



# Comparative analysis of volatile organic compounds from flowers attractive to honey bees and bumblebees

Aman Dekebo<sup>1,2\*</sup>, Min-Jung Kim<sup>1</sup>, Minwoong Son<sup>3</sup> and Chuleui Jung<sup>1,3\*</sup>

<sup>1</sup>Agricultural Science and Technology Institute, Andong National University, Andong 36729, Republic of Korea

<sup>2</sup>Department of Applied Chemistry, Adama Science and Technology University, Adama, P.O. Box 1888, Ethiopia

<sup>3</sup>Department of Plant Medicals, Andong National University, Andong 36729, Republic of Korea

## ARTICLE INFO

**Received** December 31, 2021

**Revised** January 14, 2022

**Accepted** February 14, 2022

**Published on** March 14, 2022

## \*Corresponding author

Aman Dekebo

**E-mail** amandekeb@gmail.com

Chuleui Jung

**E-mail** cjung@andong.ac.kr

**Background:** Pollinators help plants to reproduce and support economically valuable food for humans and entire ecosystems. However, declines of pollinators along with population growth and increasing agricultural activities hamper this mutual interaction. Nectar and pollen are the major reward for pollinators and flower morphology and volatiles mediate the specialized plant–pollinator interactions. Limited information is available on the volatile profiles attractive to honey bees and bumblebees. In this study we analyzed the volatile organic compounds of the flowers of 9 different plant species that are predominantly visited by honey bees and bumblebees. The chemical compositions of the volatiles were determined using a head space gas chromatography-mass spectrometry (GC-MS) method, designed to understand the plant-pollinator chemical interaction.

**Results:** Results showed the monoterpene 1,3,6-octatriene, 3,7-dimethyl-, (E) (*E*- $\beta$ -ocimene) was the dominating compound in most flowers analyzed, e.g., in proportion of 60.3% in *Lonicera japonica*, 48.8% in *Diospyros lotus*, 38.4% *Amorpha fruticosa* and 23.7% in *Robinia pseudoacacia*. *Ailanthus altissima* exhibited other monoterpenes such as 3,7-dimethyl-1,6-octadien-3-ol ( $\beta$ -linalool) (39.1%) and (5*E*)-3,5-dimethylocta-1,5,7-trien-3-ol (hotrienol) (32.1%) as predominant compounds. Nitrogen containing volatile organic compounds (VOCs) were occurring principally in *Corydalis speciosa*; 1H-pyrrole, 2,3-dimethyl- (50.0%) and pyrimidine, 2-methyl- (40.2%), and in *Diospyros kaki*; 1-triazene, 3,3-dimethyl-1-phenyl (40.5%). *Ligustrum obtusifolium* flower scent contains isopropoxycarbamic acid, ethyl ester (21.1%) and n-octane (13.4%) as major compounds. In *Castanea crenata* the preeminent compound is 1-phenylethanone (acetophenone) (46.7%).

**Conclusions:** Olfactory cues are important for pollinators to locate their floral resources. Based on our results we conclude monoterpenes might be used as major chemical mediators attractive to both honey bees and bumblebees to their host flowers. However, the mode of action of these chemicals and possible synergistic effects for olfaction need further investigation.

**Keywords:** pollinators, honey bees, bumblebees, flowers, monoterpenes,  $\beta$ -ocimene

## Introduction

Given the growing global human population, there is a need to assure food security (Khalifa et al. 2021). With this necessity, there have been many researches dealing with plant-pollinator interactions. However, consequences of plant-pollinator interactions are geographically variable (Hiraiwa and Ushimaru 2017; Johnson et al. 2017; Ollerton 2012; Ollerton 2017; Zanata et al. 2017), and this variation is still not well documented and difficult to elucidate owing to various factors associated with pollination (Ollerton 2012). While many gymnosperm plants depend on the

wind and water for their pollination (Funamoto 2019; Khalifa et al. 2021), animal pollinators including insects, bats and birds have contributed to pollination and seed production of angiosperms. Most of flowering plants are not dependent on just one pollinator, but involve a broad spectrum of pollinators. Among which bees are the major and most important contributors for the production of several leading agricultural crops worldwide (Klein et al. 2007; Proctor and Yeo 1973). It is an accepted fact that bee-pollinated crops contribute approximately one-third of the total human dietary needs.

The reason bees are significant pollinators is due to their



effectiveness and availability. Literature reports indicate that 87 global crops such as cocoa (*Theobroma cacao*), kiwi (*Actinidia deliciosa* var. *deliciosa*), passion fruit (*Passiflora edulis*), and watermelon (*Citrullus lanatus*), used directly for human consumption in the world, are bee pollinators dependent (Khalifa et al. 2021; Klein et al. 2007). Additionally, bee pollination has a considerable impact for crop quality and quantity, improving economic and dietary yields (Khalifa et al. 2021). Therefore, the decline of bees endangers the supply of crops based on insect-pollination.

Exploring the causes of the declines of bees along with their host plants requires identifying signals that mediate their interactions. Foraging bees help moving pollen from one flower to the other and at the same time getting floral rewards such as pollen, nectar, oils, and resins. For getting rewards, bees use visual, olfactory, and tactile floral cues to locate their host plants (Dobson 2017; Giurfa et al. 1996; Kevan and Lane 1985; Lacher 1964; Menzel 1985; Vareschi 1971; Whitney et al. 2009). They are also capable of learning floral signals during their foraging periods (Beekman 2005; Kaiser and De Jong 1993; Menzel et al. 1974; Smith 1991; Zhang et al. 2006). In general bees depend mainly on olfactory and visual cues during their early foraging trips with visual cues becoming increasingly important in host-plant finding for experienced bees due to their learning capability (Dobson 2017).

Volatile organic compounds from host plants are known to influence the interaction, communication, and recruitment between active and inactive workers in the colony of honey bees, bumblebees, and stingless bees (Arenas et al. 2007; Arenas et al. 2008; Díaz et al. 2007; Dornhaus and Chittka 1999; Free 1969; Getz and Smith 1987; Jakobsen et al. 1995; Johnson 1967; Koltermann 1969; Lindauer and Kerr 1960; Molet et al. 2009; Reinhard et al. 2004a; Reinhard et al. 2004b; Ribbands 1954; Wenner et al. 1969). Each bee colony might forage on different flowers and thereby acquire different odours that result in the development of colony-specific patterns of floral odours (Smith and Breed 1995). For example pheromones that contain floral scents

such as 1,8-cineole, (*E,E*)-farnesol and (*Z*)- $\beta$ -ocimene were reported to be most important in recruiting bumblebees (Granero et al. 2005). Benzaldehyde, limonene,  $\beta$ -ocimene and linalool are volatile organic compounds which are common and constitute major components of the floral scents of several species of crop plants (Farré-Armengol et al. 2020). Filella et al. (2013) assume that  $\beta$ -ocimene serves as a generalist pollinator attractant, because it was reported as the predominant volatile compound emitted by plants such as *Muscari neglectum*, *Ranunculus gramineus*, *Euphorbia flavicoma* and *Iris lutescens*. Dobson (2006) has reviewed all available data until 2006 on the use of floral scent by food-seeking bees and found that flowers visited by bees emit odour bouquets which, taken as a group, encompass a huge variety of compounds and compound blends. However, their roles in bee–plant interactions remain poorly understood. Deepening of our knowledge on how plants communicate with pollinators is the basis for more intricate applications of the information in a more sustainable agriculture.

The main aim of the current study was to identify volatile organic compounds scent bouquets, and to characterize which of these compounds might eventually be common or distinct in different flower scents of bee-pollinated plants. Our final goal is to analyze chemical compounds responsible for the attractiveness of these scents to the various bee species.

## Materials and Methods

### Collection of flowers

Bees hosting flowers were collected from the surroundings of Andong National University, Andong, Gyeongsangbuk-do (Republic of Korea), during spring and summer seasons in 2018. We collected 9 different plant species that are predominantly visited by honey bees and bumblebees in each flowering season (Table 1). The plant flowers were selected for this study based on our field observation of

**Table 1** List of 9 selected plants studied and their flower characteristics

| Family         | Plant species                 | Common name     | Code | Flower        |             |         |         |
|----------------|-------------------------------|-----------------|------|---------------|-------------|---------|---------|
|                |                               |                 |      | Color         | Shape       | Nectar* | Pollen* |
| Papaveraceae   | <i>Corydalis speciosa</i> *   | Corydalis Lilac | CS   | Yellow        | Galeate     | N       | N       |
| Ebenaceae      | <i>Diospyros lotus</i>        | Persimmon       | DL   | Yellow        | Open        | E       | E       |
| Ebenaceae      | <i>Diospyros kaki</i>         | Persimmon       | DK   | Yellow        | Open        | E       | E       |
| Oleaceae       | <i>Ligustrum obtusifolium</i> | Border privet   | LO   | White         | Tubular     | T       | T       |
| Fabaceae       | <i>Robinia pseudoacacia</i>   | Black locust    | RP   | White         | Papilionate | E       | G       |
| Fabaceae       | <i>Amorpha fruticosa</i>      | Indigo bush     | AF   | Purple        | Open        | G       | G       |
| Fabaceae       | <i>Castanea crenata</i>       | Chestnut        | CC   | White         | Open        | E       | E       |
| Simaroubaceae  | <i>Ailanthus altissima</i>    | Ailanthus       | AA   | Green         | Open        | R       | R       |
| Caprifoliaceae | <i>Lonicera japonica</i>      | Honeysuckle     | LJ   | Yellow, White | Tubular     | R       | R       |

E: excellent; G: good; T: temporary; R: rarely; DL: *Diospyros lotus*; LJ: *Lonicera japonica*; CS: *Corydalis speciosa*; AA: *Ailanthus altissima*; CC: *Castanea crenata*; DK: *Diospyros kaki*; LO: *Ligustrum obtusifolium*; RP: *Robinia pseudoacacia*.

