Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
Impact Factor (RJIF): 5.83
www.entomoljournal.com
JEZS 2025; 13(6): 40-45

Received: 24-08-2025
Accepted: 27-09-2025

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Comparative gas chromatography-mass spectrometry analysis of cuticular hydrocarbons in *Camponotus pennsylvanicus* (Hymenoptera: Formicidae) and *Dysdercus cingulatus* (Hemiptera: Pyrrhocoridae)

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DOI: https://www.doi.org/10.22271/j.ento.2025.v13.i6a.9627

Abstract
Cuticular hydrocarbons (CHCs) constitute a vital biochemical interface between insects and their environment, functioning in communication, waterproofing, and ecological adaptation. This study comparatively analyzed the qualitative and quantitative composition of CHCs in the carpenter ant *Camponotus pennsylvanicus* and the red cotton bug *Dysdercus cingulatus* using Gas Chromatography-Mass Spectrometry (GC-MS). Both species were collected from field habitats and extracted in n-hexane under controlled laboratory conditions. GC-MS profiling revealed distinct chemical patterns: *C. pennsylvanicus* exhibited a predominance of long-chain linear alkanes such as pentadecane (18.1%) and heneicosane (11.1%), while *D. cingulatus* showed a higher proportion of fatty acids and esters, including phthalic acid esters (14.4%) and octadecadienoic acid (12.1%). These compositional divergences reflect ecological differentiation, with *C. pennsylvanicus* utilizing stable saturated hydrocarbons for desiccation resistance and social communication, whereas *D. cingulatus* displays lipid-rich cuticular profiles associated with reproductive and host-plant interactions. The study provides biochemical evidence supporting the adaptive evolution of CHCs across social and phytophagous insect taxa, contributing to a broader understanding of chemical ecology in insects.

Keywords: Cuticular hydrocarbons, GC-MS analysis, *Camponotus pennsylvanicus*, *Dysdercus cingulatus*, chemical communication, ecological adaptation

Introduction
Cuticular hydrocarbons (CHCs) are the principal lipid constituents of the insect epicuticle and play multiple physiological and ecological roles ranging from waterproofing to chemical communication (Blomquist & Bagnères, 2010) [1]. These long-chain compounds, composed primarily of straight and methyl-branched alkanes, alkenes, and fatty acid derivatives, constitute the primary barrier against desiccation and microbial invasion (Lockey, 1988; Gibbs, 1998) [12, 5]. Beyond their protective function, CHCs serve as semiochemical cues mediating a broad array of intra- and interspecific interactions, including nestmate recognition, mate selection, and caste differentiation (Howard & Blomquist, 2005; van Zweden & d'Ettorre, 2010; Leonhardt *et al*., 2016) [7, 20, 11].
The composition of CHCs varies significantly among insect taxa, shaped by phylogeny, ecological niche, and behavioral complexity (Martin & Drijfhout, 2009) [4]. In eusocial Hymenoptera such as ants, CHCs are crucial for maintaining colony structure, ensuring nestmate recognition, and regulating reproductive hierarchies (Hölldobler & Wilson, 1990; Leonhardt *et al*., 2016) [8, 11]. The carpenter ant *C. pennsylvanicus*, a dominant species in forest and urban habitats, exhibits caste-specific and environmentally modulated CHC patterns (Martin & Drijfhout, 2009) [4]. These hydrocarbons also confer desiccation resistance, particularly vital for species that occupy exposed and fluctuating terrestrial microhabitats (Gibbs, 1998) [5]. Conversely, in phytophagous hemipterans such as the red cotton bug *D. cingulatus*, CHCs serve additional adaptive roles linked to host-plant interactions, aggregation behavior, and reproductive signaling (Rani & Rajasekaran, 2016) [14].

This species, a major pest of cotton and other Malvaceae, relies heavily on lipid-derived compounds for cuticular integrity and pheromonal communication. Previous reports have indicated the presence of diverse fatty acids and esters in hemipteran cuticles, often correlated with temperature and humidity tolerance (Johnson & Lee, 2017) [10]. However, few studies have directly compared the CHC composition between phylogenetically distinct social and phytophagous insects under uniform analytical conditions.

