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Cotton Plant Volatiles and Insect's Behavior

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Abstract: Volatile blend of cotton leaves extracted in Steam distillate was found to play some decisive role in insect attractions when evaluated through various field and laboratory bioassays. TLC and GC-MS analysis and identification of steam distillate of highly susceptible cotton variety S-12 and resistant variety Ravi showed some qualitative and quantitative differences. Susceptible varieties indicated the presence of greater potential of mono-terpenes ("-pinene myrcene, limonene and ocimene etc), while resistant variety Ravi possessed more $-pinene. An unidentified compound showed 96% peak intensity in S-12 and 34% in Ravi. Cis form of caryophyllene was present in S-12 while Ravi possessed trans- caryophyllene and "-terpeniolene was only present in Ravi.

Key words: Plant volatiles and insect attractions; insect plant communications; volatiles; attractants; kairomones; Gossypium ;stimulants ; insect orientations

Introduction

Some highly susceptible and resistant cotton varieties were investigated for their volatile composition and role of this volatile blend in insect's orientations to their host. Volatile compounds, which involve in insect attractions to their host plants for appropriate food, oviposition sites and shelter are mainly monoterpenes (Metcalf,1987). Monoterpenes are lypophilic in nature and derived by the condensation of mevalonic acid. These plant odorants are mainly the glandular secretions, which may act as attractants or arrestants and oviposition stimulants as reported by Keller *et al.* (1965). Chang *et al.* (1985) collected and identified air born organic compounds released by cotton flower buds from the air surrounding them. These compounds were mainly monoterpenes, "-pinene, $- pinene, $-myrcene, "-limonene and $-ocimene. Loughrin *et al.* (1998) described the importance of these plant volatiles in attracting behaviors of parasitoids and herbivores.

Material and Methods

Experimental fields (90 X 90) were planted with the cotton varieties, NIAB-86, NIAB-78, NIAB 26-N, S-12, SP-16 and Ravi (an old world cotton *G. arboreum*) at Nuclear Institute of Agriculture & Biology (NIAB) Faisalabad.

Extraction of Volatiles (Steam distillation): A simple distillation assembly was designed for volatile collection, superior in the sense of no bumping and high recovery of the volatiles. One kg. of fresh cotton leaves was ground in liquid nitrogen and put in a round bottom flask (2.5L) having 1 L of distilled water. Steam distillate was collected in a separating funnel containing 50 ml of organic solvent (diethyl ether). Separating funnel was kept in ice container. Condenser was attached to cold water circulating assembly (Julabo-VC) to minimize the losses of very low boiling point volatiles. After four hours extraction, separating funnel was detached from the assembly and shacked well by putting thumb on the stopper (due to increased internal pressure). Upper organic layer was concentrated on rotary evaporator at low temperature and without vacuum and then flushing with a very low stream of nitrogen gas up to the volume of 2ml and kept in freezer in tightly closed screw capped vials.

Field Biotests: Sticky surfaces of the orange coloured delta type traps were treated with 1 ml of steam distillate and hanged in unsprayed cotton field. These traps are commonly used as lure for male moths (Hennebery & Clayton, 1982). Control traps were also hanged by treating only with solvent (diethyl ether). After 24 hours the number of insects (mostly jassids) attracted to the treated and control traps was counted. These biotests were repeated several times from mid August to mid November, each time with newly distilled extracts.

Biotests with Standard Monoterpenes: Some monoterpene standards (Sigma), "-pinene, $-pinene, myrcene, geraniol, "-terpeniolene and a mixture of monoterpenes containing "-pinene, $-pinene and myrcene were also biotested in field in delta sticky traps at 2% concentration in diethyl ether along with control traps.

Multi Choice Biotests: Response of jassids to volatile blend of susceptible cotton variety S-12 and resistant variety Ravi were also observed in a flat, round shaped, multi chamber apparatus ,having eight compartments and a plastic lid. One compartment was treated with 0.1 ml of cotton extract and others remained untreated. Jassids were collected randomly from the field by sucking through pasture pipette and released through the hole in the center of the lid. The hole was then closed with cotton plug. Number of jassids in each chamber was counted after 4 hours.