\**Corydalis speciosa* is an herbaceous plant selected for the study based on our observation in the field this plant flower is a host for bumblebees.

frequent visitation of honey bees and bumble to these flowers. Eight of these plants were trees and only *Corydalis speciosa* is an herbaceous plant. Flowers of *Corydalis speciosa* are frequently visited by bumblebees. Flowers from each plant were collected at the flowering stage (when they were completely open and stigma and anthers were fresh and colored). The flowers were collected in the early morning and immediately transported to the gas chromatography-mass spectrometry (GC-MS) laboratory. Five randomly selected flower samples (2 g) of each were put in 20 mL headspace vials, and immediately sealed with silicone rubber septa and aluminum caps. The sample vials were then placed in the headspace tray. The headspace analysis was done using a GC-MS system which has a headspace auto-sampler, heater and agitator.

### GC-MS analysis

GC-MS analysis was done by using a GC (7890B; Agilent Technologies, Santa Clara, CA, USA) coupled with an MS (5977A Network; Agilent Technologies). The GC had an HP 5MS column (non-polar column, Agilent Technologies), 30 m × 250 µm internal diameter and 0.25 µm film thickness and injection volume was 2 µL. The carrier gas was helium flowing at a rate of 1 mL/min. The temperatures of the Headspace-GC transfer line and the injector were 160°C and 250°C, respectively and the injection mode was a split mode with split ratio 2:1. The initial oven temperature was 40°C held for 5 minutes. It was raised to 250°C at 6°C/min. The total run-time was 40 minutes. Mass spectra were recorded in electron ionization mode at 70 eV, scanning the 50–550 m/z range. Run method for library search: Threshold value 100, scan speed (u/s) 1.562, frequency (scan/sec) 2.9, cycle time (ms) 342.63, step size (m/z) 0.1. Analysis method for library search: Integration parameter peak width value 0.05, threshold value 18.0. The identification of the volatile compounds was performed by comparing the mass spectra of the compounds with those in the database of NIST11 and literature data.

### Statistical analysis

Amount of volatile of organic compounds (VOCs) can potentially be affected by the flower species. Thus, the detected amounts of VOCs of each plant were transformed to compositional proportions to compare between plant species. To classify plant species by extracted VOCs, principal component analysis (PCA) was conducted with the “prcomp” function in R. 4.1.1 (R Core Team 2021). We used proportional values (> 1%) of VOCs from each species for analysis. A hierarchical cluster analysis (HCA) was also performed to evaluate similarity between proportional chemical compositions of sampled species. Ward’s variance minimizing method was applied for this analysis (Ward, 1963) and the result was visualized as a dendrogram. The analysis was conducted using the “hclust” function in R

4.1.1 (R Core Team 2021).

## Results

In this study we analyzed the chemical composition of the floral fragrances of nine flowers of different plant species belonging to 7 families. These plants were selected based on our field observation as host flowers of mainly honey bees and bumblebees. The VOC compositions of the floral scents are shown in Table 2. We identified 173 different VOCs from 9 plant species through the GC-MS analysis.

Out of a total 173 identified VOCs, 83 VOCs, which represents at least 1% in one species, were used for PCA and HCA. Biplot created by principal two components potentially represented that VOC proportion can differentiate flower of plant species (Fig. 1). In Figure 1, 1,3,6-octatriene, 3,7-dimethyl-, (*E*) (*E*- $\beta$ -ocimene) (X68), 1H-pyrrole, 2,3-dimethyl- (X27), 1-phenylethanone (acetophenone) (X69), pyrimidine, 2-methyl- (X32), 3,7-dimethyl-1,6-octadien-3-ol ( $\beta$ -linalool) (X80), 1-triazene, 3,3-dimethyl-1-phenyl- (X16) and (5*E*)-3,5-dimethylocta-1,5,7-trien-3-ol (hotrienol) (X85) were influential in classifying plant species. In particular, compositional values of *E*- $\beta$ -ocimene were important to discriminate between flower types (rich or not) in HCA (Fig. 2, Table 2). Other major compounds (X16, X27, X69, X32, X80, and X85) detected by PCA were specifically found in each plant (Fig. 2, Table 2).

In order to compare the VOC profiles of 9 bee pollinated flowers and aggregate those with similar profiles, the hierarchical clustering method was applied as a dendrogram plot shown in Figure 2. A cut-off distance level indicated a distribution of the flowers in three homogeneous classes. The first cluster includes *Amorpha fruticosa*, *Diospyros lotus*, and *Lonicera japonica* which containing  $\beta$ -ocimene as a common and principal compound in these flowers. *Corydalis speciosa* was clustered separately due its unique nitrogen containing principal compounds, 1H-Pyrrole, 2,3-dimethyl- (50.0%) and pyrimidine, 2-methyl- (40.2%), which is absent in other flowers (Fig. S1, Table 2). The third cluster, which includes *Ailanthus altissima*, *Castanea crenata*, *Diospyros kaki*, *Ligustrum obtusifolium*, and *Robinia pseudoacacia* was classified based on their compositional proportions of  $\beta$ -ocimene (Figs. S1–3) and plant-specific compounds such as  $\beta$ -linalool, hotrienol and acetophenone. The plant species classified as second and third cluster showed generally lower compositional proportions of  $\beta$ -ocimene than the first cluster (Table 2, Fig. 2).

*Ligustrum obtusifolium* flowers scent constitutes isopropoxycarbamic acid, ethyl ester (21.1%) and octane (13.4%) as predominant compounds. Principal volatile compounds in flowers of *Robinia pseudoacacia* were acetamide-2-fluoro- (22.5%) and *E*- $\beta$ -ocimene (21.1%) *Castanea*