Comparative CHC analysis across taxonomic and ecological boundaries can therefore elucidate how evolutionary pressures shape chemical signaling strategies and cuticular adaptations (Sevgili *et al*., 2025; Hsu *et al*., 2024) [17, 9]. Such comparisons also provide insight into the biochemical basis of sociality, communication, and environmental fitness.

The present study aims to characterize and compare the cuticular hydrocarbon profiles of *C. pennsylvanicus* and *D. cingulatus* using Gas Chromatography-Mass Spectrometry (GC-MS). By integrating qualitative and quantitative analyses, we assess how CHC composition reflects ecological specialization and behavioral evolution in two functionally divergent insect taxa.

Methodology

Taxonomic Identification

Specimens of *C. pennsylvanicus* (Hymenoptera: Formicidae) and *D. cingulatus* (Hemiptera: Pyrrhocoridae) were identified using standard entomological taxonomic keys and morphological characteristics. Identification followed Borror and DeLong's Introduction to the Study of Insects (Triplehorn & Johnson, 2005) [19], True Bugs of the World (Schuh & Slater, 1995) [15], and The Ants (Hölldobler & Wilson, 1990) [8]. Diagnostic features such as body segmentation, antennal structure, wing venation, coloration, and caste morphology were examined under a stereomicroscope. Confirmation of genus and species was carried out using relevant literature (Bolton, 2020; Henry, 2017; Singh & Singh, 2005) [2, 6, 8].

Insect Collection

Camponotus pennsylvanicus

Colonies of *C. pennsylvanicus* were collected from decaying wooden structures and tree trunks within the PSGR Krishnammal College for Women campus, Coimbatore, Tamil Nadu. Worker ants were manually collected using sterile forceps and placed into clean, labeled containers. Nests were covered with thin polyethylene sheets during sampling to minimize disturbance and prevent escape. The collected ants were transported to the laboratory immediately after collection.

Dysdercus cingulatus

Adult and nymphal stages of *D. cingulatus* were collected from malvaceous weed plants present in the same field locality. The insects were handpicked and also captured using insect nets while feeding on host plants. Samples were transferred into labeled containers and transported to the laboratory for further analysis.

Hydrocarbon Extraction and GC-MS Analysis

The extraction of cuticular hydrocarbons using non-polar solvents such as n-hexane and subsequent analysis by Gas Chromatography-Mass Spectrometry (GC-MS) is a standard approach in insect chemical ecology (Blomquist & Bagnères, 2010; Martin & Drijfhout, 2009) [1, 4]. Solvent extraction enables the isolation of surface lipids without structural degradation or internal contamination (Howard & Blomquist, 2005) [7]. The use of HP-5 capillary columns and temperature ramping between 60°C and 300°C has been widely employed to achieve optimal resolution of long-chain hydrocarbons and esters (Drijfhout *et al*., 2009) [4]. Quantitative estimation through Total Ion Chromatogram (TIC) integration and compound identification based on NIST library matching are well-established procedures for assessing qualitative and quantitative CHC profiles (Leonhardt *et al*., 2016; van Zweden & d'Ettorre, 2010) [11, 20]. This standardized protocol ensures comparability of hydrocarbon spectra across diverse insect taxa and experimental conditions.

Results

Microscopic observation of the collected specimens confirmed two distinct insect taxa based on external morphological features. The first specimen exhibited a smooth, glossy blackish-brown exoskeleton with a distinct petiole separating the thorax and abdomen, elbowed antennae, and well-developed mandibles—features characteristic of the carpenter ant *C. pennsylvanicus*. The second specimen showed a bright reddish-orange body with a prominent black X-shaped marking on the hemelytra, elongated rostrum, and slender legs, confirming it as the red cotton bug *D. cingulatus*.

