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Research Article

Headspace gas chromatography-mass spectrometry (HS GC-MS) for identifying floral synomones from *Impatiens maculata* Wight. and *Impatiens cordata* Wight.: Two endemic balsams of the Southern Western Ghats

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Abstract

Headspace Gas Chromatography-Mass Spectrometry (HS GC-MS), an analytical technique commonly used in environmental science, chemical ecology, and food science, identifies volatile chemicals emitted from solid or liquid samples. Despite its utility, HS-GC-MS is seldom applied in plant science. In the present study, HS-GC-MS was used to identify key floral synomones in two rare and endemic balsam species from the Mankulam forest range of the Munnar Hills in Kerala, a region within the Southwestern Ghats of India. Synomones are plant-released volatile compounds that attract beneficial insects. HS-GC-MS analysis of fresh flowers of *Impatiens maculata* revealed the presence of hexanal, 1-hexanol, heptanal, benzaldehyde, benzoic acid methyl ester, and (3E,7E)-4,8,12-trimethyltrideca-1,3,7,11-tetraene. In contrast, analysis of *Impatiens cordata* flowers revealed 2,2-dimethyl-1,3-butanediol, 2-heptanol, benzaldehyde, methyl salicylate, and tetradecane. Many of these volatiles mediate plant-environment interactions, particularly by attracting floral visitors, suggesting that cultivating these plants could enhance the natural balance of beneficial insects.

Keywords: Synomones, Headspace Gas Chromatography, *Impatiens,* Volatiles, Floral visitors

Introduction

The genus *Impatiens*, first described by Carl Linnaeus in 1753, is a large member of the family Balsaminaceae, comprising approximately 1,107 species distributed worldwide. These species are primarily found across Southeast Asia, tropical Africa, and Madagascar (Yu, 2012; POWO, 2024). India notably hosts 314 species and 26 varieties or subspecies, with significant concentrations in the Eastern Himalayas, Northeastern states, and the Western Ghats (Prabhukumar *et al.,* 2022; Khanal *et al.,* 2023; Richard & Ravichandran, 2023; Tiwari, 2023). Species of *Impatiens* typically thrive in moist, humid habitats, ranging from sea level to 4,000 meters, often occurring in valleys and along streams, though some species can tolerate drier sites (Yu *et al.,* 2015). Despite this extensive diversity, only a handful of *Impatiens* species are widely cultivated—including *I. walleriana*, the New Guinea hybrids, *I. balsamina*, and *I. glandulifera*. The vast majority remain wild and are horticulturally underexplored (Morgan, 2007).

Flower colour and fragrance serve as major visual and chemical cues for pollinators. Floral scent, in particular, attracts and guides specific pollinators, thereby enhancing pollen transfer and reproductive success. Beyond pollination, these floral volatile organic compounds (VOCs) also play crucial roles in plant defence, interplant signalling, and interactions with herbivores and microbes (Dudareva *et al.,* 2013). Consequently, characterizing floral scent composition is vital for research in ecology, taxonomy, and chemotaxonomy.

Over recent decades, numerous analytical methods have been developed to identify floral scent constituents (Kong *et al.,* 2012). While modern volatile collection techniques typically include solvent extraction, distillation, and headspace adsorption (Scalliet *et al.,* 2008), early studies predominantly relied on solvent extraction and hydrodistillation of petals. These historical approaches frequently altered the native fragrance through heat degradation, enzymatic changes, and solvent artefacts, thus failing to accurately represent the volatiles emitted by living flowers.

Headspace adsorption techniques address these limitations by directly collecting volatiles from intact tissues, preserving natural emission patterns, and concentrating trace compounds with low olfactory thresholds (Tholl *et al.,* 2020). Among these methods, solid-phase microextraction (SPME) is widely used in headspace analysis. Its simplicity, sensitivity, solvent-free nature, and ability to facilitate rapid equilibration between analytes and the fibre coating make it particularly well-suited for *in situ* floral scent sampling (Kataoka *et al.,* 2000; Wang *et al.,* 2009).

Floral scents are complex mixtures of terpenoids, benzenoids/phenylpropanoids, fatty-acid derivatives, and N- or S-containing compounds. This complexity necessitates robust separation and accurate identification for effective profiling. Qualitative identification typically involves comparing retention times with standards and matching mass spectra to libraries (e.g., NIST and Wiley). For quantification, methods include peak-area normalization or internal/external standard calibration (Lubes & Goodarzi, 2017).

