



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

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**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*)- $\beta$ -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*-β-ocimene) (X68), 1H-pyrrole, 2,3-dimethyl- (X27), 1-phenylethanone (acetophenone) (X69), pyrimidine, 2-methyl- (X32), 3,7-dimethyl-1,6-octadien-3-ol (β-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*-β-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 β-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 β-ocimene (Figs. S1–3) and plant-specific compounds such as β-linalool, hotrienol and acetophenone. The plant species classified as second and third cluster showed generally lower compositional proportions of β-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 acetamide2-fluoro- (22.5%) and *E*-β-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	Pteridine-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-thio-	-	-	-	-	-	-	-	-	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-	-	2.6	-	-	-	-	-	-	0.3
4.803	X12	Isopropoxycarbamic acid, ethyl ester	0.5	-	-	21.1	-	-	-	-	-
4.534	X13	Ethanoic acid	-	-	7	-	-	-	-	-	-
4.648	X14	Diaminmethanone	-	-	-	-	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	-	-	-	-	-	0.17	2.2	0.6	0.7
5.947	X20	1-Octane	-	-	-	13.4	-	-	-	-	-
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	-	2.7	-	-	-	0.3	-	0.4	-
8.642	X29	1-Nonane -	-	-	-	-	-	-	-	-	-
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-Azadipiro[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	-	-	-	-	-	-	-	-	-
10.061	X37	Bicyclo[2.2.1]heptane, 2,2-dimethyl-3-methylene-, (1S)-	-	-	-	5.6	-	-	-	-	-
10.399	X38	Phenylformaldehyde	0.3	-	-	5.8	-	-	0.16	-	0.1



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	(5E)-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, E,E-	-	-	-	-	-	-	-	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	-	-	-	-	-	-	-	-	3.4
15.703	X95	Cyclopropane, trimethyl(2-methyl-1-propenylidene)-	-	-	-	-	-	0.28	-	-	-
15.943	X96	1H-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	5H-Naphtho[2,3-b]carbazole	0.3	0.5	-	4.4	-	0.68	3.74	-	-
16.224	X99	N,N-Dimethyl-N'-(10-propyl-10H-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-methoxyphenyl)-2-phenylpyrazolo[1,5-a]pyrimidine-6-carbonitrile	-	-	-	-	-	-	0.23	-	-
17.952	X108	2,4-Hexadiene, (E,Z)-	-	-	-	-	-	-	0.24	-	-
17.958	X109	3-Hexene, 2,5-dimethyl-, (E)-	-	-	-	-	-	0.21	-	-	-
17.963	X110	Cyclohexane, chloro-	-	-	-	-	-	-	-	-	0.1
17.98	X111	2-Thiophenemethanamine	-	-	-	-	-	-	-	0.2	-
18.072	X112	1,3-Pentadiene, 2-methyl-, (E)-	-	-	-	-	-	-	-	-	0.2
18.484	X113	(2-Nonyloxybenzyl)-phenylamine	-	-	-	-	-	-	1.02	-	-
18.558	X114	2-Thiazoline, 5-methyl-2-(2-pyridylmethyl)amino-	-	-	-	-	-	-	-	0.2	-
20.092	X115	(E)-Hex-3-enyl (E)-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)-1H-indole	3.1	5.5	11.8	9.2	-	0.86	3.78	-	-



