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Monitoring the Emission of Volatile Organic Compounds from Flowers of *Jasminum sambac* Using Solid-Phase Micro-extraction Fibers and Gas Chromatography with Mass Spectrometry Detection

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Solid-phase micro-extraction (SPME) was studied as a solvent free alternative method for the extraction and characterization of volatile compounds in intact and plucked flowers of *Jasminum sambac* at different day time intervals using gas chromatography (GC-FID) and gas chromatography-quadrupole mass spectrometry. The analytes identified included alcohols, esters, phenolic compounds, and terpenoids. The main constituents identified in the flower aroma using different fibers were cis-3-hexenyl acetate, (E)-β-ocimene, linalool, benzyl acetate, and (E,E)-α-farnesene. The benzyl acetate proportion decreased from morning to afternoon and then increased in evening collections. PDMS fiber showed a high proportion of (E,E)-α-farnesene in jasmine floral aroma. Among other constituents identified, cis-3-hexenyl acetate, linalool, and benzyl acetate were major aroma contributors in plucked and living flowers extracts using PDMS/DVB, Carboxen/PDMS, and DVB/Carboxen/PDMS fibers. PDMS/DVB recorded the highest emission for benzyl acetate while the (E)-β-ocimene proportion was highest in DVB/Carboxen/PDMS when compared with the rest. The highest linalool content, with increasing proportion from morning to noon, was found using mixed coating fibers. Almost negligible volatile adsorption was recorded for the polyacrylate fiber for intact flower aroma, whereas it was most effective for benzyl acetate, followed by indole under plucked conditions. Moreover, the highest amounts extracted, evaluated from the sum of peak areas, were achieved using Carboxen/PDMS, and DVB/Carboxen/PDMS. Introduction of a rapid, and solvent free SPME method for the analysis of multicomponent volatiles can be successfully employed to monitor the extraction and characterization of flower aroma constituents.

**Keywords:** *Jasminum sambac*, SPME, gas chromatography-quadrupole mass spectrometry, benzyl acetate, (E,E)-α-farnesene, cis-3-hexenyl acetate, linalool, indole.

Floral scent has an important role in the reproductive processes of many plants. Many floral volatiles fall into the terpenoid or phenylpropanoid/benzenoid classes of compounds. The terpenes derived from isoprenoids constitute the largest class of secondary products and they are also the most important precursors for phytocides. Phytocides are volatile organic compounds released by plants that resist and break up hazardous substances in air. It has been well established that phytocides can reduce dust and bacteria in the air and exposure to essential oils from trees has also been reported to lessen anxiety and depression [1].

For humans, scented flowers also constitute a commodity with strong aesthetic and emotional values. Unfortunately, floral scent has been a casualty of plant-breeding programs for the cut-flower market and ornamental plants [2]. In India, jasmine (family Oleaceae) is represented by more than 40 species [3]. Among these, *Jasminum sambac* is cultivated for commercial purposes in the whole country. *J. sambac* is an erect or scendent shrub with dropping branches, 10 cm long, broadly ovate-elliptic, obtuse or acute leaves. Flowers are whitish-pink in color, flowered cymes, flowering from February to July [3-5].

Jasmine flowers are used for the treatment of diarrhea, abdominal pain, conjunctivitis and dermatitis while leaves and roots are used for treating diarrhea, fever and as an analgesic [5]. Antimicrobial activity of *J. grandiflorum* absolute against Gram-positive and Gram-negative bacteria was reported [6]. Flower absolute of *J. sambac* has been characterized from Egypt, India and France [7-9]. Fragrance of *J. sambac* and *J. pubescens* have also been reported [10, 11]. The headspace [12], purge and trap [13] of jasmine flowers and vacuum headspace analysis of absolute [14] were also reported. Conventionally, extraction of volatile compounds from jasmine flowers is carried out by different techniques such as enfleurage, maceration, and solvent extraction [15-17].