**Table 2** Percentage composition of volatile organic compounds of bee host flowers

| RT (min) | No. | Compounds                                                                                                                                                        | CS   | DL   | DK   | LO   | RP   | AF   | CC   | AA   | LJ  |
|----------|-----|------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|------|------|------|------|------|------|------|-----|
| 1.787    | X1  | Bis(dipentylcarbamodithioato-S,S')-                                                                                                                              | -    | -    | -    | -    | -    | -    | -    | 1.0  | -   |
| 1.81     | X2  | 8-Morpholino-4-cycloocten-1-one oxime                                                                                                                            | 0.7  | -    | -    | -    | -    | -    | -    | -    | -   |
| 1.861    | X3  | 3 $\beta$ ,21 $\alpha$ -diacetoxo-18,22,22-trimethyl-17,27,29,30-tetranor-c-homoolean-14-ene                                                                     | -    | -    | -    | -    | -    | -    | -    | -    | 1.1 |
| 1.873    | X4  | Peridine-8-oxide, 6-aldoximino-2-amino-4(3H)-oxo-                                                                                                                | -    | -    | -    | -    | -    | 1.16 | -    | -    | -   |
| 1.879    | X5  | Acetamide, N-isoxazolo[5,4-b]pyridin-3-yl                                                                                                                        | -    | 3.8  | 4.6  | -    | 3.8  | -    | -    | -    | -   |
| 1.884    | X6  | 1,3,3-trimethyl-2-[(1E,3E,5E,7E,9E,11E,13E,15E,17E)-3,7,12,16-tetramethyl-18-(2,6,6-trimethylcyclohexen-1-yl)octadeca-1,3,5,7,9,11,13,15,17-nonaenyl]cyclohexene | -    | -    | -    | -    | -    | -    | 0.89 | -    | -   |
| 2.474    | X7  | Acetamide, 2-amino-N-ethyl-2-thioxo-                                                                                                                             | -    | -    | -    | -    | -    | -    | -    | -    | 1.8 |
| 2.537    | X8  | Ethyl ethanoate                                                                                                                                                  | -    | -    | -    | -    | -    | -    | -    | -    | 2.7 |
| 2.622    | X9  | (Z),(Z)-2,4-Hexadiene                                                                                                                                            | -    | -    | -    | -    | -    | -    | -    | 10.9 | -   |
| 4.105    | X10 | Hydrazine, 1,2-dimethyl-                                                                                                                                         | -    | -    | -    | 4.9  | -    | -    | -    | 0.3  | -   |
| 4.494    | X11 | Hydrazine, 1,1-dimethyl-                                                                                                                                         | -    | -    | -    | -    | -    | -    | -    | -    | 0.3 |
| 4.803    | X12 | Isopropoxycarbamic acid, ethyl ester                                                                                                                             | 0.5  | 2.6  | -    | 21.1 | -    | -    | -    | -    | -   |
| 4.534    | X13 | Ethanoic acid                                                                                                                                                    | -    | -    | 7    | -    | -    | -    | -    | -    | -   |
| 4.648    | X14 | Diaminomethanone                                                                                                                                                 | -    | -    | -    | -    | 2.2  | -    | -    | -    | -   |
| 5.209    | X15 | Propane, 2-chloro-2-nitro-                                                                                                                                       | -    | 18.0 | -    | -    | -    | -    | -    | -    | -   |
| 5.255    | X16 | 1-Triazene, 3,3-dimethyl-1-phenyl-                                                                                                                               | -    | -    | 40.5 | -    | -    | -    | -    | 2.2  | -   |
| 5.363    | X17 | Acetamide, 2-fluoro-                                                                                                                                             | -    | -    | -    | -    | 22.5 | -    | -    | -    | -   |
| 5.421    | X18 | Heptane, 2,4-dimethyl-                                                                                                                                           | -    | -    | -    | -    | -    | 6.76 | -    | -    | -   |
| 5.609    | X19 | Ethyl carbonochloridate                                                                                                                                          | -    | -    | -    | -    | -    | -    | -    | -    | 1.5 |
| 5.947    | X20 | 1-Octane                                                                                                                                                         | -    | -    | -    | 13.4 | -    | 0.17 | 2.2  | 0.6  | 0.7 |
| 6.594    | X21 | 1H-Imidazole, 1,4-dimethyl-                                                                                                                                      | -    | -    | -    | -    | -    | -    | 1.08 | -    | -   |
| 6.811    | X22 | Furan-2-carbaldehyde                                                                                                                                             | -    | -    | -    | -    | -    | -    | 0.2  | -    | -   |
| 7.263    | X23 | 4-Hexen-1-ol, (Z)-                                                                                                                                               | -    | -    | -    | -    | -    | -    | 0.39 | -    | -   |
| 7.275    | X24 | 3-Hexen-1-ol, (Z)-                                                                                                                                               | -    | -    | -    | 4.7  | -    | 0.19 | -    | -    | 5   |
| 7.698    | X25 | 1-Hexanol                                                                                                                                                        | -    | -    | -    | -    | -    | -    | -    | -    | 1.8 |
| 8.213    | X26 | 1,3,5,7-Cyclooctatetraene                                                                                                                                        | -    | -    | -    | -    | -    | -    | 0.38 | 0.8  | -   |
| 8.551    | X27 | 1H-Pyrrole, 2,3-dimethyl-                                                                                                                                        | 50   | -    | -    | -    | -    | -    | -    | -    | -   |
| 8.596    | X28 | Heptanal                                                                                                                                                         | -    | -    | -    | -    | -    | -    | -    | 0.4  | -   |
| 8.642    | X29 | 1-Nonane -                                                                                                                                                       | -    | 2.7  | -    | -    | -    | 0.3  | -    | -    | -   |
| 8.654    | X30 | 2-Furanmethanol                                                                                                                                                  | -    | -    | -    | -    | -    | -    | 0.27 | -    | -   |
| 8.671    | X31 | 2(3H)-Furanone, dihydro-4,5-dimethyl-                                                                                                                            | -    | -    | -    | -    | -    | -    | -    | -    | 0.2 |
| 8.722    | X32 | Pyrimidine, 2-methyl-                                                                                                                                            | 40.2 | -    | -    | -    | -    | -    | -    | -    | -   |
| 9.369    | X33 | 9-Azadispiro[3.1.3.0]nonane                                                                                                                                      | -    | 4.2  | -    | -    | -    | -    | -    | -    | -   |
| 9.432    | X34 | 2-Propanone, 1-(1-cyclohexen-1-yl)-                                                                                                                              | -    | -    | 2.5  | -    | -    | -    | -    | -    | -   |
| 9.609    | X35 | Oxime-, methoxy-phenyl-                                                                                                                                          | -    | 4.7  | 10.9 | 1.3  | 4.3  | -    | -    | -    | 0.2 |
| 9.621    | X36 | (1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene                                                                                                                     | -    | -    | -    | 5.6  | -    | -    | -    | -    | -   |
| 10.061   | X37 | Bicyclo[2.2.1]heptane, 2,2-dimethyl-3-methylene-, (1S)-                                                                                                          | -    | -    | -    | 5.8  | -    | -    | -    | -    | -   |
| 10.399   | X38 | Phenylformaldehyde                                                                                                                                               | 0.3  | -    | -    | -    | -    | -    | 0.16 | -    | 0.1 |

Table 2 Continued

| RT (min) | No. | Compounds                                                                     | CS  | DL   | DK   | LO  | RP   | AF   | CC    | AA  | LJ   |
|----------|-----|-------------------------------------------------------------------------------|-----|------|------|-----|------|------|-------|-----|------|
| 10.708   | X39 | 2-Furancarboxaldehyde, 5-methyl-                                              | -   | -    | -    | -   | -    | -    | 0.63  | -   | -    |
| 10.851   | X40 | 2H-Pyran, 2-ethenyltetrahydro-2,6,6-trimethyl-                                | -   | -    | -    | -   | -    | -    | 1.17  | -   | -    |
| 10.948   | X41 | Bicyclo[4.1.0]hept-3-ene, 3,7,7-trimethyl-                                    | -   | -    | -    | -   | 1.0  | -    | -     | -   | -    |
| 10.908   | X42 | Benzene, 1-azido-4-methyl-                                                    | -   | -    | -    | -   | -    | -    | -     | -   | 0.4  |
| 10.937   | X43 | (1S,5S)-6,6-Dimethyl-2-methylenebicyclo[3.1.1]heptane                         | -   | -    | -    | 2.2 | -    | 0.5  | -     | -   | -    |
| 10.965   | X44 | 1-Hexene, 3,5,5-trimethyl-                                                    | -   | -    | -    | -   | -    | 0.13 | -     | -   | -    |
| 11.154   | X45 | 1-Octen-3-ol                                                                  | -   | -    | -    | -   | 8.1  | 1.41 | -     | -   | -    |
| 11.274   | X46 | 1-Pentanol, 2,2,4-trimethyl-                                                  | -   | -    | -    | -   | -    | 0.29 | -     | -   | -    |
| 11.36    | X47 | 1-Hepten-6-one, 2-methyl-                                                     | -   | -    | -    | -   | -    | -    | -     | -   | 0.2  |
| 11.492   | X48 | 1,6-Octadiene, 7-methyl-3-methylene-                                          | -   | -    | -    | -   | -    | 4.68 | -     | -   | 0.1  |
| 11.503   | X49 | Furan, 2-pentyl-                                                              | -   | -    | -    | -   | 1.0  | -    | -     | -   | -    |
| 11.566   | X50 | 5-Hepten-2-ol, 6-methyl-                                                      | -   | -    | -    | -   | -    | -    | -     | -   | 0.2  |
| 11.789   | X51 | Octanal                                                                       | -   | -    | -    | -   | -    | 0.2  | 0.87  | -   | -    |
| 11.944   | X52 | 3,6-Bis(N-dimethylamino)-9-ethylcarbazole                                     | 0.2 | -    | -    | -   | -    | -    | 3.36  | -   | -    |
| 11.95    | X53 | 7H-Dibenzolb,glcarbazole, 7-methyl-                                           | -   | -    | -    | -   | -    | 0.85 | -     | -   | -    |
| 11.955   | X54 | trans-4-(2-(5-Nitro-2-furyl)vinyl)-2-quinolinamine                            | -   | -    | -    | -   | 0.4  | -    | -     | -   | -    |
| 11.978   | X55 | 1,3,8-p-Menthatriene                                                          | -   | -    | -    | -   | -    | -    | -     | 1.0 | 3.1  |
| 11.99    | X56 | 3-Hexen-1-ol, acetate, (E)-                                                   | -   | -    | -    | 8.1 | -    | -    | -     | -   | -    |
| 12.184   | X57 | 1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-                               | -   | -    | -    | -   | -    | -    | 0.25  | -   | -    |
| 12.447   | X58 | cis-5,8,11,14,17-Eicosapentaenoic acid                                        | -   | -    | -    | -   | -    | -    | -     | 0.3 | -    |
| 12.47    | X59 | Pyridine, 3-methyl-                                                           | 0.7 | -    | -    | -   | -    | -    | -     | -   | -    |
| 12.539   | X60 | (4R)-1-methyl-4-prop-1-en-2-ylcyclohexene                                     | -   | -    | -    | 2.2 | -    | -    | 1.6   | 0.2 | -    |
| 12.573   | X61 | 2,4,6-Octatriene, 2,6-dimethyl-                                               | -   | -    | -    | -   | -    | 0.43 | -     | -   | 0.3  |
| 12.756   | X62 | Undecane, 4,7-dimethyl-                                                       | -   | -    | -    | -   | -    | 0.1  | -     | -   | -    |
| 12.762   | X63 | Eicosane                                                                      | -   | -    | -    | 1   | -    | -    | -     | -   | -    |
| 12.876   | X64 | 1,3,6-Octatriene, (Z,E)-                                                      | -   | -    | -    | -   | -    | -    | 1.8   | -   | -    |
| 12.939   | X65 | 2-phenylacetaldehyde                                                          | -   | -    | -    | -   | -    | -    | -     | 0.6 | -    |
| 13.048   | X66 | Phenylmethanol                                                                | -   | -    | -    | -   | -    | -    | 0.59  | -   | 1.1  |
| 13.163   | X67 | 4,7,7-trimethylbicyclo[4.1.0]hept-2-ene                                       | -   | -    | -    | -   | -    | 8.61 | -     | -   | -    |
| 13.168   | X68 | 1,3,6-Octatriene, 3,7-dimethyl-, (E) -                                        | -   | 48.8 | 11.1 | 1.3 | 23.7 | 38.4 | 0.94  | 1.3 | 60.3 |
| 13.54    | X69 | 1-phenylethanone                                                              | -   | -    | -    | -   | -    | -    | 46.68 | -   | -    |
| 13.603   | X70 | Ethanone, 2-hydroxy-1,2-diphenyl-, oxime                                      | -   | -    | -    | -   | -    | -    | -     | 0.5 | -    |
| 13.684   | X71 | 2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-                                    | -   | -    | -    | -   | -    | -    | -     | -   | 0.3  |
| 13.809   | X72 | 2-hydroxy-1,2-diphenylethanone                                                | -   | -    | -    | -   | -    | -    | 2.26  | -   | -    |
| 13.82    | X73 | 5-(3,3-dimethyloxiran-2-yl)-3-methylpent-1-en-3-ol                            | -   | -    | -    | -   | -    | -    | -     | 1.9 | -    |
| 13.821   | X74 | 1-Octanol                                                                     | -   | -    | -    | -   | -    | 0.45 | -     | -   | -    |
| 13.826   | X75 | 2-Furanmethanol, 5-ethenyltetrahydro- $\alpha$ , $\alpha$ ,5-trimethyl-, cis- | -   | -    | -    | -   | 0.9  | -    | -     | -   | -    |
| 14.032   | X76 | 3-Ethyl-1,5-octadiene                                                         | -   | -    | -    | -   | -    | -    | -     | -   | 0.5  |
| 14.238   | X77 | 3,7,7-trimethylbicyclo[4.1.0]hept-2-ene                                       | -   | -    | -    | -   | -    | -    | -     | -   | -    |
| 14.255   | X78 | Ethyl 2-(5-methyl-5-vinyltetrahydrofuran-2-yl)propan-2-yl carbonate           | -   | -    | -    | -   | -    | 0.27 | 1.82  | -   | -    |