T.L.C. Analysis of Volatiles: Thin layer chromatographic plates of thickness 0.25 mm (20 x 20 cm) were prepared with silica gel GF-254 and activated at 100^0C for 24hrs. T.L.C. of cotton extracts (steam distillate) of various varieties, viz. Ravi, SP-16, NIAB-86, NIAB-78, NIAB-26N, and S-12 was performed by applying 10Fl of each extract in the form of spots on 0.25mm thin layer plates and 20Fl in the form of streaks on preparative layer plates of thickness 0.5 mm(Fig.3). Plates were then developed in 15% ethyl acetate /n-hexane. The T.L.C. tank was tightly closed by applying vacuum grease and was kept in cold chamber to avoid the losses of the volatiles at room temperature. After 30 minutes, the plates were immediately sprayed with reagent anisaldehyde: sulfuric acid: acetic acid (0.5 ml: 1 ml: 50 ml). Separated spots were then became visualized after heating the sprayed plates at 100^0C for 15 minutes (Stahl and Kaltenbach, 1961). Volatile compounds are difficult to analyze because they are colorless, lipophilic, occur in low concentrations and isomerize and rearrange themselves when exposed to sunlight or air. Therefore chromatography should perform with much care for reproducible results.

GC-MS of Steam Distillate: Two cotton varieties, highly susceptible variety S-12 and resistant variety Ravi were selected for their volatile analysis. Steam distillate of cotton varieties was subjected to gas liquid chromatographic analysis and mass spectroscopic studies. Steam distillate (1.0 Fl) was injected to gas chromatograph (JEOL-5890A) having HP-5 capillary column. Oven temperature was programmed as 70^0C/min. Injector temperature was 270^0C and carrier gas used was helium. Gas chromatograph was connected with JEOL, JMS-HX 110 mass spectrometer. Peaks obtained were identified by comparing their mass spectra with library reference spectra (Benchtop / PBM version 3.1 and wiley Registary 6th edition), available in HEJ Institute of Chemistry, University of Karachi, Pakistan.

The data was subjected to analysis of variance followed by DMR.test on microcomputer using MSTATE software package.

Results and discussion

Results obtained from the field biotests of the cotton plant volatiles (present in steam distillate of cotton leaves) indicate the presence of some chemical signals for the insects. (Fig.1,2a). Insect orientations to their host may also involve several other factors as air currents, light attractions and random encounter. But in our studies the traps treated with steam distillate always attracted a significant number of insects than control traps indicating some absolute role of volatiles in insect attractions besides all other factors. The number of insects attracted in biotests performed from August to September was found to be greater than the tests performed from October to November (Fig.2a). This may possibly be due to the higher rate of production of volatiles in full blooming season (from 60 to 90 days after planting). The peak of insects in field is also observed from mid August to mid September. Volatile production decreases with plant maturity that may in turn decreases the concentration of specific compounds in volatile blend, which may be responsible for insect attraction.

The results (Fig.2a) showed that the highly susceptible varieties S-12 and NIAB-26N attracted significantly greater number of insects, 89.2 ±4.87 and 87.0 ±4.70. In field biotests the susceptible variety NIAB-78 attracted comparatively less number of insects (jassids) being 71.2 ±3.76, although insect per leaf data in field showed the greater number of jassids on this variety. On the other hand NIAB-26N which attracts more insects in field biotests showed less number of jassids in insect per leaf data. These behavioral differences for plants and their volatile blend may possibly be attributed to the physical or morphological characteristics of these plants. NIAB-26N having comparatively more villous substrate than NIAB-78 is less preferred by the jassids because jassids generally avoid the hairy substrate. The other susceptible and Semi resistant or moderate varieties also attracted significantly greater number of insects than the resistant variety Ravi. This behavioral difference of the insect orientation for S-12 and Ravi was also observed in multi choice biotests. In multi choice apparatus the number of insects in treated chamber was significantly and persistently higher than the control chambers (Fig.2b). Mean number of jassids attracted to S-12 extract was significantly higher, being 27.8 ± 2.21 insects than the control chambers for respective biotests. Insignificant differences were observed for all the control chambers for mean number of insects with 4.7 to 6.3 ± 0.94 to ± 1.40. Biotests performed with steam