Solid-phase micro-extraction is an advanced technique for extraction of volatiles and eliminates complicated
Intervals. Day time using different polarity fibers at different time extraction of volatiles of the intact and plucked flowers in constituents. Moreover, the present report deals with SPME technique to monitor the emission of individual plucked flowers, and iii) to demonstrate the ability of the variations of volatile compounds emitted by intact and compounds (BVOCs), ii) to monitor the dynamic characterization and quantify the biogenic volatile organic SPME analysis during day time has not been reported. The exists on the composition of compositions are worth pursuing. In India, the SPME needs to be carried out. Hence, investigations on floral aromatic plants is still an area where more research work needs to be carried out. Hence, investigations on floral compositions are worth pursuing. In India, the SPME method is poorly utilized to date and only a single report exists on the composition of J. sambac [32], whereas SPME analysis during day time has not been reported. The aims of the study in this communication are i) to characterize and quantify the biogenic volatile organic compounds (BVOCs), ii) to monitor the dynamic variations of volatile compounds emitted by intact and plucked flowers, and iii) to demonstrate the ability of the SPME technique to monitor the emission of individual constituents. Moreover, the present report deals with extraction of volatiles of the intact and plucked flowers in day time using different polarity fibers at different time intervals.

The percentage composition and RI values of released volatiles with different fibers in day time intervals are listed in order of elution from a DB-5 capillary column (Table 1). A total of 23 constituents of J. sambac flower aroma were identified. Among these, the major proportion of the fragrance was comprised of cis-3-hexenyl acetate, (E)-ß-ocimene, linalool, benzyl acetate and (E,E)-a-farnesene (ca. 80%). Unlike a previous report [32], benzyl acetate and linalool were found predominant in the intact flower rather than the plucked flower aroma.

For the 100 µm PDMS fiber, terpenoids contributed 63.7%, followed by phenylpropanoids (22.1%) and non-terpenoid constituents (9.1%). (E,E)-a-Farnesene was recorded with the highest proportion when compared with other fibers. Constituents recorded in good proportions were benzyl acetate, linalool and phenethylacetate in intact flowers, and benzyl acetate, linalool and (E)-ß-ocimene in plucked flower aroma.

The green leaf volatile, cis-3-hexenyl acetate, was one of the major constituent of the jasmine fragrance. Due to its high volatility, cis-3-hexenyl acetate was identified more in the HS-SPME of flowers in comparison with essential oil, concrete and absolute [8, 28]. Conversely, cis-3-hexenyl benzoate was observed more in conventional methods. An unexpected emission pattern was observed for benzyl acetate in intact flowers; a higher proportion was recorded in the morning and evening with the lowest proportion in the afternoon, while the DVB/PDMS fiber (partially crosslinked) registered the highest content (48.0%) in the evening (Figure 1). Apart from this, a gradual increase in linalool content from morning to afternoon was recorded with mixed coating fibers.

With the Carboxen/PDMS fiber (partially crosslinked), afternoon samples showed good emission of volatile compounds, comprised of linalool, (E,E)-a-farnesene and indole. In addition, cis-3-hexenyl acetate and benzyl acetate were major aroma contributors in the evening. Similarly, benzyl acetate, (Z)-ß-ocimene, and (E,E)-a-farnesene were the major constituents identified in plucked flower aroma.

With the DVB/Carboxen/PDMS fiber (highly crosslinked), linalool forms a higher proportion (25.7%, 30.1% and 37.8%, respectively) in the intact flowers. Besides linalool, (E)-ß-ocimene was found in good proportions. The plucked flowers contained benzyl acetate, linalool and (E,E)-a-farnesene as major aroma contributors. Using polyacrylate (PA), benzyl acetate (35.3%) and indole (15.7%) were found in higher amounts as compared with (E,E)-a-farnesene, linalool and cis-3-hexenyl acetate in plucked flowers. Moreover, SPME fibers have shown the best adsorption for ß-myrcene, limonene, (Z)-ß-ocimene, (E)-ß-ocimene and phenylethylacetate and these are entirely new entities reported in J. sambac through the SPME technique for the first time.