Table 2 Continued

| RT (min) | No.  | Compounds                                                                                       | CS  | DL  | DK   | LO  | RP   | AF   | CC   | AA   | LJ  |
|----------|------|-------------------------------------------------------------------------------------------------|-----|-----|------|-----|------|------|------|------|-----|
| 14.582   | X79  | 1,3,7-Octatriene, 3,7-dimethyl-                                                                 | -   | 1.5 | -    | 2.6 | 16.6 | -    | -    | -    | 0.1 |
| 14.587   | X80  | 3,7-Dimethyl-1,6-octadien-3-ol                                                                  | -   | -   | -    | -   | -    | -    | -    | 39.1 | -   |
| 14.57    | X81  | 1,6-Octadien-3-ol, 3,7-dimethyl-                                                                | -   | -   | -    | -   | -    | -    | 6.61 | -    | -   |
| 14.667   | X82  | 1-Methoxy-3-hydroxymethylheptane                                                                | -   | -   | -    | -   | -    | -    | 1.19 | -    | -   |
| 14.679   | X83  | Nonanal                                                                                         | -   | -   | -    | -   | -    | 1.46 | -    | -    | -   |
| 14.69    | X84  | 7-Octen-1-ol, 3,7-dimethyl-, (S)-                                                               | -   | -   | -    | -   | -    | -    | -    | -    | 0.3 |
| 14.7     | X85  | (5 <i>E</i> -3,5-dimethylocta-1,5,7-trien-3-ol                                                  | -   | -   | -    | -   | -    | -    | -    | 32.1 | -   |
| 14.702   | X86  | 1-Cyclohexylnonene                                                                              | -   | 1.1 | -    | 4.3 | -    | -    | -    | -    | -   |
| 15.028   | X87  | 2-Phenylethan-1-ol                                                                              | -   | -   | -    | -   | 0.8  | 0.94 | -    | -    | 1.6 |
| 15.039   | X88  | Fluoren-9-ol, 3,6-dimethoxy-9-(2-phenylethynyl)-                                                | -   | -   | -    | -   | 2.6  | -    | 0.72 | -    | -   |
| 15.045   | X89  | 1,7-Octadien-3-one, 2-methyl-6-methylene-                                                       | -   | -   | -    | -   | 3.5  | -    | -    | -    | -   |
| 15.182   | X90  | 2,6-Dimethyl-1,3,5,7-octatetraene, <i>E,E</i> -                                                 | -   | -   | -    | -   | -    | -    | -    | 0.8  | -   |
| 15.262   | X91  | Octanoic acid, methyl ester                                                                     | -   | -   | -    | -   | -    | -    | -    | -    | 0.2 |
| 15.36    | X92  | Bicyclo[3.1.0]hex-2-ene, 4,4,6,6-tetramethyl-                                                   | -   | -   | -    | -   | -    | 9.2  | -    | -    | -   |
| 15.405   | X93  | 1,4-Cyclohexadiene, 3-ethenyl-1,2-dimethyl-                                                     | -   | -   | -    | -   | -    | -    | -    | 2.6  | -   |
| 15.531   | X94  | 2-phenylacetone nitrile                                                                         | -   | -   | -    | -   | -    | -    | -    | -    | 3.4 |
| 15.703   | X95  | Cyclopropane, trimethyl(2-methyl-1-propenylidene)-                                              | -   | -   | -    | -   | -    | 0.28 | -    | -    | -   |
| 15.943   | X96  | 1 <i>H</i> -Indole, 1,3-dimethyl-5,6-dimethoxy-2-(3,5-dimethoxyphenyl)-                         | -   | -   | -    | -   | -    | -    | 0.21 | 0.4  | -   |
| 16.006   | X97  | Cyclobutane, methylene-                                                                         | -   | -   | -    | -   | -    | -    | 0.2  | -    | -   |
| 16.218   | X98  | 5 <i>H</i> -Naphtho[2,3- <i>b</i> ]carbazole                                                    | 0.3 | 0.5 | -    | 4.4 | -    | 0.68 | 3.74 | -    | -   |
| 16.224   | X99  | <i>N,N</i> -Dimethyl- <i>N'</i> -(10-propyl-10 <i>H</i> -acridin-9-ylidene)-benzene-1,4-diamine | -   | -   | -    | -   | 0.5  | -    | -    | -    | -   |
| 16.401   | X100 | Benzoic acid, ethyl ester                                                                       | -   | -   | -    | -   | -    | -    | -    | -    | 0.4 |
| 16.498   | X101 | 3-Decyn-1-ol                                                                                    | -   | -   | -    | -   | -    | -    | -    | 0.6  | -   |
| 16.561   | X102 | 4-(4-Chlorophenyl)-2,6-diphenylpyridine                                                         | -   | -   | -    | -   | -    | -    | 0.15 | -    | -   |
| 16.905   | X103 | 2-(4-methylcyclohex-3-en-1-yl)propan-2-ol                                                       | -   | -   | -    | -   | -    | -    | 3.55 | 0.4  | -   |
| 17.534   | X104 | 6-Methyl-2-pyridinecarbaldehyde                                                                 | -   | -   | -    | -   | 0.6  | -    | -    | -    | -   |
| 17.546   | X105 | 3-Cyclohexene-1-acetaldehyde, $\alpha$ ,4-dimethyl-                                             | -   | -   | -    | -   | -    | -    | 0.56 | -    | -   |
| 17.872   | X106 | 3,7-dimethyloct-6-en-1-ol                                                                       | -   | -   | -    | -   | -    | 0.13 | -    | -    | 0.1 |
| 17.855   | X107 | 5-Amino-7-(4-methoxy-phenyl)-2-phenyl-pyrazolo[1,5- <i>a</i> ]pyrimidine-6-carbonitrile         | -   | -   | -    | -   | -    | -    | 0.23 | -    | -   |
| 17.952   | X108 | 2,4-Hexadiene, ( <i>E,Z</i> )-                                                                  | -   | -   | -    | -   | -    | -    | 0.24 | -    | -   |
| 17.958   | X109 | 3-Hexene, 2,5-dimethyl-, ( <i>E</i> )-                                                          | -   | -   | -    | -   | -    | 0.21 | -    | -    | -   |
| 17.963   | X110 | Cyclohexane, chloro-                                                                            | -   | -   | -    | -   | -    | -    | -    | -    | 0.1 |
| 17.98    | X111 | 2-Thiophenemethanamine                                                                          | -   | -   | -    | -   | -    | -    | -    | 0.2  | -   |
| 18.072   | X112 | 1,3-Pentadiene, 2-methyl-, ( <i>E</i> )-                                                        | -   | -   | -    | -   | -    | -    | -    | -    | 0.2 |
| 18.484   | X113 | (2-Nonyloxy-benzyl)-phenyl-amine                                                                | -   | -   | -    | -   | -    | -    | 1.02 | -    | -   |
| 18.558   | X114 | 2-Thiazoline, 5-methyl-2-(2-pyridylmethyl)amino-                                                | -   | -   | -    | -   | -    | -    | -    | 0.2  | -   |
| 20.092   | X115 | ( <i>E</i> )-Hex-3-enyl ( <i>E</i> )-2-methylbut-2-enoate                                       | -   | -   | -    | -   | -    | -    | -    | -    | 3.7 |
| 20.229   | X116 | 3-Methyl-2-butenic acid, octadecyl ester                                                        | -   | -   | -    | -   | -    | -    | -    | -    | 1.9 |
| 20.315   | X117 | 1-hexyl-3-(naphthalen-1-ylmethyl)-1 <i>H</i> -indole                                            | 3.1 | 5.5 | 11.8 | 9.2 | -    | 0.86 | 3.78 | -    | -   |

