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Characterizing the volatile organic profile of *Chinocossus acronyctoides* (Lepidoptera: Cossidae) larvae through Solid-Phase Micro extraction-Gas Chromatography-Mass Spectrometry (SPME-GCMS)

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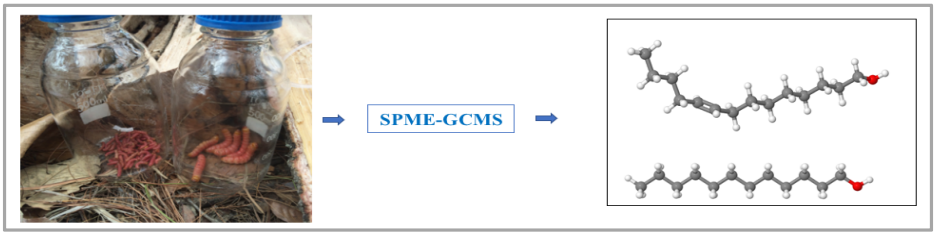
ABSTRACT

Plants and animals emit diverse array of Volatile Organic Compounds (VOCs) which plays an important role in their ecology. Many insects use scents as a way of deterring predators. Hence, we decided to investigate the VOCs composition of Chinocossus acronyctoide smoth larvae, a Cossid species. The VOCs composition of Chinocossus acronyctoides moth larvae were identified through a coupled Solid-Phase Micro extraction (SPME) with gas chromatography mass spectrometry (GC-MS). This study allowed the identification of major VOCs involves behind the pungent and aromatic nature of the larvae of Chinocossus acronyctoides. A comparative analysis results that in the younger stage larvae or second instar, 3 major organic compounds were identified while in the older stage or fifth instar larvae, 4 major VOCs were identified. Each sample had a varying VOCs profile. In all the two stages the compounds present were the fatty acid class of alcohol, acetate and aldehyde. Present study represents the first comparative analysis between the different stages of Chinocossus acronyctoides moth larvae as well provides the composition details of VOCs in this Cossid species particularly.

Graphical Abstract:



SPME-GCMS Analysis of the two different stage larvae of *Chinocossus acronyctoides*.

Keywords: Molecular docking, DFT (density functional theory), ADME/T, Halogen.

INTRODUCTION

Volatile organic compounds (VOCs) generally are lipophilic compounds with a high vapour pressure at room temperature and standard atmospheric pressure [**1**]. They are solid and liquid carbon-based substances that enter the gaseous phase by vaporization at 20°C and 0.01 kPa [**2**]. VOCs are a subject of interest across various domains, including food and fragrance assessment, environmental and atmospheric investigations, industrial uses security, as well as medical and life sciences [**3**]. VOCs emitted by insects have garnered significant attention due to their diverse roles in insect ecology and behaviour. These compounds are involved in various aspects of insect life, including chemical communication [**4**], mating [**5**], foraging [**6**], and defence mechanisms [**7**]. After the discovery of the first insect pheromone [**8**], Bombykol a sex pheromone (IUPAC name (10E, 12Z)-hexadeca-10, 12-dien-1-ol) from female silkworm moth, *Bombyx mori* researchers have started extensively studying the VOCs of lepidopteran insects [**9**]. Generally, volatile compounds in the form of sex pheromones produced by female moths are complex mixtures of straight-chain acetates, aldehydes, and alcohols, with 10–12 carbon atoms and up to three unsaturation's [**10**]. Studies from other cossid species also reveals that larvae commonly produce fatty-acid derivatives, similar to the sex pheromone compounds produced by adult female moths [**11**].

The Volatile Organic Compounds produced by *Chinocossus acronyctoides* larvae possess a strong pungent, aromatic smelling compound which is believed to be a defence mechanism to protect itself from predator-prey [**12**] and due to this smelly nature of the larvae the family Cossidae groups are sometimes called as goat moths [**13**]. The larvae are seen boring primarily on Oak tree, *Quercus serrata* as its host. When the larvae are younger the smelly nature of the VOCs are more prominent as compared to the older stages however, the adult moths do not possess any smelly characteristics. These odours are comprised of VOCs that exist in a gaseous state therefore, the use of gas chromatography mass spectrometry (GCMS) for characterizing odour is very valuable as it operates in the gas phase. Using GCMS, a large mixture of VOCs can be separated and identify in order to improve the understanding of the chemical composition of the odour released by the source [**14**]. The aim of this work was to identify and establish the specific VOCs profiles in the two different stages of the *Chinocossus acronyctoides* larvae. The volatiles from the two stages, younger stage or 2nd instar and older stage or 5th instar were extracted by using SPME and solvent extraction technique.