Since, the selection of an appropriate fiber was based on the volatility of phytoncides, the amount of the compounds extracted depends on their different affinities for the fiber and on the competition phenomenon. Thus, a non-polar fiber like PDMS extracts, mainly, non-polar compounds ((E,E)-a-farnesene), while a more polar one, such as PA showed an affinity for extracting more polar compounds, mainly phenols and alcohols. Mixed phase, such as
Table 1: Relative compositions (%) of *Jasminum sambac* floral emissions using five different polarity fibers at different day time intervals using SPME-GC-FID.

<table>
<thead>
<tr>
<th>Constituents</th>
<th>RI</th>
<th>PDMS</th>
<th>PDMS/DVB</th>
<th>Carboxen/PDMS</th>
<th>DVB/Carboxen/PDMS</th>
<th>PA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
<td>II</td>
<td>III</td>
<td>IV</td>
<td>I</td>
<td>II</td>
</tr>
<tr>
<td>β-Myrcene</td>
<td>992</td>
<td>0.4</td>
<td>-</td>
<td>-</td>
<td>0.6</td>
<td>0.3</td>
</tr>
<tr>
<td>cis-3-Hexenyl acetate</td>
<td>1008</td>
<td>7.7</td>
<td>3.9</td>
<td>0.5</td>
<td>26.0</td>
<td>10.2</td>
</tr>
<tr>
<td>Limonene</td>
<td>1030</td>
<td>0.1</td>
<td>-</td>
<td>-</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Benzyl alcohol</td>
<td>1035</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.4</td>
<td>0.6</td>
</tr>
<tr>
<td>(Z)-β-Ocimene</td>
<td>1037</td>
<td>0.4</td>
<td>-</td>
<td>-</td>
<td>0.5</td>
<td>0.2</td>
</tr>
<tr>
<td>(E)-β-Ocimene</td>
<td>1047</td>
<td>9.8</td>
<td>2.8</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Methyl benzoate</td>
<td>1096</td>
<td>0.3</td>
<td>0.5</td>
<td>-</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Linalool</td>
<td>1102</td>
<td>14.6</td>
<td>12.3</td>
<td>3.5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Phenylethyl alcohol</td>
<td>1110</td>
<td>0.5</td>
<td>0.2</td>
<td>0.5</td>
<td>9.0</td>
<td>0.5</td>
</tr>
<tr>
<td>Benzyl nitrite</td>
<td>1136</td>
<td>0.4</td>
<td>0.6</td>
<td>1.8</td>
<td>0.8</td>
<td>0.8</td>
</tr>
<tr>
<td>Benzyl acetate</td>
<td>1165</td>
<td>16.8</td>
<td>19.3</td>
<td>2.6</td>
<td>13.6</td>
<td>16.0</td>
</tr>
<tr>
<td>Methyl salicylate</td>
<td>1194</td>
<td>0.6</td>
<td>0.5</td>
<td>-</td>
<td>0.4</td>
<td>0.2</td>
</tr>
<tr>
<td>Pheny lethyl acetate</td>
<td>1256</td>
<td>2.7</td>
<td>4.9</td>
<td>2.3</td>
<td>19.7</td>
<td>1.8</td>
</tr>
<tr>
<td>Indole</td>
<td>1292</td>
<td>1.3</td>
<td>6.0</td>
<td>3.9</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Phenylethyl benzoate</td>
<td>1294</td>
<td>0.4</td>
<td>0.4</td>
<td>0.6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Methyl anthranilate</td>
<td>1330</td>
<td>0.4</td>
<td>1.1</td>
<td>1.6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>trans-Caryophyllene</td>
<td>1418</td>
<td>0.3</td>
<td>0.1</td>
<td>-</td>
<td>0.1</td>
<td>-</td>
</tr>
<tr>
<td>Germacrene D</td>
<td>1480</td>
<td>0.8</td>
<td>0.4</td>
<td>0.8</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Germacrene A</td>
<td>1503</td>
<td>0.2</td>
<td>0.3</td>
<td>0.6</td>
<td>8.0</td>
<td>-</td>
</tr>
<tr>
<td>(E,E)-α-Farnesene</td>
<td>1507</td>
<td>36.5</td>
<td>36.4</td>
<td>55.3</td>
<td>41.2</td>
<td>-</td>
</tr>
<tr>
<td>δ-Cadinene</td>
<td>1524</td>
<td>0.2</td>
<td>0.1</td>
<td>0.8</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>cis-3-Hexenyl benzoate</td>
<td>1568</td>
<td>0.1</td>
<td>0.3</td>
<td>0.6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Germacrene D-4-ol</td>
<td>1574</td>
<td>0.4</td>
<td>1.0</td>
<td>3.1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Terpenoids</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phenyl Propanoids</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