Gas chromatography–mass spectrometry (GC-MS) is a widely adopted platform for analyzing volatile organic compounds (VOCs) in fields such as plant volatile analysis, food aroma profiling, and chemotaxonomy (Dudareva *et al.,* 2013; Lubes & Goodarzi, 2017). Its indispensability arises from its powerful combination of effective chromatographic separation with mass-spectral identification, resulting in high sensitivity, reproducibility, and accuracy (Vallarino *et al.,* 2018; Tholl *et al.,* 2020). This synergistic approach allows GC-MS to reliably detect trace compounds and provide structural insights through fragmentation and library matching.

This study used direct static Headspace Gas Chromatography–Mass Spectrometry (GC-MS) to investigate the floral volatile compounds of two *Impatiens* species (*Impatiens maculata* and *Impatiens cordata*), characterizing their floral scent chemical compositions and evaluating interspecific differences. These findings may provide insights into pollination ecology, species discrimination, and chemotaxonomy within *Impatiens*.

I. maculata (Figure 1) is a perennial herb endemic to the southern Western Ghats, occurring along montane rivers and streams in Kerala and Tamil Nadu (Morgan, 2007). Its axillary, many-flowered glabrous racemes bear 2.5-3 cm flowers that range from white to lilac or pale pink. Bracts and pedicels are glabrous and pinkish-brown to pale pink. Lateral sepals are two, ovate, acute, and glabrous; the suborbicular dorsal petal is slightly keeled adaxially with an emarginate apex. Lateral united petals are two-lobed (basal lobes ovate and smaller than the striated, orbicular distal lobes). The lower sepal is cymbiform, glabrous, white to pale pink, and the spur is slender, prominently incurved, glabrous, and white or pale pink (Hareesh *et al.,* 2022).

I. cordata (Figure 2) is an annual herb endemic to the southern Western Ghats, found on montane stream banks. It resupinates inflorescences arise from leaf axils in subumbellate arrangements resembling racemes or fascicles. Flowers are bisexual and zygomorphic, borne on pedicels with bracts that may be persistent or caducous. Typically, there are three imbricate sepals (rarely five); two lateral sepals are small, flat, green, linear-lanceolate to ovate, while the large posterior sepal is petaloid, navicular or sac-like. Five free petals are showing red, scarlet, purple, greenish, or white hues. The standard (upper petal) is flat, keeled, or hooded, often lobed; lateral petals (wings) are paired and may be entire or lobed, sometimes with a basal appendage (Hareesh *et al.,* 2022).

Materials and methods

Fresh flowers of *Impatiens maculata* and *Impatiens cordata* at full bloom were collected from the Mankulam Forest Range, Munnar Hills, Kerala (10°05’04” N, 76°57’02” E; 999 m above mean sea level). Immediately after collection, the samples were transported in airtight polypropylene bags to the laboratory and processed without delay.

Sample preparation

Approximately 1.0 g of fresh flower material was finely chopped (approximately 0.5 × 0.5 mm pieces) and

Figure 1: (a-c) Habit of *Impatiens macualata*

Figure 2: (a-c) Habit of *Impatiens cordata*

transferred to 20 mL headspace vials, which were then sealed with PTFE/silicone septa. No pretreatment was applied before analysis.

HS GC-MS analysis

Headspace Gas Chromatography–Mass Spectrometry (HS-GC–MS) analysis was performed using a QP2020 NX Gas Chromatograph Mass-Spectrometer (Shimadzu, Kyoto) coupled with an HS-20 headspace autosampler (90-vial capacity). An SH-Rxi-1 ms capillary column (30 m × 0.25 mm id., 0.25 µm film thickness) was used for separation.

For headspace extraction, sealed vials were incubated at 65 °C for 10 min with agitation (shaking level 3 and then pressurized to 60 kPa before injection into the GC system.

The oven temperature was programmed to increase from 50 °C to 200 °C at a rate of 10 °C min^{-1}. Electron impact ionization was performed at 70 eV. Mass spectra were recorded and processed using GC-MS solution software (version 4.0, Shimadzu).

Results and discussion

Chemical profiling of *Impatiens* floral volatiles revealed distinct chemical markers that not only dictate insect visitation patterns but also determine the nature of plant-pollinator dynamics—whether mutualistic or repellent. This profiling, conducted using Headspace Gas Chromatography-Mass Spectrometry (GC-MS), unveiled significant differences in the volatile organic compound (VOC) profiles among the evaluated *Impatiens* species, encompassing various chemical classes such as aldehydes, alcohols, esters, alkenes, and hydrocarbons.