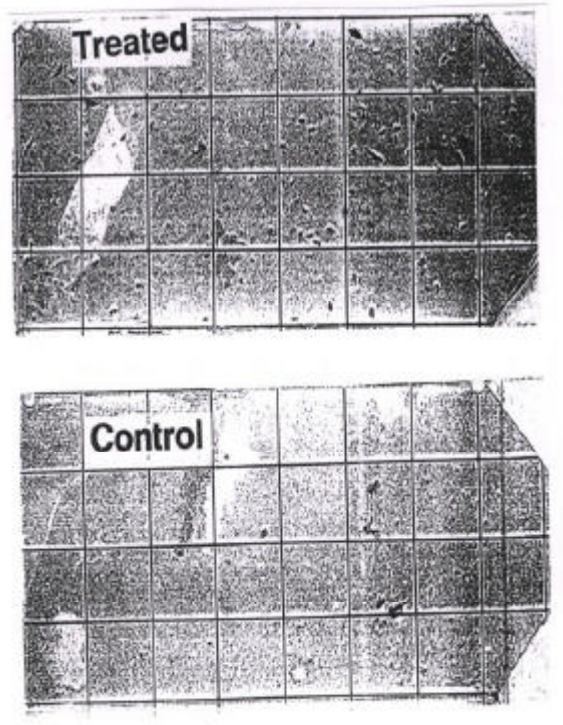

Fig. 1: Treated and control traps showing a significant difference for the insects attracted. **Treated:** Traps treated with steam distillate of cotton leaves (highly susceptible variety, S12) **Control:** Traps treated only with ether

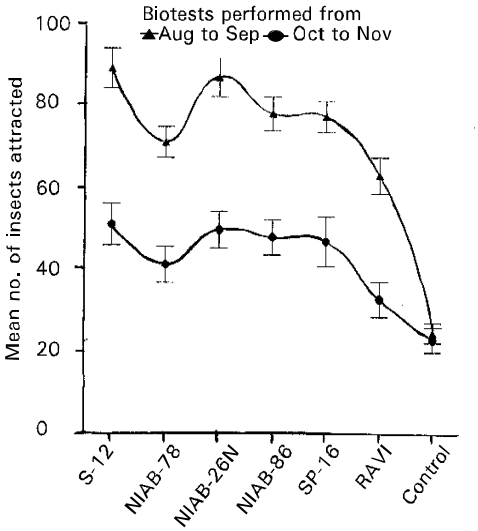

Fig. 2a: Field biotests of cotton extracts used as bait for insects, representing the significant difference of insect attractions for susceptible and resistant cotton varieties and decreasing potential of volatile blend with maturity

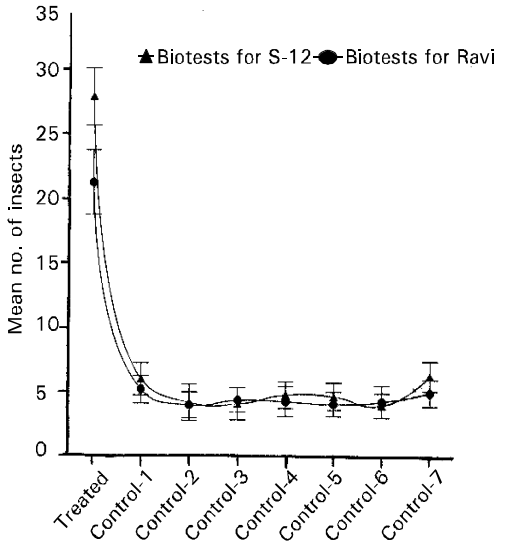

Fig. 2b: Biotests of cotton extracts (S-12 and Ravi) in multi choice apparatus, showing a significant difference of mean number of insects attracted to both treated chambers and also treated and control chambers.