Fig 1: Dorsal view of *Camponotus pennsylvanicus* under stereomicroscope showing the smooth, dark brown exoskeleton and distinct head capsule with elbowed antennae.

Fig 2: Lateral view of *Camponotus pennsylvanicus* illustrating the constricted petiole connecting the thorax and gaster, characteristic of the genus Camponotus.

Fig 3: Dorsal view of *Dysdercus cingulatus* showing the bright red-orange coloration and the prominent black X-shaped marking on the hemelytra.

Fig 4: Enlarged dorsal view of *Dysdercus cingulatus* highlighting the membranous hind wings, elongated rostrum, and symmetrical thoracic pattern typical of the species.

Gas Chromatography-Mass Spectrometry (GC-MS) analysis revealed distinct cuticular hydrocarbon (CHC) profiles for *C. pennsylvanicus* and *D. cingulatus*. Each chromatogram displayed species-specific peaks corresponding to alkanes, fatty acids, esters, and unsaturated hydrocarbons. Compounds were identified based on mass spectral data and expressed as a percentage of total ion chromatogram (TIC) area.

GC-MS of *C. pennsylvanicus*: The GC-MS chromatogram of *C. pennsylvanicus* (Fig. 1) indicated a predominance of straight-chain alkanes, particularly pentadecane (18.11%), undecane (12.24%), and heneicosane (11.14%) (Table 1). Moderate proportions of esters such as bis(2-ethylhexyl) phthalate (13.38%) and isopropyl myristate (6.14%), and the fatty acid tetradecanoic acid (3.57%) were also recorded. Minor components included hexadecane, eicosane, and heptadecane (<3%).

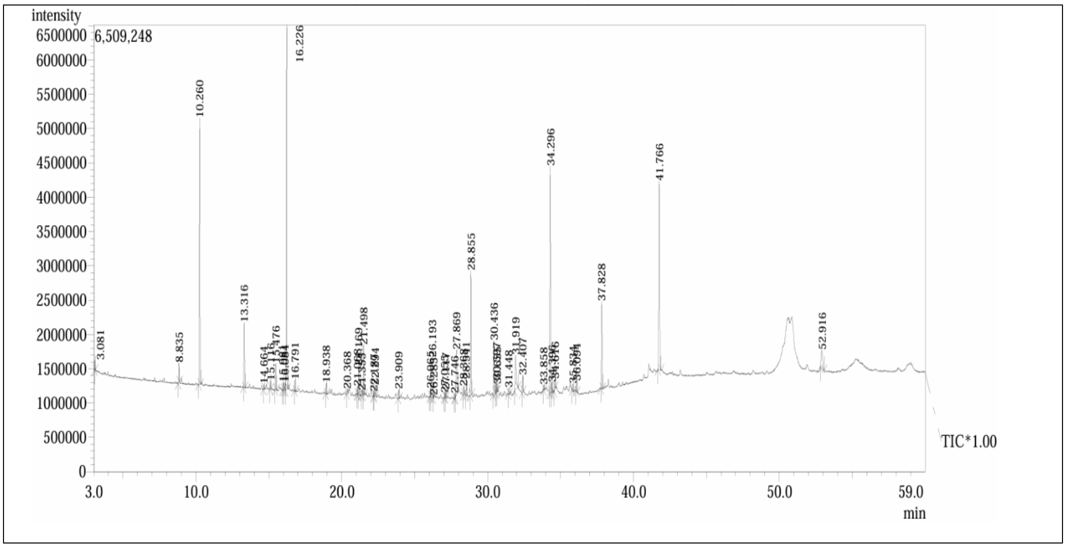

Fig 5: GC-MS chromatogram of *C. pennsylvanicus* cuticular hydrocarbon extract

The dominance of long-chain saturated hydrocarbons suggests their functional significance in nestmate recognition, cuticular waterproofing, and colony communication.

Table 1: Major hydrocarbon compounds identified in *C. pennsylvanicus*.