Comparative analysis of floral volatile emission profiles

The *Impatiens* species investigated exhibit distinct volatile chemical profiles, reflecting an evolutionary trade-off between attracting pollinators and synthesizing insect deterrents.

I. maculata

Headspace GC-MS analysis of *I. maculata* identified six volatile compounds: hexanal (an aldehyde), 1-hexanol (an alcohol), heptanal (an aldehyde), benzaldehyde (an aldehyde), methyl benzoate (an ester), and (3E,7E)-4,8,12-trimethyltrideca-1,3,7,11-tetraene (an alkene). Among these, methyl benzoate was the predominant floral volatile. All six compounds are known to be detectable by *Apis mellifera* and lepidopteran pollinators, suggesting a synergistic function as synomones in floral attraction (Table 1). Field observations confirmed honeybees and butterflies as the principal floral visitors and effective pollinators of *I. maculata*. The prominence of methyl benzoate, widely recognized as a floral volatile associated with pollinator attraction and insect semiochemical communication (Feng & Zhang, 2017; Zhu *et al.,* 2019), supports its proposed role as a crucial semiochemical mediating plant–pollinator interactions (Figure 3).

I. cordata

In contrast, *I. cordata* exhibited a distinct metabolic profile, releasing five volatile compounds: methyl salicylate (ester), 2-heptanol (alcohol), tetradecane (hydrocarbon), 2,2-dimethyl-1,3-butanediol (alcohol), and benzaldehyde (aldehyde). Of these, methyl salicylate (MeSA) was the predominant volatile emitted (Table 2). Despite this characteristic chemical signature, particularly the dominance of MeSA, no pollinators were observed visiting *I. cordata*

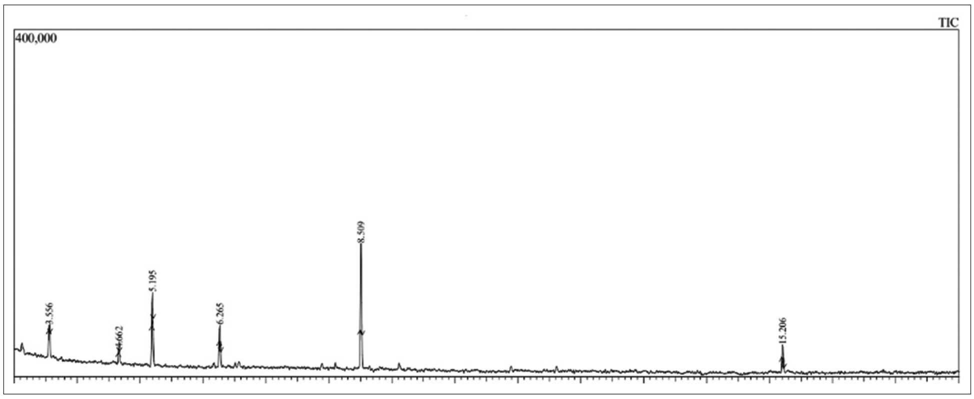

Figure 3: HS GC-MS chromatogram of *I. maculata*

Table 1: Compounds identified in *I. maculata* through HS GC-MS

Peak	Retention Time	Area	Compound Name	Area %
1	3.556	2995	Hexanal	1.81
2	4.662	2329	Hexanol	1.41
3	5.195	24761	Heptanal	14.96
4	6.265	18665	Benzaldehyde	11.28
5	8.509	99587	Benzoic acid, Methyl Ester	60.18
6	15.206	17156	(3E,7E)-4,8,12-Trimethyltrideca-1,3,7,11-tetraene	10.37