Table 1: Field biotests of monoterpenes used as bait for insects

Monoterpenes	Mean No. of insects attracted	± SD
"-pinene	52.0a	5.05
$-pinene	25.2c	2.62
Myrcene	45.9b	4.65
"-Terpeniolene.	23.7c	3.86
Geraniol	22.0c	3.89
Mixture (Mono terpene)	56.1a	4.31
Control**	21.3c	2.27

Coefficient of variation (CV) = 11.31%, LSD Value = 4.506.
*Numbers followed by the similar letters do not differ significantly at 0.05% probability level by DMRT
**(Traps treated only with ether)

distillate of cotton variety Ravi showed less number of insects attracted than S-12 extract but significantly more than control chambers. Chambers treated with Ravi extract showed 21.2 ± 2.49 insects while control chambers showed mean number of insects, 4.0 to 5.2 ± 0.95 to ± 1.25. The number of insects per biotest was also found to be greater in control chambers (2 and 7), which were adjacent to the treated chamber. These adjacent chambers may acquire the volatile smell and confuse the insects to come in. All these results of field and laboratory biotests strongly justify the role of volatiles in insect attractions. These results are revealed by the findings of many workers as Elzen *et al.* (1986) and Chang *et al.* (1988). They established the positive correlations between cotton plant volatiles and insect attractions by performing various kinds of bioassays including wind tunnel flight responses, laboratory olfactometer and multi choice olfactometer bioassays.

All of these workers identified the volatile compounds either by GC- MS of airborn volatiles surrounding the cotton plants, dynamic head space volatiles or steam distillate of cotton leaves. The compounds identified and reported were mainly mono-terpenes as "-pinene, $-pinene, myrcene, limonene, $-ocimene caryophyllene and humulene etc. These reported compounds along with some other compounds were also identified in steam distillate of cotton varieties S-12 and Ravi (Table 2, Fig.4). The compounds "-pinene, myrcene, cis-3-hexanol, cis- caryophyllene, trans-caryophyllene, "-humulene, and spathulenol were identified with 100% overlap with the lib. reference spectra. The compounds $-pinene, dl-limonene,

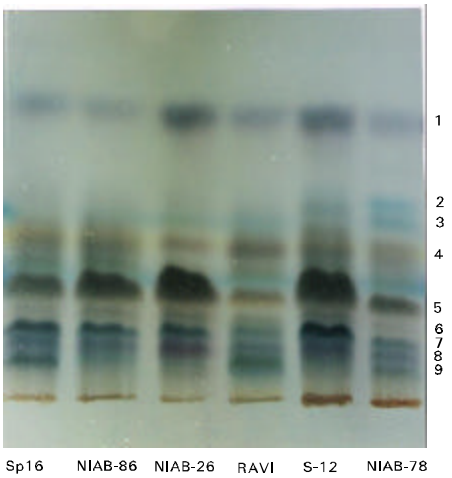

Fig. 3: Thin layer chromatographic profile of steam distillate of cotton varieties showing varietal differences for fraction 1 and 5 being more in more susceptible varieties S-12 and NIAB-26N.

Table 2: Relative peak intensities of compounds identified in steam distillate of cotton varieties S-12 and Ravi

Compounds	Retention time (min)	Peak Intensity(%)* S-12	Ravi
"-pinene	1.24	30	10
$-pinene	1.59	26	54
Myrcene	1.99	24	5
dl-lmonene	2.37	30	7
$-ocimene	3.12	55	12
2,4-hexadiene-l-ol	3.25	15	11
Cyclo propyl methyl ketone	6.40	25	16
Cis-hexanol	7.44	28	12
Unknown	8.28	24	15
"-terpeniolene	14.51	-	7
Trans-caryophyllene	15.39	-	6
Cis-caryophyllene	15.56	18	-
"-humulene	19.13	10	10
$-bisabolene	23.02	10	10
"-santalol	39.41	10	10
Unknown	40.00	8	8
Spathulenol	42.40	14	14
Unknown	43.52	96	34
Unknown	45.37	15	10
Veridiflorol	47.06	10	5
Unknown	49.45	10	5
Unknown	52.00	10	5
Dehydrohumulinic acid	54.45	35	22
Hexadecanoic acid	74.00	15	-

* % Peak intensities, measured directly from the chromatographic profiles of both cotton varieties. Qualitative and quantitative differences are shown for both, resistant (Ravi) and susceptible (S-12) varieties.