MATERIALS AND METHODS

Insect sample collection: *Chinocossus acronyctoides* larvae were collected from the field site at Kidima-Kohima, Nagaland, India. GPS position for latitude is 25° 33'33" N and longitude is 94°10'50" E.

Volatile extraction: The volatiles of the two different stage of *Chinocossus acronyctoides* larvae, old and young were collected by the technique solid-phase extraction and solvent extraction. At the time of collecting the organic volatiles both the two different stage larvae samples were kept enclosed in a sterilised chamber to avoid unnecessary contamination.

Solvent extraction: Solvent extraction was performed by using hexane as the solvent [**15**]. 10g of each of the larval stages (old and young) was used to extract the Volatile Organic Compounds. Each of the samples were exposed in hexane for 10 min in approximately 25 mL of hexane. Later the extracts were filtered using the Whatman filter paper. The filtrate was concentrates by evaporating the solvent using a slow stream of ultra-high purity nitrogen gas.

Solid-phase extraction: Solid-phase extraction (SPE) was performed using preconditioned polydimethylsiloxane (PDMS) tubes procured from Carl Roth (Rotilabo®–silicone tube). PDMS

tubes of 1.5 mm inner diameter and 3.5 mm outer diameter were cut into 5 mm pieces and soaked for 4 hr. in 1:1 mixture of acetonitrile and methanol. They were then dried using ultra-high-purity nitrogen gas and conditioned in a Gerstel Tube Conditioner by heating over the stream of nitrogen gas at 4 bar constant pressure. The entire process was repeated twice before using for extraction as performed by Nair *et al* [16]. For the extraction of the larvae volatiles two PDMS tubes were exposed to the required stage larvae for 10 min. Then later the tubes were then removed and stored in labeled, sterilized 0.5 mL amber glass vials ready for GC analysis.

GC-MS analysis: Organic Volatiles extracted from two different stages of *Chinocossus acronyctoides* larvae samples were separated and identified using an Agilent 7890B gas chromatograph coupled with a 5977A MSD mass spectrometer. An HP-5 MS column (30 m × 0.25 mm id, 0.25 µm film thickness) was used with helium as the carrier gas at a flow rate of 1 mL min^{-1}. The column oven was kept at 40°C min^{-1}, increased to 180°C at a rate of 5°C min^{-1}, and finally increased to 270°C with a 5 min holding temperature in the second ramp at 25°C min^{-1}. The transfer line between the GC and MS was maintained at 250°C, whereas the source and quadrupole temperatures were 230 and 150°C, respectively. Ionization was performed in electron impact mode with ionization energy of 70 eV. GC-MS acquisition was performed using Agilent MassHunter Workstation software B.07.02.1938, and qualitative analysis was assessed by MassHunter Qualitative Analysis Version B.07.00. Each GC chromatogram was also compared to a corresponding blank control to check for contaminants. Blank controls were empty sterilized glass vials exposed to similar environmental conditions and extraction procedures as the samples.

RESULTS AND DISCUSSION

The study reveals that, a total of 7 major Volatile Organic Compounds (VOCs) were identified from SPME-GCMS analysis from the two different stages of *Chinocossus acronyctoides* larvae. 3 Volatile Organic Compounds from the younger stage larvae and 4 Volatile Organic Compounds from the older stage larvae. The identified compounds present in the younger stage larvae were the saturated fatty acids of alcohol, acetate and aldehyde while in the older stage the compounds were the unsaturated fatty acid of alcohol and acetate. The details of the identified compounds are outline in the table 1.