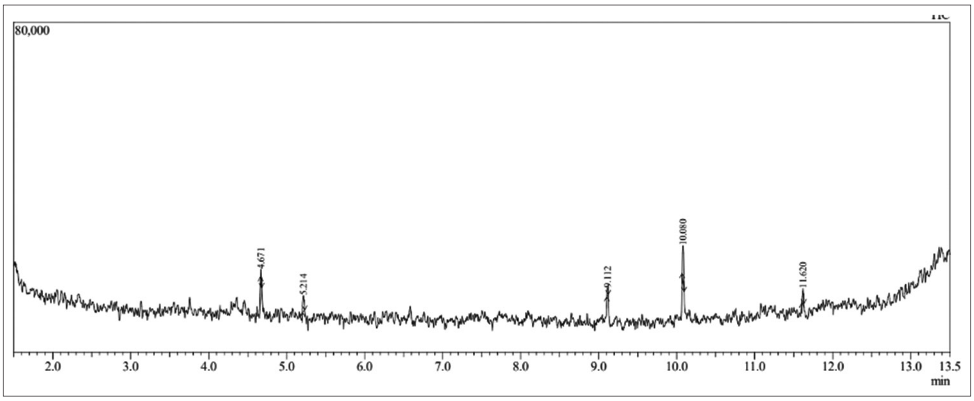

Figure 4: HS GC-MS chromatogram of *I. cordata*

Table 2: Compounds identified in *I. cordata* through HS GC-MS

Peak	Retention Time	Area	Compound Name	Area %
1	4.671	899	2,2-Dimethyl-1,3-butanediol	6.60
2	5.214	3253	2-Heptanol	23.88
3	9.112	983	Benzaldehyde	7.22
4	10.080	5871	Methyl salicylate	43.10
5	11.620	2617	Tetradecane	19.21

during monitoring. While MeSA is known as an herbivore-induced plant volatile (HIPV) that functions as an indirect defense mechanism, its effect on foraging insects is highly context-specific. Experimental data, for instance, shows that high concentrations of MeSA significantly depress pollinator visitation rates. This finding aligns with Mayer (1997)'s early reports of localized honeybee foraging depression when MeSA was evaluated as a potential repellent (Figure 4).

Genus-wide divergence and pollinator specialization

The chemical strategies observed in species like *I. maculata* and *I. cordata* illustrate broader metabolic patterns across the genus *Impatiens*. For instance, *Impatiens parviflora*, *I. glandulifera*, and *I. capensis* all produce geraniol and linalool, utilizing these as specialized olfactory signals to selectively recruit Apidae family members (bumblebees and honeybees) (Jakubska-Busse *et al.,* 2023).

However, *I. parviflora* uniquely modifies its metabolism by processing linalool into specialized monoterpene derivatives, specifically linalool oxide and 8-hydroxy linalool (Jakubska-Busse *et al.,* 2023). These oxygenated monoterpenes are highly attractive to Diptera, explaining why syrphid flies serve as their primary visitors and effective pollinators (Jakubska-Busse *et al.,* 2023).

Sukumaran *et al.* (2025) further highlight this genus-wide macro-evolutionary divergence in secondary metabolism. Their whole-plant GC-MS characterization of *Impatiens minor* identified twelve major bioactive volatile compounds within its ethanol fraction. This complex metabolic pool suggests a broad range of adaptations, potentially extending beyond pollinator recruitment to include defense against diverse environmental pressures. Such a bioactive and antimicrobial reservoir warrants further investigation.

Synthesis of volatile-mediated behaviors

Comparative data shows that even subtle shifts in plant volatile compounds, such as the transition from a methyl benzoate system (*I. maculata*) to a methyl salicylate system (*I. cordata*), profoundly alter plant-insect interactions. For instance, *I. maculata* uses methyl benzoate for synomonal pollinator attraction, while *I. cordata* employs methyl salicylate for chemical deterrence and to depress foraging.

This target-specific recruitment strategy is also evident in *I. parviflora*, which synthesizes linalool derivatives (oxides) to selectively attract syrphid flies. Meanwhile, *I. glandulifera* and *I. capensis* utilize geraniol and linalool to maintain broad hymenopteran recruitment. Ultimately, these specialized chemical signals enable insects to evaluate their microenvironments through sensory structures, providing a molecular basis for pollinator sorting and the maintenance of local biodiversity.

Conclusion

The primary compounds identified in both species are notable for their reported roles as attractants of species-specific pollinators. These compounds may, therefore, mediate interactions among the plants and pollinators, herbivores and their natural enemies, other plants, and microorganisms. Given that this is the first study of its kind on these two species, further structural validation of the compounds could yield significant insights into how these phytoconstituents act as synomones. Furthermore, mass cultivation of these plants may promote a more balanced ecosystem for beneficial insects.

Author contributions

Sivadasan Abhilash contributed to the conceptualization, investigation, and formal analysis of this study, as well as data curation and the initial draft of the manuscript. A. Resmi and Priyanjana Prabhakar contributed to the methodology and validation and reviewed and edited the manuscript. Shibu P. Varghese oversaw the project and its administration and reviewed and edited the manuscript. A. K. Sreekala provided resources and supervision and reviewed and edited the manuscript. All authors approved the final manuscript.

Ethical approval statement

This study adhered to established ethical standards and guidelines. No human or animal subjects were involved, and all plant materials were sourced with appropriate ethical approval and consideration.

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