S. No.	Compound	Chemical Class	Relative Abundance (%)
1	Pentadecane	Alkane	18.11
2	Undecane	Alkane	12.24
3	Heneicosane	Alkane	11.14
4	Bis(2-ethylhexyl) phthalate	Ester	13.38
5	Isopropyl myristate	Ester	6.14
6	Tetradecanoic acid	Fatty acid	3.57

GC-MS of *D. cingulatus*

The CHC profile of *D. cingulatus* exhibiting a higher concentration of fatty acids and esters (Fig. 2, Table 2). Major compounds included phthalic acid di(6-methylhept-2-yl) ester (14.42%), (9E,11E)-octadecadienoic acid (12.10%), and eicosanoic acid (10.79%). Additional constituents such as isopropyl myristate (8.53%), 1-tetracosene (7.95%), and heneicosanoic acid (6.20%) were also recorded.

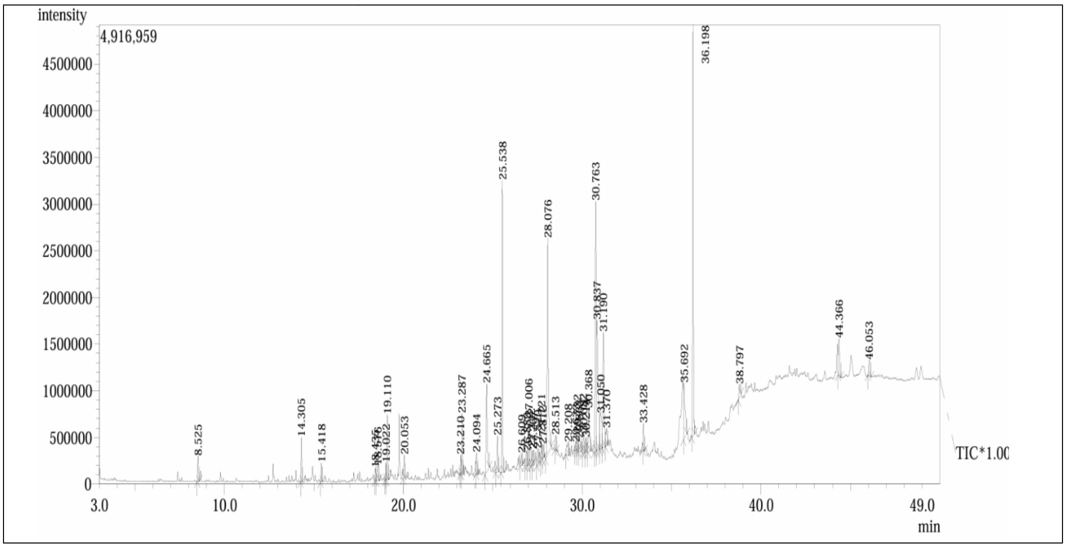

Fig 6: GC-MS chromatogram of *D. cingulatus* cuticular hydrocarbon extract

This fatty acid-enriched composition indicates enhanced cuticular flexibility and potential involvement in semiochemical signaling related to reproduction and host-plant interactions.

Table 2: Major hydrocarbon compounds identified in *D. cingulatus*.

S. No.	Compound	Chemical Class	Relative Abundance (%)
1	Phthalic acid di(6-methylhept-2-yl) ester	Ester	14.42
2	(9E,11E)-Octadecadienoic acid	Fatty acid	12.10
3	Eicosanoic acid	Fatty acid	10.79
4	Isopropyl myristate	Ester	8.53
5	1-Tetracosene	Unsaturated hydrocarbon	7.95
6	Heneicosanoic acid	Fatty acid	6.20

Comparative analysis of GC-MS

The comparative analysis (Table 3) demonstrated clear interspecific differences. *C. pennsylvanicus* showed a hydrocarbon-dominated profile within the C_{11}-C_{21} range, while *D. cingulatus* exhibited a lipid-rich mixture characterized by higher fatty acid and ester content. The total ion area was greater for *D. cingulatus* (97,641,196) than *C. pennsylvanicus* (84,197,506), indicating a higher diversity or concentration of extractable lipids.