Table 1. Major VOCs identified in the 2nd and 5th instar larvae. The blue region depicts the compounds identified from 2nd instarlarvae and orange region for the compounds identified in 5th instar larvae

S. No.	Name of the VOCs from Larvae	Molecular formula	Mol.Mass (SPME-GCMS)	Retention Time (Min)
1	Dodecanol	$C_{12}H_{26}O$	186.34	21.5
2	Dodecanal	$C_{12}H_{24}O$	184.183	23.0
3	Dodeceyl acetate	$C_{14}H_{26}O_2$	226.359	26.0
1	9,11-dodecenol	$C_{12}H_{24}0$	184.318	22.5
2	9-tetradecenol	$C_{14}H_{28}O$	212.317	23.0
3	9,11-dodecenyl acetate	$C_{14}H_{26}O_2$	226.355	25.4
4	11-dodecenyl acetate	$C_{14}H_{26}O_2$	226.355	26.0

In the figure 1A and 1B, shows the chromatogram of the SPME-GCMS analysis of the two different stage larvae. In figure 1A, peak “a” corresponds to compound dodecanol, “b”dodecanal, “c” dodeceyl acetate and figure 1B “p” denotes for compound 9,11-dodecenol,“q” for 9-tetradecenol, “r” for 9,11-dodecenyl acetate and “s” for 11-dodecenyl acetate. The structure of the Volatile Organic Compounds from *Chinocossus acronyctoides* larvae are shown in figure 2. In the younger stage (2nd instar) larvae, the identified compounds are saturated and their structures are a straight chains fatty acid class of alcohol, aldehyde and acetate. While in the older stage (5thinstar) larvae the structures are more complex as compared to the younger stage sample. The compounds are unsaturated straight chain fatty acid meaning their structures comprises of at least one double bond in the identified compounds.

Unlike the younger stage the aldehyde compounds are not seen to be the major compounds but only the alcohol and acetate compounds. This larvae VOCs which are fatty acids, believed to derive semiochemistry as was also reported by many **[17-18]**.

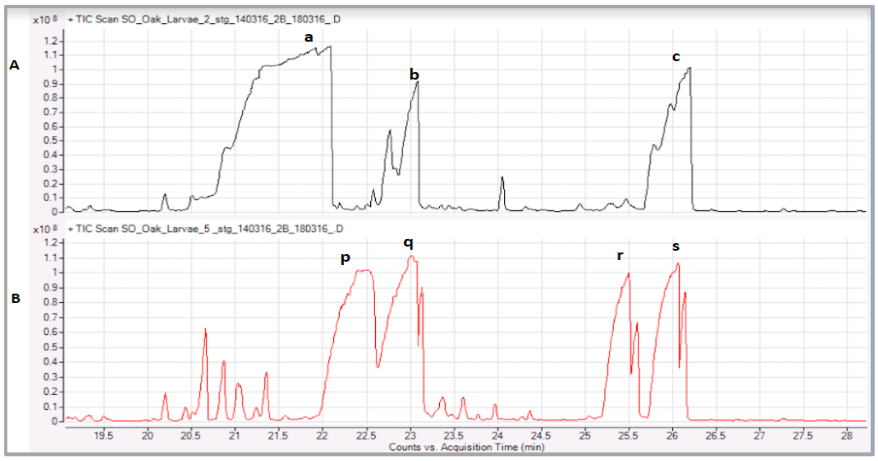


Figure 1A and 1B. SPME-GCMS chromatogram showing the different VOCs profiles in the larvae of *Chinocossus acronyctoides.*