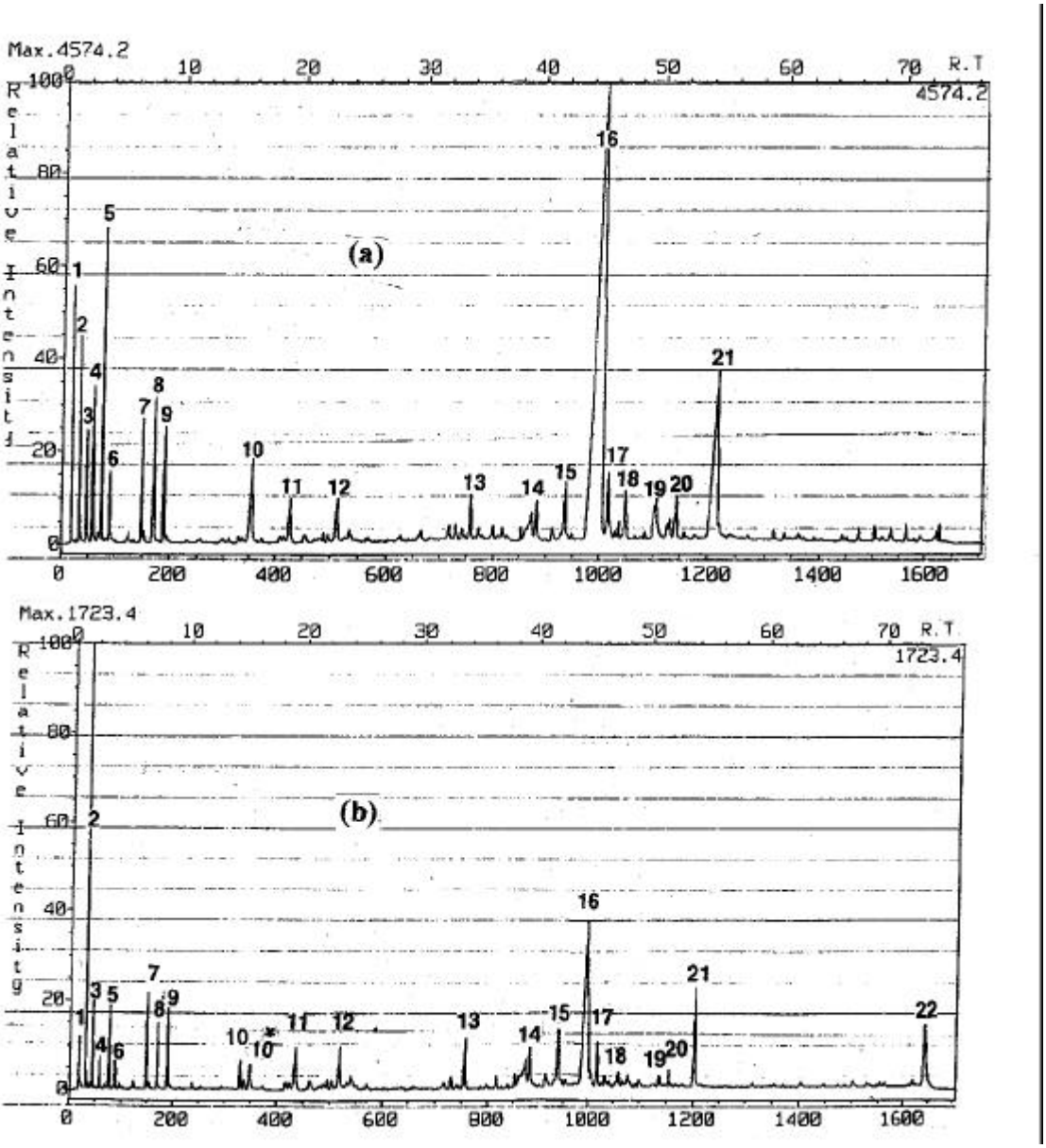

Fig. 4: Gas chromatogram of steam distillate of susceptible cotton variety S-12 (a) showing greater potential for volatiles than resistant variety Ravi (b). Qualitative differnce for "-terpeniolene (peak 10) and hexa decanoic acid (peak 22) which is absent in S-12. Ravi possessed trans-caryophyllene and S-12, cis-caryophyllene (peak10*).

$-ocimene, 2, 4-hexadiene-1-ol, cyclo methyl propyl ketone, and "-terpeniolene, were identified with 98 to 99% overlap while $-bisabolene, "-santalol, and veridiflorol were identified with 90 to 92% overlap with library reference spectra. Dehydro humulinic acid and hexadecanoic acid were identified with 83 to 86% overlap. 6 Peaks (9, 14, 16,17, 19 and 20) at retention times 8.28, 40.00, 43.52,45.37, 49.45, and 52.00 min. were remained unidentified. Peak intensities for the compounds mentioned (Table 2) were directly taken from the chromatograms just to compare the relative peak intensities in cotton varieties S-12 and Ravi. Obvious quantitative differences were observed in these insect susceptible and insect resistant varieties. The overall concentration of volatiles was higher in S-12 except $-pinene, which was present at higher percentage in Ravi. This greater potential of volatiles in S-12, also revealed by the thin layer chromatographic profile of the steam distillate of Ravi and S-12 (Fig.3). The role of monoterpenes in insect-plant communication was also revealed by the field bioassays of some mono terpene standards. Alpha-pinene, myrcene and their mixture along with $-pinene attracted significant number of insects than control traps (Table 1). Geraniol that was

absent in both the cotton extracts showed the number of insects similar to control. $-pinene which was found to be in higher concentration in resistant variety Ravi and "-terpeniolene which was absent in S-12 also showed number of insects similar to control traps. Hexadecanoic acid and "-terpeniolene were only present in Ravi. S-12 contained cis-caryophyllene while Ravi contained trans caryophyllene. These qualitative