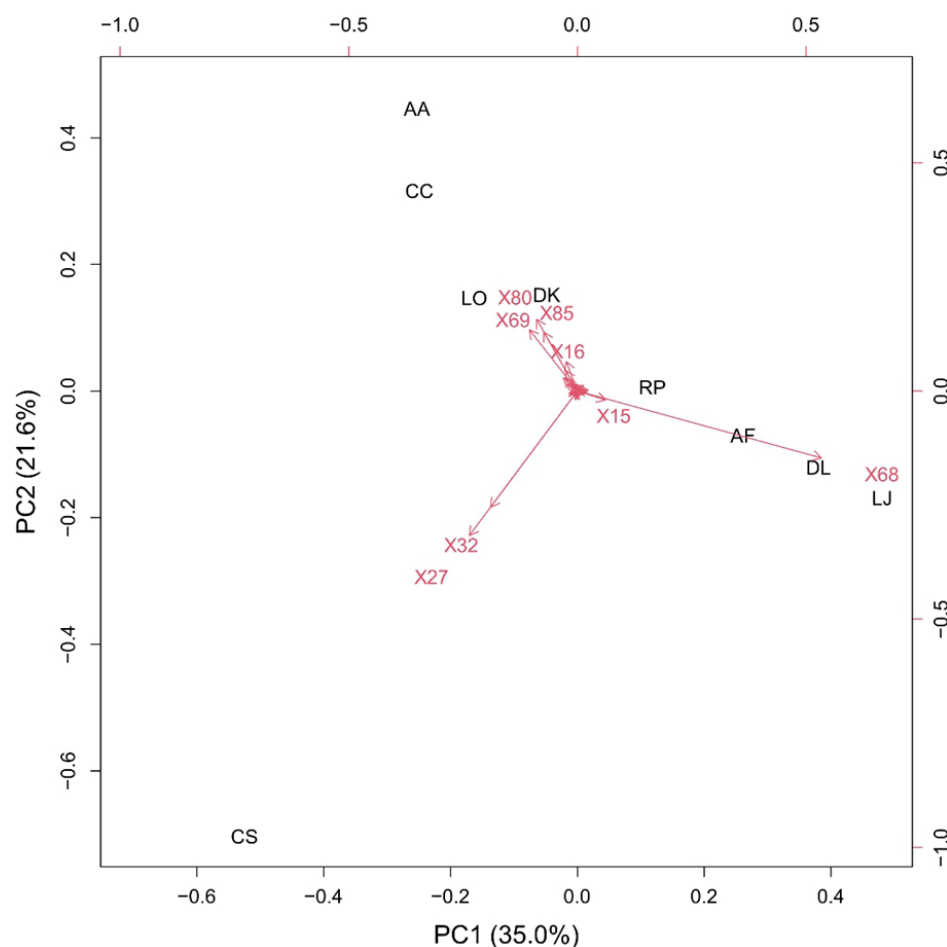
Table 2 Continued

[illegible]

Table 2 Continued

| RT (min) | No.  | Compounds                                                                                  | CS  | DL  | DK  | LO | RP  | AF   | CC   | AA  | LJ  |
|----------|------|--------------------------------------------------------------------------------------------|-----|-----|-----|----|-----|------|------|-----|-----|
| 25.133   | X152 | 2,6,10,14-Hexadecatetraen-1-ol, 3,7,11,15-tetramethyl-, acetate, (E,E,E)-                  | -   | -   | -   | -  | 0.6 | -    | -    | -   | -   |
| 25.425   | X153 | Isolongifolene, 9,10-dehydro-                                                              | -   | -   | -   | -  | -   | 0.16 | -    | -   | -   |
| 25.717   | X154 | 2,2,4a-trimethyl-8-methylidene-2a,3,4,5,6,7-hexahydro-1H-cyclobuta[[i]indene               | -   | -   | -   | -  | -   | 0.83 | -    | -   | 0.6 |
| 25.774   | X155 | 2-Nitrophenyl n-butyrate                                                                   | 0.3 | -   | -   | -  | -   | -    | -    | -   | -   |
| 26.152   | X156 | 1H-3a,7-Methanoazulene, 2,3,4,7,8,8a-hexahydro-3,6,8,8-tetramethyl-, [3R-(3a,3ab,7b,8aa)]- | -   | -   | -   | -  | -   | 0.09 | -    | -   | -   |
| 26.369   | X157 | 1,2-Bis(bicyclo[2.2.1]hept-2-yl)ethane                                                     | -   | -   | -   | -  | 0.4 | -    | -    | -   | -   |
| 26.398   | X158 | (1R,2R,5S)-2,6,8-tetramethyltricyclo[5.3.1.0 <sup>1,5</sup> ]undec-8-ene                   | -   | -   | -   | -  | -   | 0.11 | -    | -   | -   |
| 26.461   | X159 | 2-Isopropenyl-4a,8-dimethyl-1,2,3,4,4a,5,6,7-octahydronaphthalene                          | -   | -   | -   | -  | -   | -    | -    | -   | 0.1 |
| 26.638   | X160 | 1,1,4,7-tetramethyl-1a,2,3,4,5,6,7,7b-octahydrocyclopropa[e]azulene                        | -   | -   | -   | -  | -   | 0.12 | -    | -   | -   |
| 27.239   | X161 | Dibutanoylmorphine                                                                         | -   | -   | -   | -  | -   | 0.35 | 0.96 | -   | -   |
| 27.245   | X162 | 2,4(1H,3H)-Quinolinedione, 3-benzoyl-3-(phenylmethyl)-                                     | 0.4 | 0.8 | 1.4 | -  | -   | -    | -    | -   | -   |
| 27.25    | X163 | Pyrazole, 3-tert-butyl-5-(4-chlorophenyl)-1-(4-nitrophenyl)-                               | -   | -   | -   | -  | 1.1 | -    | -    | -   | 0.1 |
| 30.088   | X164 | 3β-Acetoxy-16-isothiocyatanopregn-5-en-20-one                                              | 0.2 | -   | -   | -  | -   | 0.11 | -    | -   | -   |
| 30.054   | X165 | Cholestan-7-one, cyclic 1,2-ethanediyl acetal, (5α-)-                                      | -   | -   | -   | -  | -   | -    | 1.51 | -   | -   |
| 30.867   | X166 | 3-Amino-4-methylbenzoic acid                                                               | 0.3 | -   | -   | -  | -   | -    | -    | -   | -   |
| 31.616   | X167 | methyl hexadecanoate                                                                       | -   | -   | -   | -  | -   | -    | -    | -   | 0.7 |
| 32.629   | X168 | 9,10-Methanoanthracen-11-ol, 9,10-dihydro-9,10,11-trimethyl-                               | 0.1 | -   | -   | -  | -   | -    | -    | -   | -   |
| 35.318   | X169 | Phenylacetic acid, 2-(1-adamantylo)ethyl ester                                             | 0.1 | -   | -   | -  | -   | -    | -    | -   | -   |
| 37.086   | X170 | Thiocyanic acid, 5α-cholestan-3β-yl ester                                                  | -   | -   | -   | -  | -   | -    | 0.41 | -   | -   |
| 39.112   | X171 | 5-Methyl-2-phenylindolizine                                                                | -   | -   | -   | -  | -   | -    | 0.32 | -   | -   |
| 39.243   | X172 | Benzo[H]quinoline, 2,4-dimethyl-                                                           | -   | -   | -   | -  | -   | -    | -    | 0.3 | -   |

CS: *Corydalis speciosa*; DL: *Diospyros lotus*; DK: *Diospyros kaki*; LO: *Ligustrum obtusifolium*; AF: *Amorpha fruticosa*; CC: *Castanea crenata*; AA: *Ailanthus altissima*; LJ: *Lonicera japonica*; RP: *Robinia pseudoacacia*.



**Fig. 1** Biplot created by principal component analysis (PCA) using compositional proportions (> 1%) of VOCs from plant species. Xn represents the chemical number assigned to each VOC in Table 2. VOCs: volatile of organic compounds; AF: *Amorpha fruticosa*; DL: *Diospyros lotus*; LJ: *Lonicera japonica*; CS: *Corydalis speciosa*; AA: *Ailanthus altissima*; CC: *Castanea crenata*; DK: *Diospyros kaki*; LO: *Ligustrum obtusifolium*; RP: *Robinia pseudoacacia*.



**Fig. 2** Dendrogram created by hierarchical cluster analysis (HCA) using compositional proportions (> 1%) of VOCs from plant species. VOCs: volatile of organic compounds; AF: *Amorpha fruticosa*; DL: *Diospyros lotus*; LJ: *Lonicera japonica*; CS: *Corydalis speciosa*; AA: *Ailanthus altissima*; CC: *Castanea crenata*; DK: *Diospyros kaki*; LO: *Ligustrum obtusifolium*; RP: *Robinia pseudoacacia*.