Unsaturated hydrocarbons such as 1-tetracosene were observed exclusively in *D. cingulatus*, suggesting roles in volatile signaling. Trace phthalate esters detected in both species were considered laboratory artifacts unrelated to biological processes.

Table 3: Comparative features of cuticular hydrocarbons in *C. pennsylvanicus* and *D. cingulatus*.

Parameter	*C. pennsylvanicus*	*D. cingulatus*
Total Ion Area	84,197,506	97,641,196
Dominant Compounds	Pentadecane (18.1%), Undecane (12.2%), Heneicosane (11.1%)	Phthalic acid ester (14.4%), Octadecadienoic acid (12.1%), Eicosanoic acid (10.8%)
Major Chemical Class	Straight-chain alkanes	Fatty acids and esters
Unsaturated Hydrocarbons	Trace	Present (≈8%)
Fatty Acid Content	≈6.7%	≈21%
Biological Interpretation	Hydrocarbon-rich, communication and waterproofing	Lipid-rich, reproductive and ecological adaptation

Overall, *C. pennsylvanicus* exhibited a stable CHC profile dominated by saturated hydrocarbons, reflecting its eusocial structure and environmental adaptability. *D. cingulatus* presented a more dynamic and diverse lipid spectrum dominated by fatty acids and unsaturated esters, indicative of reproductive and host-plant communication roles.

Discussion

The GC-MS profiles of *C. pennsylvanicus* and *D. cingulatus* revealed distinct chemical compositions that correspond closely with their ecological and behavioral differentiation. *C. pennsylvanicus* exhibited a predominance of long-chain saturated hydrocarbons such as pentadecane, undecane, and heneicosane-compounds known to function in nestmate recognition, colony cohesion, and desiccation resistance (Martin & Drijfhout, 2009; van Zweden & d'Ettorre, 2010) [4, 20]. These chemically stable, non-volatile alkanes form a consistent surface signature critical for eusocial communication and survival in open terrestrial environments (Blomquist & Bagnères, 2010) [1]. In contrast, *D. cingulatus* displayed a higher abundance of fatty acids and esters, notably octadecadienoic and eicosanoic acids, which are commonly associated with pheromonal activity and cuticular plasticity in phytophagous insects (Howard & Blomquist, 2005; Johnson & Lee, 2017) [7, 10]. The detection of unsaturated hydrocarbons such as 1-tetracosene further supports their role in short-range volatile signaling, facilitating mating and host-plant aggregation (Leonhardt *et al*., 2016) [11].

The marked compositional divergence between the two species underscores the influence of evolutionary and ecological pressures on CHC biosynthesis. Social hymenopterans like *C. pennsylvanicus* favor structurally simple, saturated hydrocarbons that ensure stability and uniformity within colonies, while hemipterans such as *D. cingulatus* exhibit chemically dynamic lipid mixtures suited for reproductive signaling and environmental adaptability (Gibbs, 1998; Drijfhout *et al*., 2009) [5, 4]. Such differentiation aligns with broader evidence that CHC diversity reflects adaptive responses to communication demands and microclimatic challenges across insect taxa (Hsu *et al*., 2024; Sevgili *et al*., 2025) [9, 17]. The comparative outcomes of this study thus provide biochemical evidence linking CHC variation to social organization, ecological specialization, and evolutionary divergence among insects.

Conclusion

The comparative GC-MS analysis revealed clear chemical differentiation between *Camponotus pennsylvanicus* and *Dysdercus cingulatus*. The ant exhibited a hydrocarbon-rich profile typical of eusocial communication and desiccation resistance, while the bug showed a fatty acid- and ester-dominant composition linked to reproductive and ecological interactions. These findings highlight how cuticular hydrocarbon diversity mirrors ecological adaptation and behavioral evolution among insect taxa.

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