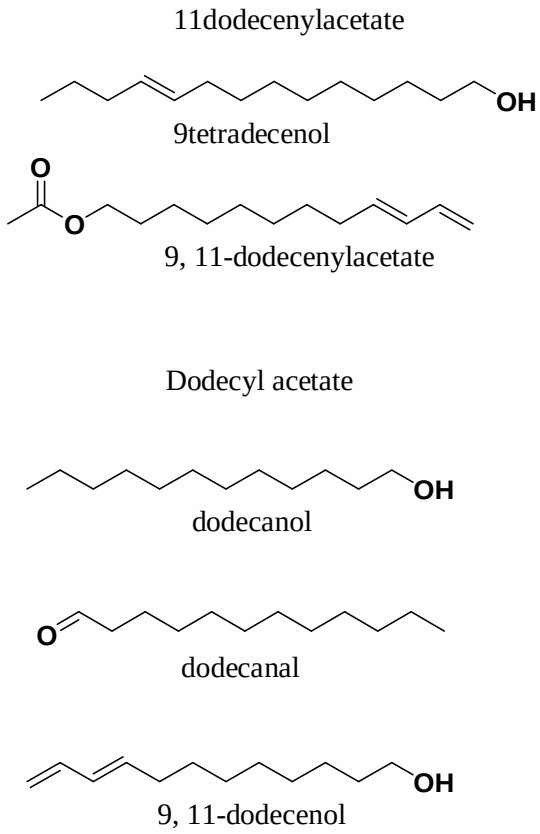


Figure 2. Structures of the identified compounds from the two different stage larvae of *Chinocossus acronyctoides.*

Similar compounds were identified from the solvent (hexane) extracts of the larvae as in SPME method. Some compounds like nonanal, decanal were also identified in the solvent extract which were earlier described. The two compounds were known to attract conspecific larvae while the similar blend was also reported to attract larval parasitoids [**19, 20**]. Dodecyl acetate and dodecanol compounds were also reported to be a major larvae component [**21**] of *Chilecomadiavaldiviana* which is another cossid species of the same family. Another species, larvae of *Chilecomadiamoorei*, dodecyl acetate and 11-dodecenylacetate were also found to be the major compounds of the larvae which were also found in our larvae hexane extract. The functions of these VOCs are not very well known but it's been speculated that they might possess antimicrobial properties or that they are used by gravid female adults at the moment of choosing an oviposition site [**21**].

CONCLUSION

The Volatile Organic Compounds (VOCs) that makes up the pungent aromatic smelly nature of the *Chinocossus acronyctoides* were identified. It revealed interesting findings regarding the composition and potential functions of these compounds. Two stages of larvae were examined, with a total of 7 major VOCs identified from our larval species. In the younger stage larvae, saturated fatty acids of alcohol, acetate, and aldehyde were predominant whereas, in the older stage larvae, it exhibited unsaturated fatty acids of alcohol and acetate. This suggests a developmental shift in the chemical composition of the larvae as it matures in to an adult. Notably, the compounds identified in the hexane solvent extract mirrored those obtained through SPME, indicating the reliability and consistency of the extraction methods. Some of the several compounds that were identified, such as nonanal and decanal, have been reported previously associated with attracting conspecific larvae and larval parasitoids, suggesting a potential role in intra and interspecies communication. Additionally, dodecyl acetate and dodecanol, which were also found in our larvae extract of *Chinocossus acronyctoides* were also reported as major components in other cossid species. This highlights the potential similarities in semiochemical profiles across related species. The precise function of the identified VOCs remains unclear, but hypotheses include antimicrobial properties or their use by gravid female adults in selecting oviposition sites. Further research is warranted to elucidate the specific roles and ecological significance of these compounds in the life history and behaviors of *Chinocossus acronyctoides* and related species. A few limitations in our study by not experimenting on bioassays to study the insect larvae behaviors with the identified compounds and the inability to synthesized the identified compounds for mass production to test in field studies for the insect management studies but overall, this study contributes valuable insights into the chemical ecology of cossid moths and highlights the importance of VOCs in mediating ecological interactions within insect communities

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REFERENCES

[1]. M. M. Lo, Z. Benfodda, R. Molinié, P. Meffre, Volatile Organic Compounds Emitted by Flowers: Ecological Roles, Production by Plants, Extraction, and Identification, *Plants*, **2024**, 13(3), 417-432. https://doi.org/10.3390/plants13030417.

[2]. E. Pagans, X. Font, A. Sánchez, Emission of volatile organic compounds from composting of different solid wastes: Abatement by biofiltration, *Journal of Hazardous Materials*, **2006**, 179–186. https://doi.org/10.1016/J.JHAZMAT.2005.09.017.