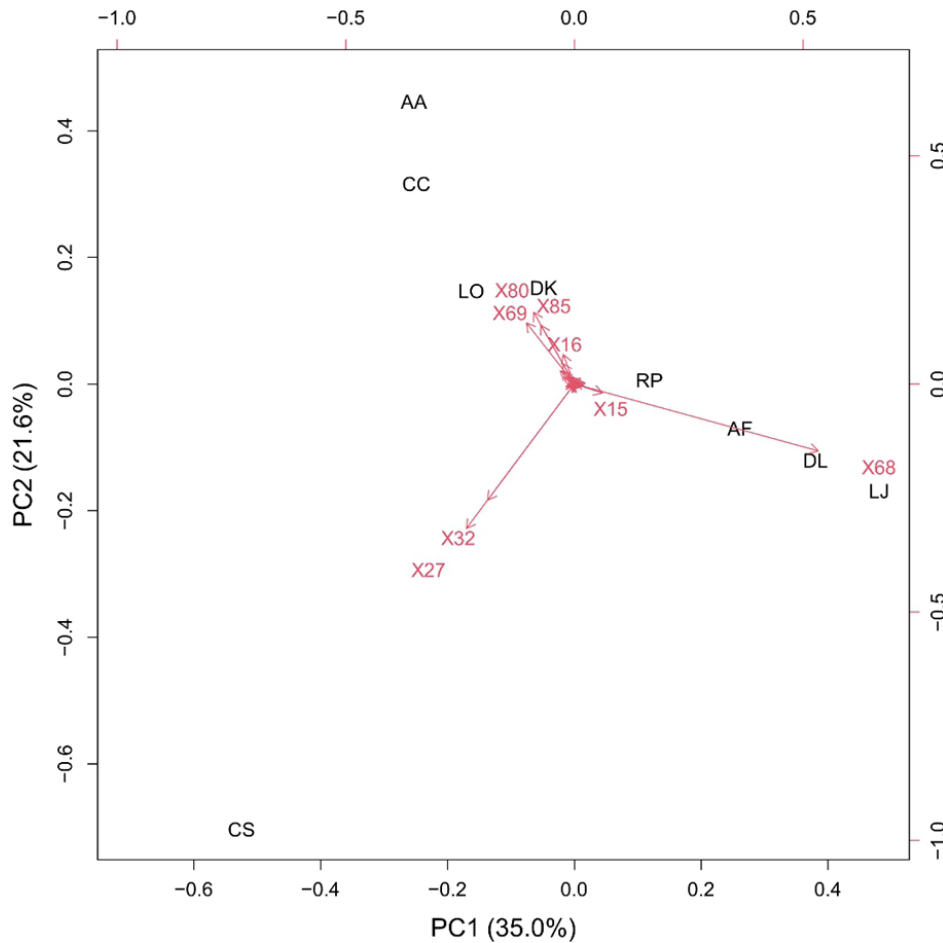


Table 2 Continued

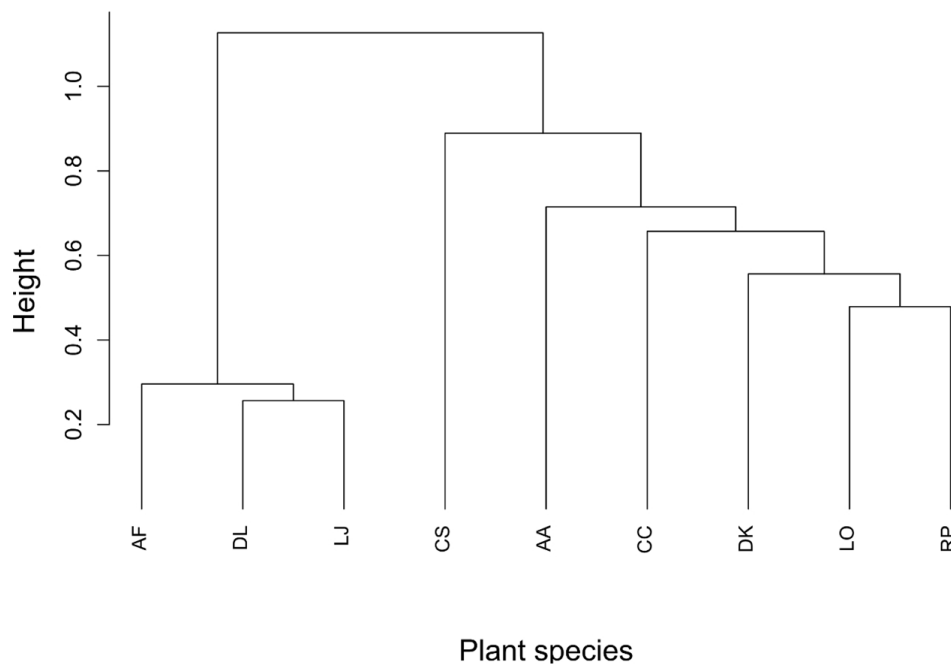
RT (min)	No.	Compounds	CS	DL	DK	LO	RP	AF	CC	AA	LJ
25.133	X152	2,6,10,14-Hexadecatetraen-1-ol, 3,7,11,15-tetramethyl-, acetate, (E,E,E)-	-	-	-	-	0.6	-	-	-	-
25.425	X153	Isolongifolene, 9,10-dehydro-	-	-	-	-	-	0.16	-	-	-
25.717	X154	2,2,4a-trimethyl-8-methylidene-2a,3,4,5,6,7-hexahydro-1H-cyclobuta[[i]indene	-	-	-	-	-	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-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-isothiocyanatopregn-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-adamantyloxy)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 monoterpenes [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-bicyclosesquiphellandrene), 4,7-dimethyl-1-(propan-2-yl)-1,2,3,4,4a,5-hexahydronaphthalene (*cis*-muurolo-3,5-diene), tricyclo[4.4.0.0<sup>2,7</sup>]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, isoledene)].

## 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/10.1186/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

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### 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.

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### 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.

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