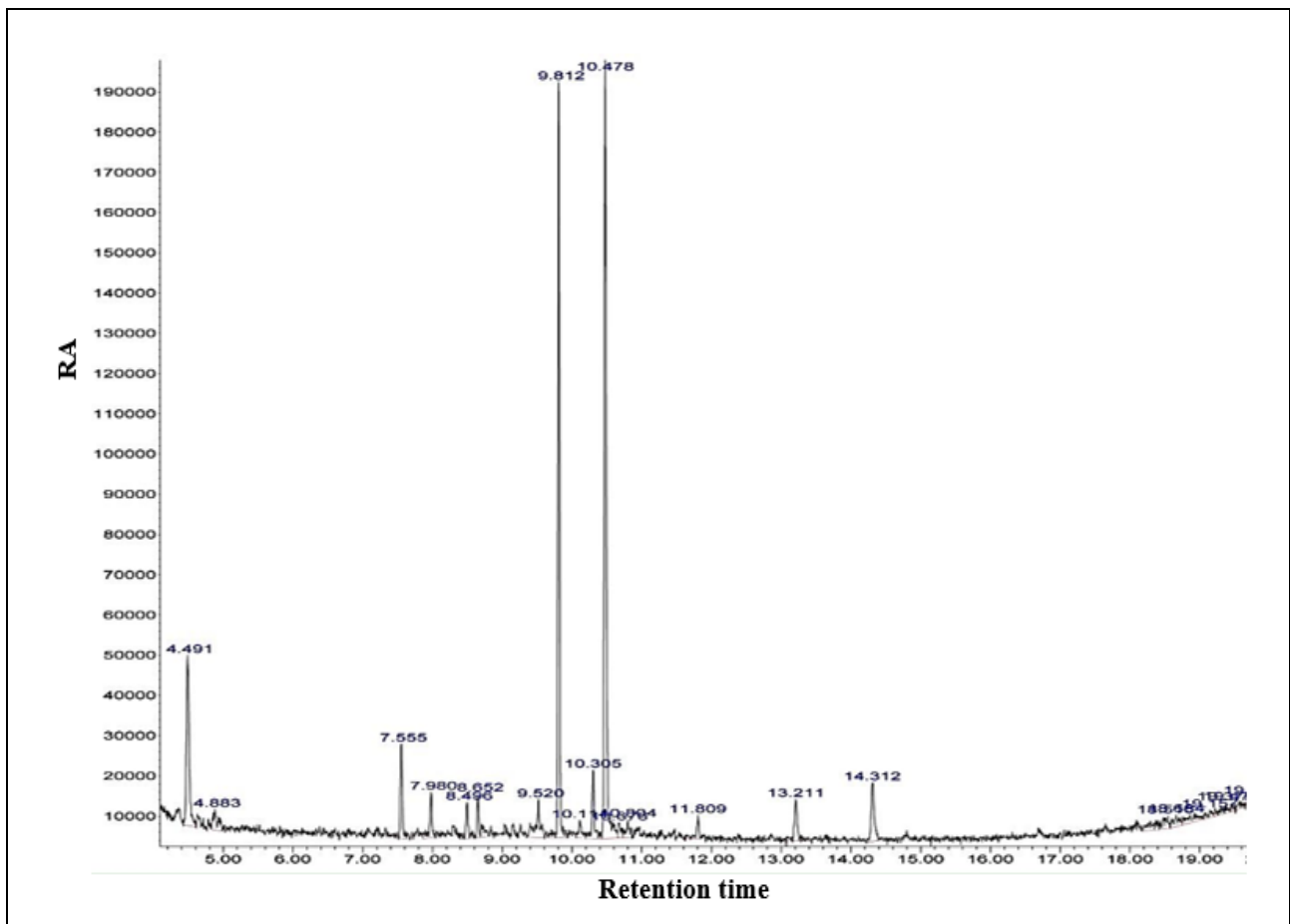
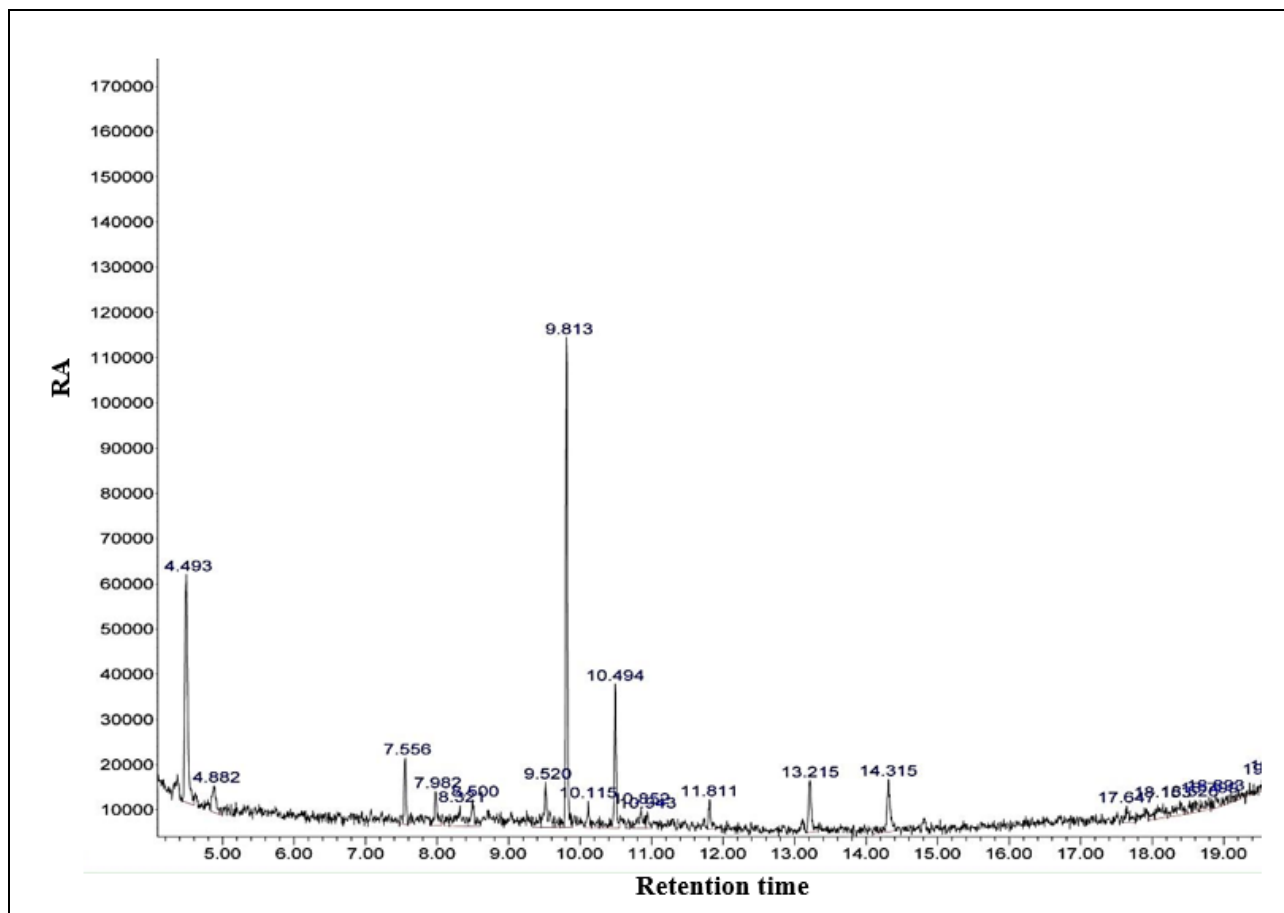


Fig 5: Volatile compound identified from LRA-5166



**Fig 6:** Volatile compound identified from TCH-1819

## Conclusion

The present experiment provides information on different volatile compounds present in cotton genotypes which shows varying levels of resistance against whitefly. This finding can be used in future to identify *B. tabaci* repellents and attractants that could be used as tools of IPM of *B. tabaci* and other whitefly pests. Further study is needed to evaluate each of these volatiles for its effect on the behavior of *B. tabaci* to find out which has the most adverse effect on *B. tabaci* as a repellent or an attractant.

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