RI: experimental retention indices on the DB-5 capillary column relative to homologous series of *n*-alkane (C8–C25) hydrocarbons (Polyscience Corp. Niles IL); flower emission monitoring was conducted at different day time intervals: plucked jasmine flowers I: 9.30 am; intact jasmine flower II: 9.30 am; III: 12.30 pm and IV: 3.30 pm; b: mass spectrum; c: Sigma Standard; –, not detected; t: trace < 0.1%, fibers are PDMS: polydimethylsiloxane, PDMS/DVB: polydimethylsiloxane/divinylbenzene; Carboxen/PDMS: Carboxen/polydimethylsiloxane; DVB/Carboxen/PDMS: divinylbenzene/Carboxen/polydimethylsiloxane; PA: polyacrylate.

Figure 2: Comparison of four SPME fibers for cis-3-hexenyl acetate content at different day time intervals

Figure 3: Comparison of four SPME fibers for linalool content at different day time intervals

Figure 4: Comparison of four SPME fibers for benzyl acetate content at different day time intervals

Figure 5: Comparison of four SPME fibers for (E,E)-α-farnesene content at different day time intervals
Carboxen/PDMS, DVB/Carboxen/PDMS and PDMS/DVB present medium polarity, reducing the discrimination towards very nonpolar and polar volatile compounds. Thus, these are preferred over “single fibers” (PDMS or PA) for multicomponent analysis.

Thus, we conclude that the influence of the polarity and extraction mechanism of the SPME fiber affects total extraction, which supports the current study results (Figures 2-5). Previously, CAR/PDMS was reported to have the highest sensitivity for the recovery of honey volatiles with the lowest dispersion for polyacrylate [28]. In several communications, it has been emphasized that the indole proportion itself influences the quality of jasmine and plays an important role in modulating the floral odors of the other ingredients [33]. Indole has been reported as one of the major compounds in concrete of Egyptian jasmine [9]. Since, a very low indole proportion was observed in our samples, this species is indole poor and is comparable with headspace SPME studies on J. sambac from India [32].

Terpenes emitted from flowers have high enough vapor pressure under normal atmospheric conditions to allow significant release into the air. As a consequence, there would be more accumulation of terpenoids in the environment. Hence, skilled use of SPME in extraction is noteworthy. All the mixed phases were able to disperse most of the J. sambac volatiles, while lowest dispersion results were obtained for the PA fiber.

In conclusion, mixed phases in this study showed better results when compared with “single fibres”, such as PDMS and PA. This fact was supported by the same normalized extraction efficiency of DVB/Carboxen/PDMS and PDMS/DVB [34]. PDMS/DVB has already been a fiber of choice for the analysis of volatiles from infant milk powder and coffee volatiles [35-36], due to its good performance. Moreover, better results were obtained in the present study when extraction was carried out using DVB/Carboxen/PDMS, which supports the earlier report [37]. Polyacrylate fiber is reported to be good for extraction of phenols and alcohols [38]. Hence, good proportions of benzyl alcohol, benzyl nitrile, benzyl acetate, methyl salicylate, phenethyl acetate, indole, phenethyl benzoate and methyl anthranilate in our present study are evident.

Experimental

**Plant material:** Jasminum sambac flowers were collected at bloom stage in May 2010 from the CIMAP farm, Lucknow. Identification (Voucher specimen no: 14515) was made by Dr. SC Singh, Botany Department, CIMAP, Lucknow.