[3]. R. Epping, M. Koch, On-Site Detection of Volatile Organic Compounds (VOCs), *Molecules*, **2023**, 28(4), 1598-1617. https://doi.org/10.3390/molecules28041598.
[4]. E. Pichersky, J. Gershenzon, The formation and function of plant volatiles: perfumes for pollinator attraction and defense, *Current Opinion in Plant Biology*, **2002**,5(3), 237–243.
[5]. H. Xu, T. C. J. Turlings, Plant Volatiles as Mate-Finding Cues for Insects. *Trends in Plant Science*, **2018**,23, 100–111. https://doi.org/10.1016/j.tplants.2017.11.004.
[6]. N. Zjacic, M. Scholz, The role of food odor in invertebrate foraging, *Genes, Brain and Behavior*, **2022**, 21(2), e12793. https://doi.org/10.1111/gbb.12793.
[7]. T. Eisner, R. P. Grant, Toxicity, Odor Aversion, and Olfactory Aposematism, *Science*, **1981**,213, 476–476. https://doi.org/10.1126/science.7244647.
[8]. V. A. Butenandt, R. Beckmann, D. Stamm, Hecker Erich, Über den Sexual-Lockstoff des Seidenspinners Bom byx mori. Reindarstellung und K onstitution, *Z Naturforschung B*, **1959**, 283–284.
[9]. A. M. El-Sayed, *The Pherobase-Database of Insect Pheromones and Semiochemicals* (Ashraf M. El-Sayed, 2003).
[10]. T. Ando, S. Inomata, M. Yamamoto, Lepidopteran Sex Pheromones, **2004**, 239, 51–96. https://doi.org/10.1007/b95449.
[11]. R. Trave, L. Garanti, A. Marchesini, M. Pavan, Sulla naturachimica del secretoodorosodella larva del Lepidottero Cossus cossus L. *Chim. Ind.*, 1**996**, 11, 1167–1176.
[12]. M. Pavan, M. Valcurone Dazzini, *Considerazion general sulleFunzioni di Difesachimicane Lepidotteri*, Inst. Ent. agr. Univ. Pavia, **1976**.
[13]. Don Herbison-Evans Crossley Stella, COSSIDAE of Australia, **2014**. https://lepidoptera.butterflyhouse.com.au/coss/cossidae.html.
[14]. C. Cannon, S. Stejskal, K. A. Perrault, The volatile organic compound profile from Cimexlectularius in relation to bed bug detection canines. *Forensic Chemistry*, **2020**, 18, 100214. https://doi.org/10.1016/J.FORC.2020.100214.
[15]. S. Sheppard, Methods in Chemical Ecology. Chemical Methods, *Journal of Environmental Quality*, **1999**, 28, 2032–2033. https://doi.org/10.2134/JEQ1999.00472425002800060048X.
[16]. J. V. Nair, P. V. Shanmugam, S. D. Karpe, U. Ramakrishnan, S. Olsson, An optimized protocol for large-scale in situ sampling and analysis of volatile organic compounds, *Ecology and Evolution*, **2018**, 8, 5924–5936. https://doi.org/10.1002/ece3.4138.
[17]. W. L. Roelofs, Chemistry of sex attraction, *Proceedings of the National Academy of Sciences*, **1995**, 92, 44–49. https://doi.org/10.1073/pnas.92.1.44.
[18]. W. L. Roelofs, A. P. Rooney, Molecular genetics and evolution of pheromone biosynthesis in Lepidoptera, *Proceedings of the National Academy of Sciences*, **2003**, 100, 9179–9184. https://doi.org/10.1073/pnas.1233767100a.
[19]. Z. Jumean, T. Unruh, R. Gries, G. Gries, Mastrusridibundus parasitoids eavesdrop on cocoon-spinning codling moth, Cydiapomonella, larvae, *Naturwissenschaften*, **2005**, 92, 20–25. https://doi.org/10.1007/s00114-004-0581-1.
[20]. Z. Jumean, R. Gries, T. Unruh, E. Rowland, G. Gries, Identification of the Larval Aggregation Pheromone of Codling Moth, Cydiapomonella. *Journal of Chemical Ecology*, **2005**, 31, 911–924. https://doi.org/10.1007/s10886-005-3552-x.
[21]. J. Bergmann, K. Lopez, G. Buono-Core, Identification and synthesis of some fatty acid derivatives from larvae of *Chilecomadiaval diviana* (Lepidoptera: Cossidae), *Natural Product Research*, **2007**, 21, 473–480. https://doi.org/10.1080/14786410601129986.