*crenata* has acetophenone (46.68%) as the principal compound (Table 2, Fig. S2).

*Amorpha fruticosa* was identified to have the most diverse VOCs of the 9 plants through this study (Fig. S2, Ta-

ble 2). This species was classified as a  $\beta$ -ocimene rich group, but also have other monoterpene [4,7,7-trimethylbicyclo[4.1.0]hept-2-ene (4-carene), 3,7,7-trimethylbicyclo[4.1.0]hept-2-ene (2-carene), 1,6-octadiene, 7-methyl-3-

methylene ( $\beta$ -myrcene), (1S,5S)-6,6-dimethyl-2-methylenebicyclo[3.1.1]heptane ( $\beta$ -pinene)] and sesquiterpenes (1S,2S)-1-ethenyl-1-methyl-2-(prop-1-en-2-yl)-4-(propan-2-ylidene)cyclohexane ( $\gamma$ -elemene), 4,10-dimethyl-7-(propan-2-yl)tricyclo[4.4.0.0]dec-3-ene ( $\alpha$ -cubebene), (1S,6S,7S,8S)-1,3-dimethyl-8-propan-2-yltricyclo[4.4.0.0<sup>2,7</sup>]dec-3-ene ( $\alpha$ -copaene), 1-methyl-5-methylidene-8-(propan-2-yl)tricyclo[5.3.0.0]decane ( $\beta$ -bourbonene), 1H,1aH,2H,3H,4H,4aH,5H,6H,7bH-cyclopropa[e]azulene ( $\alpha$ -gurjunene), (1S,6R,7R,8S)-1-methyl-3-methylidene-8-(propan-2-yl)tricyclo[4.4.0.0]decane ( $\beta$ -ylangene), (1R,4E,9S)-4,11,11-trimethyl-8-methylidenebicyclo[7.2.0]undec-4-ene (caryophyllene), 4-methyl-7-methylidene-1-(propan-2-yl)-1,2,3,4,4a,5,6,7-octahydronaphthalene ((+)-epi-bicyclosquiphellandrene), 4,7-dimethyl-1-(propan-2-yl)-1,2,3,4,4a,5-hexahydronaphthalene (*cis*-muurola-3,5-diene), tricyclo[4.4.0.02,7]decane, 1-methyl-3-methylene-8-(1-methylethyl)-, (1R,2S,6S,7S,8S)-rel ( $\beta$ -copaene), 5,5,11,11-tetramethyltricyclo[6.2.1.0]undeca-2,6-diene (neoisolongifolene), 1,6-dimethyl-4-propan-2-yl-1,2,3,7,8,8a-hexahydronaphthalen (epizonarene), 1,3,6,10-dodecatetraene, 3,7,11-trimethyl-, (*E,E*) ( $\alpha$ -farnesene), naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-, (1a,4aa,8aa) ( $\gamma$ -muurolene), naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1a,4aa,8aa)- ( $\alpha$ -muurolene), isolongifolene, 9,10-dehydro, 1H-3a,7-methanoazulene, 2,3,4,7,8,8a-hexahydro-3,6,8,8-tetramethyl-, [3R-(3a,3ab,7b,8aa)]- (cedrene), (1R,2R,5S)-2,6,6,8-tetramethyltricyclo[5.3.1.0<sup>1,5</sup>]undec-8-ene (7-epi-  $\alpha$ -cedrene, isodedene)].

## Discussion

Our results showed there are differences in the emission of floral volatile organic compounds among the nine bees host flowers investigated in this study. However, monoterpene, *E*- $\beta$ -ocimene was the principal component in volatilities of *Lonicera japonica* (60.3%) *Diospyros lotus* (48.8%), *Amorpha fruticosa* (38.4%), and *Robinia pseudoacacia* (23.7%) (Table 2, Figs. S1–3). Similar studies also indicated that *Ranunculus acris* (Farré-Armengol et al. 2013), *Mirabilis jalapa* (Effmert et al. 2005), snapdragon (*Antirrhinum majus*) (Dudareva et al. 2003) flowers have a floral scent dominated by  $\beta$ -ocimene. It was reported that *E*- $\beta$ -ocimene acts as a brood pheromone of bees like *Apis mellifera* and by sending signals to worker bees to carry out foraging activities, food collection, storage and processing for colony and larvae (Maisonasse et al. 2010). *E*- $\beta$ -ocimene was also identified to act as primer pheromone on worker bees by inhibiting maturation of their ovaries. Emission of *E*- $\beta$ -ocimene by honey bee larvae may prevent workers (especially nurses) from utilizing food into egg production instead of taking care of the larvae (Maisonasse et al. 2009).

Several reports indicate a role of  $\beta$ -ocimene in pollinator attraction in addition to its high occurrence in floral scents. Research conducted on  $\beta$ -ocimene shows it can effectively attract honey bees and bumblebees (Granero et al. 2005; Pecetti et al. 2002). We believe the reason for the honey bees' attraction to flowers which have  $\beta$ -ocimene as a major volatile organic compound might be due to their correlating the aroma of the flower's volatiles with those of their brood pheromone in the hive. There was a report that emission rates of trans- $\beta$ -ocimene from the flowers of snapdragon and *Satsuma mandarin* vary depending on the floral growth stage. Maximum emissions were recorded when flowers were fully open, but decreased emissions were observed in later stages until fruit development (Dudareva et al. 2003; Shimada et al. 2005). Therefore, it is assumed that emitting  $\beta$ -ocimene might be a strategy of the plant such as the first cluster in Figure 2 for attracting bees for pollination during its flowering period.

Dötterl and Vereecken (2010) reviewed floral scent eliciting positive behavioral responses in bee species such as *Apis mellifera*, *Bombus terrestris*, and *Euglossini* sp. These workers reviewed mainly monoterpenes that showed positive responses to bee species. In the current study, *Ailanthus altissima* also contained monoterpenes such as  $\beta$ -linalool (39.1%) and hotrienol (32.1%) as predominant compounds. Similar studies indicated linalool is a principal compound in *Daphne mezereum* (95%). Linalool might be a general attractant of solitary bees, acting both as a food and a sexual attractant of male bees (Borg-Karlson et al. 1996). It was also reported that  $\beta$ -linalool was a major compound in alfalfa (Fabaceae) floral volatile eliciting a positive behavioral response to *Apis mellifera* (Henning and Teuber 1992). We believe that this might be the reason for the frequent visits of *Ailanthus altissima* by honey bees. Solitary bees and bumble-bees produce linalool in their mandibular glands, i.e., a pheromone that causes the males to aggregate (Borg-Karlson et al. 1996). Ocimene was reported to elicit positive responses to *Bombus terrestris* and linalool elicited positive responses to *Apis mellifera* (Dötterl and Vereecken 2010), observations that support our findings.

Major components in VOC of *Corydalis speciosa*, *Diospyros kaki*, and *Ligustrum obtusifolium* are nitrogen containing compounds, namely 1H-pyrrole, 2,3-dimethyl- (50.0%) and pyrimidine, 2-methyl- (40.2%) in *Corydalis speciosa* and 1-triazene, 3,3-dimethyl-1-phenyl (40.5%) in *Diospyros kaki* and isopropoxycarbamic acid, ethyl ester (21.1%) in *Ligustrum obtusifolium*. Nitrogen bearing compounds such as phenylacetonitrile amounted to 0%–11.8% and 0%–6.8% in male and female inflorescences of *Salix caprea* (Dötterl et al. 2014). Additionally, the nitrogenous compound indole was determined as 0–1.5% and 0.4–3.2% in male and female inflorescences of *Salix caprea* (Dötterl et al. 2014).

Although not identified as ‘major’, some volatile organic compounds in the current study were reported to elicit positive behavioral responses in experienced bees or those for whom experience was not investigated (Dötterl and Vereecken 2010). These compounds include benzyl alcohol identified in the flower scent of *Castanea crenata* and *Lonicera japonica* and were previously reported to elicit positive response in *Apis mellifera* (Dötterl and Vereecken 2010; Williams and Whitten 1983). 3-Carene identified in *Robinia pseudoacacia*. (*E,E*)- $\alpha$ -farnesene and phenylethyl alcohol identified in flowers of *Lonicera japonica*, *Robinia pseudoacacia* and *Amorpha fruticosa* were reviewed to elicit positive responses of *Apis mellifera*.

Floral emission of volatiles is a complex phenomenon that leads mixtures of organic compounds, having a high variable constituents and high relative amounts of compounds. Both these two aspects may play important roles in pollinators, especially insects’ attraction. It has been noted that some monoterpenes including  $\beta$ -pinene and (*E*)- $\beta$ -ocimene (Gong et al. 2015) may elicit strong antennae responses in some insects. D-Limonene, Benzaldehyde and O-cymene, the three compounds in male flowers that were significantly attractive and  $\alpha$ -pinene repellent to honey bees (Fernandes 2019). In the current study even though it is not major, *Ligustrum obtusifolium*, *Castanea crenata*, and *Ailanthus altissima* constitute D-limonene in the relative percentage of 2.2%, 1.6%, and 0.2%, respectively. The amount of this compound in these plant flowers might be sufficient to attract the bee species which need further studies.