**Intact flowers:** Four mature flowers in full bloom were trapped in a designed glass chamber with the help of a polystyrene film. An equilibrium time (time needed to reach the equilibrium between sample and above headspace) of 15 min was set for field conditions. The injection port temperature, as well as the desorption time, were optimized to ensure that volatiles were totally desorbed from the fiber. Memory effect of each fiber was excluded with the blank analysis performed after each run. Then, the conditioned SPME fiber was introduced into the chamber and was exposed to the sample headspace for 15 min (extraction time). Following sampling, the fiber was retracted and removed from the chamber. Each SPME sampling was conducted in triplicate in a day at 9.30 am, 12.30 pm and 3.30 pm and the fibers were immediately thermally desorbed in the S/SL injector for 10 min at 250 °C. The whole procedure was repeated on the next day for the remaining fibers.

**Plucked flowers:** Mature flowers in full bloom were plucked at 9.00 am from the same plant that was used earlier for intact flowers, and transferred directly into a designed glass chamber and allowed to equilibrate for 30 min. Then, the conditioned SPME fiber was introduced into the chamber and exposed to the sample headspace for 30 min (extraction time). Following sampling, the fibers were retracted and removed from the chamber and the fibers immediately thermal desorbed in the S/SL injector for 10 min at 250°C.

**Fiber calibration:** All 4 fibers were calibrated using authentic linalool. R^2 values for linalool for all the fibers were 0.9909 (PDMS), 0.9944 (PDMS/DVB), 0.9991 (Carboxen/PDMS), and 0.9832 (DBV/Carboxen/PDMS).

**Solid-phase micro-extraction (SPME) fibers:** A manual SPME holder and 4 fibers of different polarity and extraction mechanism (Supleco Co., Bellefonte, PA, USA) were used for the extraction study: PDMS (100 µm Polydimethylsiloxane; non-polar), PA (85µm Polyacrylate; high polarity), PDMS/DVB (65µm Polydimethylsiloxane/Carboxen; medium polarity), DVB/Carboxen/PDMS (50/30µm Divinylbenzene/Carboxen/ Polydimethylsiloxane; medium polarity) and Carboxen/PDMS (85µm Carboxen/Polydimethylsiloxane; medium polarity). The fibers were conditioned at the manufacturer’s recommended conditioning temperature before analysis.

**SPME-GC-FID and SPME-GC-MS analyses:** For capillary GC, a Varian CP-3800 gas chromatograph was used, fitted with a DB-5 column (30 m x 0.25 mm i.d., film thickness 0.25 µm). The oven column temperature ranged from 60–240°C, programmed at 3°C/min, with a final hold time of 10 min, using H2 as carrier gas at 1.0 mL/min constant flow, a split ratio of 1:40, and injector and detector (FID) temperatures of 250°C and 280°C, respectively. GC-MS utilized a PerkinElmer AutoSystem XL GC interfaced with a Turbomass Quadrupole mass spectrometer fitted with an Equity-5 fused silica capillary column (60 m x 0.32 mm i.d., film thickness 0.25 µm; Supelco Bellefonte, PA, USA). The oven temperature program ranged from 70–250°C, programmed at 3°C/min,
Volatile organic compounds from flowers of *Jasminum sambac* with initial and final hold time of 2 min, carrier gas He at 10 psi constant pressure, a split ratio of 1:15; injector, transfer line and source temperatures were 250°C; ionization energy 70 eV; mass scan range 40-450 amu. Characterization was achieved on the basis of retention time, elution order, relative retention index using a homologous series of n-alkanes (C₈-C₂₅ hydrocarbons, Polyscience Corp. Niles IL), co-injection with standards in a GC-FID capillary column supplied from Aldrich and Fluka, mass spectra library search (NIIST/EPA/NH version 2.1 and Wiley registry of mass spectral data 7th edition) and by comparing with mass spectral literature data [39]. The relative amounts of individual components were calculated based on GC peak areas without using correction factors.

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