difference may contribute to the less number of insects attracted to Ravi because very small differences in the molecular structure can result in different biological effects as studied by Sharma & Saxena (1974) that optical isomers (-)-limonene was a fly attractant and (+)-limonene was fly deterrent. Number of insects attracted in individual monoterpenes (Table 1) was not as high as in cotton extracts indicating that not only the quality and quantity of the volatile compounds are important for insect attractions but their cumulative effect or/synergistic effect with other plant compounds is of great importance. An other important role of these volatile compounds is recently been observed by various workers, Tingle *et al.* (1990) and Jennifer (1999). They reported that infested plants emit a volatile blend, which serve as olfactory cues for the natural enemies of the insects. Compounds reported are mostly similar to the compounds identified in our studies. It may be possible that greater potential of volatile compounds in susceptible varieties may be the result of comparatively more insect infestation on these plants in comparison to resistant one. It can be concluded from the results of volatile studies that cotton plant volatiles definitely contribute to the insect orientations to their host plants mostly the un substituted monoterpenes, "-pinene, myrcene, limonene and ocimene play an important role in insect attraction while $-pinene and "-terpeniolene serve partially to insect resistance. The unidentified broad peak (No-16) present in GLC profile of S-12 , reported earlier (communications, 1979) may also be responsible for the insect attractions because the results of field biotests (Fig.2a) indicate more insect attractions in the varieties having more concentration of this compound(compound No-5 in TLC profile of steam distillate ,Fig.3). Ravi and NIAB-78 which posses less amount of this compound as well as the monoterpenes ("-pinene ,myrcene and limonene) also showed less insect attraction in field biotest.

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