Additionally, apart from a species-specific response, relative percentages in blends may play a crucial role and even non-active compounds can enhance the attractiveness of other volatiles by lowering the active threshold dose of active ones (Chen and Song 2008).

## Conclusions

Insects use both olfactory and visual cues to find flowers (Raguso and Willis 2002), offering pollinators decoupled cues or a combination of both cues. Some insects respond more to visual (Balkenius et al. 2006; Dötterl et al. 2011; Omura and Honda 2005; Roy and Raguso 1997) and others more to olfactory cues (Dötterl et al. 2011), but it would seem that in many species a combination of both cues is needed to elicit specific behavioral responses. The study results from the visual, olfactory and combined experiments on *Salix caprea* by (Dötterl et al. 2014) showed that visual and olfactory cues are also important for naive honey bees to find their host. Bees distinctively respond to decoupled olfactory and visual cues of the flower, but olfactory cues were more attractive than visual cues alone, but most honey bees were attracted to a combination of both

cues rather than either of them alone.

Based on our results we conclude that monoterpenes might be used as major chemical mediators for honey bees and bumblebees in locating their host flowers. Among monoterpenes, *E*- $\beta$ -ocimene was found as a common and major compound in most plant flowers that we analyzed. However, whether scents of major compounds or synergistic effects of mixtures of compounds identified in the flowers are responsible for olfaction needs to be further investigated.

## Supplementary Information

Supplementary information accompanies this paper at <https://doi.org/10.5141/jee.21.001>

**Fig. S1.** Chromatographic profiles of *Diospyros lotus*, *Diospyros kaki*, and *Corydalis speciosa*. **Fig. S2.** Chromatographic profiles of *Robinia pseudoacacia*, *Amorpha fruticosa*, and *Castanea crenata*. **Fig. S3.** Chromatographic profiles of *Ligustrum obtusifolium*, *Ailanthus altissima*, and *Lonicera japonica*.

### Abbreviations

VOC: Volatile of Organic compounds

GC-MS: Gas chromatography-mass spectrometry

HP 5MS: HP-5 5% Diphenyl/95% Dimethylpolysiloxane

EI: Electron ionization

NIST: The National Institute of Standards and Technology

### Acknowledgements

We appreciate Prof. Victor Benno Meyer-Rochow for English correction and proof reading of the manuscript.

### Authors’ contributions

AD and CJ designed the study together. AD submitted the samples for analysis and analyzed the data and wrote the manuscript. MK and MS did the statistical analysis and wrote the manuscript. CJ reviewed and edited the manuscript. Both authors read and approved the final manuscript.

### Funding

This work was partly supported by National Research Foundation of Korea (NRF-2018R1A6A1A03024862) and Rural Development Administration (RDA, PJ0157462021).

### Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author (Prof. Chuleui Jung) on reasonable request.

### Ethics approval and consent to participate

Not applicable.

## Consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

## References

- Arenas A, Fernández VM, Farina WM. Floral odor learning within the hive affects honeybees' foraging decisions. *Naturwissenschaften*. 2007;94(3):218–22. <https://doi.org/10.1007/s00114-006-0176-0>.
- Arenas A, Fernández VM, Farina WM. Floral scents experienced within the colony affect long-term foraging preferences in honeybees. *Api-dologie*. 2008;39(6):714–22. <https://doi.org/10.1051/apido:2008053>.
- Balkenius A, Rosén W, Kelber A. The relative importance of olfaction and vision in a diurnal and a nocturnal hawkmoth. *J Comp Physiol A Neuroethol Sens Neural Behav Physiol*. 2006;192(4):431–7. <https://doi.org/10.1007/s00359-005-0081-6>.
- Beekman M. How long will honey bees (*Apis mellifera* L.) be stimulated by scent to revisit past-profitable forage sites? *J Comp Physiol A Neuroethol Sens Neural Behav Physiol*. 2005;191(12):1115–20. <https://doi.org/10.1007/s00359-005-0033-1>.
- Borg-Karlson AK, Unelius CR, Valterová I, Nilsson LA. Floral fragrance chemistry in the early flowering shrub *Daphne mezereum*. *Phytochemistry*. 1996;41(6):1477–83. [https://doi.org/10.1016/0031-9422\(95\)00801-2](https://doi.org/10.1016/0031-9422(95)00801-2).
- Chen C, Song Q. Responses of the pollinating wasp *Ceratosolen solmsi* marchali to odor variation between two floral stages of *Ficus hispida*. *J Chem Ecol*. 2008;34(12):1536–44. <https://doi.org/10.1007/s10886-008-9558-4>.
- Díaz PC, Grüter C, Farina WM. Floral scents affect the distribution of hive bees around dancers. *Behav Ecol Sociobiol*. 2007;61(10):1589–97.
- Dobson HE. Floral volatiles in insect biology. In: Bernays EA, editor. *Insect-plant interactions*. 5th ed. Boca Raton: CRC press; 2017. p. 47–82.
- Dobson HE. Relationship between floral fragrance composition and type of pollinator. In: Dudareva N, Pichersky E, editors. *Biology of floral scent*. Boca Raton: CRC press; 2006. p. 147–98.
- Dornhaus A, Chittka L. Evolutionary origins of bee dances. *Nature*. 1999;401(6748):38. <https://doi.org/10.1038/43372>.
- Dötterl S, Glück U, Jürgens A, Woodring J, Aas G. Floral reward, advertisement and attractiveness to honey bees in dioecious *Salix caprea*. *PLoS One*. 2014;9(3):e93421. <https://doi.org/10.1371/journal.pone.0093421>.
- Dötterl S, Milchreit K, Schäffler I. Behavioural plasticity and sex differences in host finding of a specialized bee species. *J Comp Physiol A Neuroethol Sens Neural Behav Physiol*. 2011;197(12):1119–26. <https://doi.org/10.1007/s00359-011-0673-2>.
- Dötterl S, Vereecken N. The chemical ecology and evolution of bee-flow-er interactions: a review and perspectives. *Can J Zool*. 2010;88(7):668–97. <https://doi.org/10.1139/Z10-031>.
- Dudareva N, Martin D, Kish CM, Kolosova N, Gorenstein N, Fäldt J, et al. (E)-beta-ocimene and myrcene synthase genes of floral scent bio-synthesis in snapdragon: function and expression of three terpene synthase genes of a new terpene synthase subfamily. *Plant Cell*. 2003;15(5):1227–41. <https://doi.org/10.1105/tpc.011015>.
- Effmert U, Große J, Röse US, Ehrig F, Kägi R, Piechulla B. Volatile composition, emission pattern, and localization of floral scent emission in *Mirabilis jalapa* (Nyctaginaceae). *Am J Bot*. 2005;92(1):2–12. <https://doi.org/10.3732/ajb.92.1.2>.
- Farré-Armengol G, Fernández-Martínez M, Filella I, Junker RR, Peñuelas J. Deciphering the biotic and climatic factors that influence floral scents: a systematic review of floral volatile emissions. *Front Plant Sci*. 2020;11:1154. <https://doi.org/10.3389/fpls.2020.01154>.
- Farré-Armengol G, Filella I, Llusia J, Peñuelas J. Floral volatile organic compounds: between attraction and deterrence of visitors under global change. *Perspect Plant Ecol Evol Syst*. 2013;15(1):56–67. <https://doi.org/10.1016/j.ppees.2012.12.002>.
- Fernandes N, Silva FAN, de Aragão F, Zocolo GJ, Freitas BM. Volatile organic compounds role in selective pollinator visits to commercial melon types. *J Agric Sci*. 2019;11(3):93–108. <https://doi.org/10.5539/jas.v11n3p93>.
- Filella I, Primante C, Llusia J, Martín González AM, Seco R, Farré-Armengol G, et al. Floral advertisement scent in a changing plant-pollinators market. *Sci Rep*. 2013;3(1):3434. <https://doi.org/10.1038/srep03434>.
- Free J. Influence of the odour of a honeybee colony's food stores on the behaviour of its foragers. *Nature*. 1969;222(5195):778. <https://doi.org/10.1038/222778a0>.
- Funamoto D. Plant-pollinator interactions in East Asia: a review. *J Pollinat Ecol*. 2019;25(6):46–68. [https://doi.org/10.26786/1920-7603\(2019\)532](https://doi.org/10.26786/1920-7603(2019)532).
- Getz WM, Smith KB. Olfactory sensitivity and discrimination of mixtures in the honeybee *Apis mellifera*. *J Comp Physiol*. 1987;160(2):239–45. <https://doi.org/10.1007/BF00609729>.
- Giurfa M, Vorobyev M, Kevan P, Menzel R. Detection of coloured stimuli by honeybees: minimum visual angles and receptor specific contrasts. *J Comp Physiol A*. 1996;178(5):699–709. <https://doi.org/10.1007/BF00227381>.
- Gong WC, Chen G, Vereecken NJ, Dunn BL, Ma YP, Sun WB. Floral scent composition predicts bee pollination system in five butterfly bush (*Buddleja*, Scrophulariaceae) species. *Plant Biol (Stuttg)*. 2015;17(1):245–55. <https://doi.org/10.1111/plb.12176>.
- Granero AM, Sanz JM, Gonzalez FJ, Vidal JL, Dornhaus A, Ghani J, et al. Chemical compounds of the foraging recruitment pheromone in bumblebees. *Naturwissenschaften*. 2005;92(8):371–4. <https://doi.org/10.1007/s00114-005-0002-0>.
- Henning JA, Teuber LR. Combined gas chromatography-electroantennogram characterization of alfalfa floral volatiles recognized by honey bees (Hymenoptera: Apidae). *J Econ Entomol*. 1992;85(1):226–32. <https://doi.org/10.1093/jee/85.1.226>.
- Hiraiwa MK, Ushimaru A. Low functional diversity promotes niche changes in natural island pollinator communities. *Proc Biol Sci*. 2017;284(1846):20162218. <https://doi.org/10.1098/rspb.2016.2218>.
- Jakobsen H, Kristjánsson K, Rohde B, Terkildsen M, Olsen CE. Can social bees be influenced to choose a specific feeding station by adding

- the scent of the station to the hive air? *J Chem Ecol.* 1995;21(11):1635-48. <https://doi.org/10.1007/BF02033666>.
- Johnson DL. Communication among honey bees with field experience. *Anim Behav.* 1967;15(4):487-92. [https://doi.org/10.1016/0003-3472\(67\)90048-6](https://doi.org/10.1016/0003-3472(67)90048-6).
- Johnson SD, Moré M, Amorim FW, Haber WA, Frankie GW, Stanley DA, et al. The long and the short of it: a global analysis of hawkmoth pollination niches and interaction networks. *Funct Ecol.* 2017;31(1):101-15. <https://doi.org/10.1111/1365-2435.12753>.
- Kaiser L, De Jong R. Multi-odour memory influenced by learning order. *Behav Processes.* 1993;30(2):175-83. [https://doi.org/10.1016/0376-6357\(93\)90007-E](https://doi.org/10.1016/0376-6357(93)90007-E).
- Kevan PG, Lane MA. Flower petal microtexture is a tactile cue for bees. *Proc Natl Acad Sci U S A.* 1985;82(14):4750-2. <https://doi.org/10.1073/pnas.82.14.4750>.
- Khalifa SA, Elshafiey EH, Shetaia AA, El-Wahed AAA, Algethami AF, Musharraf SG, et al. Overview of bee pollination and its economic value for crop production. *Insects.* 2021;12(8):688. <https://doi.org/10.3390/insects12080688>.
- Klein AM, Vaissière BE, Cane JH, Steffan-Dewenter I, Cunningham SA, Kremen C, et al. Importance of pollinators in changing landscapes for world crops. *Proc Biol Sci.* 2007;274(1608):303-13. <https://doi.org/10.1098/rspb.2006.3721>.
- Koltermann R. [Learning and forgetting processes in the honey bee—demonstrated using scent exercises]. *Z Vgl Physiol.* 1969;63(3):310-34. German. <https://doi.org/10.1007/BF00298165>.
- Lacher V. [Electrophysiological studies on individual receptors for smell, carbon dioxide, air humidity and temperature on the antennae of worker bees and drones (*Apis mellifica* L.)]. *Z Vgl Physiol.* 1964;48:587-623. German. <https://doi.org/10.1007/BF00333743>.
- Lindauer M, Kerr WE. Communication between the workers of stingless bees. *Bee World.* 1960;41(2):29-41. <https://doi.org/10.1080/0005772X.1960.11095309>.
- Maisonnasse A, Lenoir JC, Beslay D, Crauser D, Le Conte Y. E- $\beta$ -ocimene, a volatile brood pheromone involved in social regulation in the honey bee colony (*Apis mellifera*). *PLoS One.* 2010;5(10):e13531. <https://doi.org/10.1371/journal.pone.0013531>.
- Maisonnasse A, Lenoir JC, Costagliola G, Beslay D, Crauser D, Plettner E, et al. E- $\beta$ -ocimene a new volatile primer pheromone that inhibits worker ovary development in honey bees. Paper presented at: International Union for the Study of Social Insects - French Section; 2009 Sep 2-4; Bondy, France. Tours: UIEIS; 2009. hal-02755737.
- Menzel R, Erber J, Masuhr T. Learning and memory in the honeybee. In: Barton Browne L, editor. *Experimental analysis of insect behaviour*. Berlin: Springer; 1974. p. 195-217.
- Menzel R. Learning in honey bees in an ecological and behavioral context. *Fortschr Zool.* 1985;31:55-74.
- Molet M, Chittka L, Raine NE. How floral odours are learned inside the bumblebee (*Bombus terrestris*) nest. *Naturwissenschaften.* 2009;96(2):213-9. <https://doi.org/10.1007/s00114-008-0465-x>.
- Ollerton J. Biogeography: are tropical species less specialised? *Curr Biol.* 2012;22(21):R914-5. <https://doi.org/10.1016/j.cub.2012.09.023>.
- Ollerton J. Pollinator diversity: distribution, ecological function, and conservation. *Annu Rev Ecol Evol Syst.* 2017;48:353-76. <https://doi.org/10.1146/annurev-ecolsys-110316-022919>.
- Omura H, Honda K. Priority of color over scent during flower visitation by adult *Vanessa indica* butterflies. *Oecologia.* 2005;142(4):588-96. <https://doi.org/10.1007/s00442-004-1761-6>.
- Pecetti L, Tava A, Felicioli A, Pinzauti M, Piano E. Effect of three volatile compounds from lucerne flowers on their attractiveness towards pollinators. *Bull Insectol.* 2002;55(1-2):21-7.
- Proctor M, Yeo P. *The pollination of flowers*. New York: Taplinger; 1973.
- R Core Team. R: a language and environment for statistical computing. Vienna: R Foundation for Statistical Computing; 2021.
- Raguso RA, Willis MA. Synergy between visual and olfactory cues in nectar feeding by naive hawkmoths, *Manduca sexta*. *Anim Behav.* 2002;64(5):685-95.
- Reinhard J, Srinivasan MV, Guez D, Zhang SW. Floral scents induce recall of navigational and visual memories in honeybees. *J Exp Biol.* 2004a;207(Pt 25):4371-81. <https://doi.org/10.1242/jeb.01306>.
- Reinhard J, Srinivasan MV, Zhang S. Olfaction: scent-triggered navigation in honeybees. *Nature.* 2004b;427(6973):411. <https://doi.org/10.1038/427411a>.
- Ribbands CR. Communication between honeybees. I: The response of crop-attached bees to the scent of their crop. *Proc R Entomol Soc Lond Ser A Gen Entomol.* 1954;29(10-12):141-4. <https://doi.org/10.1111/j.1365-3032.1954.tb01187.x>.
- Roy BA, Raguso RA. Olfactory versus visual cues in a floral mimicry system. *Oecologia.* 1997;109(3):414-26. <https://doi.org/10.1007/s004420050101>.
- Shimada T, Endo T, Fujii H, Hara M, Omura M. Isolation and characterization of (E)-beta-ocimene and 1,8 cineole synthases in *Citrus unshiu* Marc. *Plant Sci.* 2005;168(4):987-95. <https://doi.org/10.1016/j.plantsci.2004.11.012>.
- Smith BH, Breed MD. The chemical basis for nestmate recognition and mate discrimination in social insects. In: Cardé RT, Bell WJ, editors. *Chemical ecology of insects 2*. New York: Chapman & Hall; 1995. p. 287-317.
- Smith BH. The olfactory memory of the honeybee *Apis Mellifera*: I. Odorant modulation of short- and intermediate-term memory after single-trial conditioning. *J Exp Biol.* 1991;161(1):367-82. <https://doi.org/10.1242/jeb.161.1.367>.
- Vareschi E. [Odor discrimination in the honey bee—single cell recordings and behavioral responses]. *Z Vgl Physiol.* 1971;75(2):143-73. German. <https://doi.org/10.1007/BF00335260>.
- Ward JH. Hierarchical grouping to optimize an objective function. *J Am Stat Assoc.* 1963;58(301):236-44.
- Wenner AM, Wells PH, Johnson DL. Honey bee recruitment to food sources: olfaction or language? *Science.* 1969;164(3875):84-6. <https://doi.org/10.1126/science.164.3875.84>.
- Whitney HM, Kolle M, Andrew P, Chittka L, Steiner U, Glover BJ. Floral iridescence, produced by diffractive optics, acts as a cue for animal pollinators. *Science.* 2009;323(5910):130-3. <https://doi.org/10.1126/science.1166256>.
- Williams NH, Whitten WM. Orchid floral fragrances and male euglossine bees: methods and advances in the last sesquidecade. *Biol Bull.* 1983;164(3):355-95. <https://doi.org/10.2307/1541248>.
- Zanata TB, Dalsgaard B, Passos FC, Cotton PA, Roper JJ, Maruyama

- PK, et al. Global patterns of interaction specialization in bird-flower networks. *J Biogeogr.* 2017;44(8):1891-910. <https://doi.org/10.1111/jbi.13045>.
- Zhang S, Schwarz S, Pahl M, Zhu H, Tautz J. Honeybee memory: a honeybee knows what to do and when. *J Exp Biol.* 2006;209(Pt 22):4420-8. <https://doi.org/10.1242/jeb.02522>.