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Volatile organic compounds in the habitat of the escamolera ant (*Liometopum apiculatum* Mayr) in Zacatecas, Mexico

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ABSTRACT

Volatile organic compounds (VOCs) take part in the biological processes of insects; however, these compounds have not been determined for genus *Liometopum*. The objective of this study was to identify the variability of the VOCs found in the *Liometopum apiculatum* habitat during the exploitation season. During the 2017 preseason and season, 35 air samples were collected from the nests of five *L. apiculatum* colonies established in crassicaule scrub vegetation; additionally, another 35 samples were taken from their foraging sites. Using a gas chromatograph with an electronic nose detector, the VOCs were identified with the Kovats index. In addition, a principal component analysis (PCA) was carried out to evaluate the intensity variability per season. Fourty-eight VOCs were identified in the *L. apiculatum* habitat. The most significant VOCs included: saturated hydrocarbon (17%), aldehydes (17%), alcohols (15%), and esters (10%). PCA accounted for 79.5% (PC1=53.8 and PC2=25.7) of the intensity variability of the VOCs in the habitat between seasons. The *escamol* season was characterized by the 3-methyl-3-sulfonyl butan-1-ol, 2-Methylbutanoic acid, and trimethylamine. This profile of the VOCs in the *L. apiculatum* habitat is a pioneer work and has future implications for the conservation and sustainable exploitation of the *escamolera* ant.

Keywords: Formicidae, hydrocarbon, edible insect, semiochemical.

INTRODUCTION

Ants (Himenoptera: Formicidae) are eusocial organisms characterized by their work hierarchy, cooperation, and an advanced caste system (López-Riquelme and Ramón, 2010). Their specific functions are determined by a highly sophisticated communication system (Peeters, 2019; Slavković and Bendahmane, 2023), divided into radial (light perception), mechanical (tactile or auditory), or chemical (smell and taste). Ants rely mainly on chemical communications, depending on the nature of the habitat, the ecological characteristics, the space, and the social groups of each species (Batey *et al.*, 2020; Paterson and Adams, 2022). The chemical communication system of the ants detects semiochemical compounds,

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which are responsible for transmiting information among organisms. These compounds include pheromones (intra-specific communication) and allelochemicals (inter-specific communication). Allelochemicals are divided into kairomones (which benefit the receptor), allomones (which benefit the emitter), sinomonas (mutually benefitial), and apneumonas (which are produced by the environment) (López-Riquelme y Ramón, 2010; Alavez-Rosas *et al.*, 2023). They are mainly composed of Volatile Organic Compounds (VOCs) (Batey *et al.*, 2020), expressed as smells or flavours (Welzel *et al.*, 2018).

Combined with stimulus provided by environmental elements (such as temperature), VOCs promote warning, defence, dispersion, recognition, attraction, reproduction, development, and foraging mechanisms (Cruz-Labana *et al.*, 2023; Dupont *et al.*, 2023). VOCs in ants can be recognized through chromatographic analyses, because they can be reproduced in a determined species or population (Acevedo, 2020; Alagappan *et al.*, 2021; Gordon, 2021). However, the behavioral response and function of VOCs depend on the volatility and the released amount (Fujiwara-Tsujii *et al.*, 2006; Cruz-Labana *et al.*, 2023).

All over the world, ants are used for medicinal and cultural purposes, as weather predictors or food. Six ant species are consumed in Mexico (Guzmán-Mendoza *et al.*, 2016): chicatana or de San Juan (*Atta mexicana* S. and *A. cephalotes* L.), mielera (*Myrmecosistus melliger* W. and *M. mexicanus* W.) and escamolera (*Liometopum occidentale* var. *luctuosum* and *L. apiculatum* Mayr) (Lara-Juarez *et al.*, 2015; García-Sandoval, 2022; Ángeles-Tovar, 2022). The escamolera ant (*Liometopum apiculatum* Mayr) has a high nutritional content and economic value (Reyes-Hernández *et al.*, 2021). The larvae or pupae of the reproductive caste are commonly known as escamoles (Lara-Juárez *et al.*, 2015). Escamoles are harvested every year in the wild and it changes depending on the geographical region (Ángeles-Tovar *et al.*, 2022). The sales of escamoles are an economic alternative in arid and semiarid regions of Mexico (García-Sandoval *et al.*, 2022). Consequently, the presence and survival of the escamolera ant in the wild is endangered, as a result of the overexploitation of their nests and the contamination of their habitats (Figueroa-Sandoval *et al.*, 2018). Although *L. apiculatum* is a species of economic interest and biocultural value, there is a lack of information about the VOCs involved in the reproductive stage of the ants and the role they play in the production of escamoles. This information is fundamental for the sustainable handling of *L. apiculatum* (Cruz-Labana *et al.*, 2023).

Identifying the VOCs in the *L. apiculatum* habitat would generate information about the networks of chemical signs used by this species in its physiological processes. In addition, it would enable the evaluation of the feasibility of breeding these edible insects under controlled systems. Therefore, the objective of this study was to identify the VOCs in the *L. apiculatum* habitat established in crassicaule scrub vegetation, as well as their variability during the 2017 escamol production preseason and season, in the Unidad de Manejo para la Conservación de la Vida Silvestre (UMA) El Milagro, Villa González Ortega, Zacatecas.

MATERIALS AND METHODS

The study was carried out in the Unidad para la Conservación, Manejo y Aprovechamiento Sustentable de la Vida Silvestre (UMA) El Milagro (22° 37' 46.41" N, 101° 56' 25.36" W, 2208 m.a.s.l.), It occupies an area of 302.9 ha in the Villa González

Ortega municipality, Zacatecas, Mexico. The dominant vegetation of the area is crassicaule scrub (Rzedowski, 2006) and the climate is semi-temperate arid temperate (BSkw'), with 12-18 °C mean annual temperatures (García, 2004).

Five *L. apiculatum* colonies were located and monitored in the UMA, where they are exploited to extract the escamoles. The foraging sites of the colonies were determined based on the number of paths and the average distance reported by Rafael-Valdez *et al.* (2017). The ant nests were differentiated according to the recommendations made by Hernández-Roldán *et al.* (2017). Air samplings from the nests and the foraging sites were taken from each colony. The sampling ports were buried into the nests with a 45° gradient; the screw cap of the ½-inch wide and 80-cm long PVC TuboPlus® sampling ports was located at ground level. The air samplings of the foraging sites were carried out at ground level in the pricklypear (*Opuntia* spp.), maguey (*Agave* spp.), or palm (*Yucca* spp.) substrates, taking into account the higher impact and activity of the worker ants (Kaspari, 2003).

From January to June 2017, air samplings were taken on a monthly basis, between 7:00 and 10:00 am, while in April —when escamoles can be found in the nests—, the sampling was taken every 15 days. A total of 70 samplings were collected: n=35 nest sampling and n=35 foraging sites sampling. In order to describe the VOCs of each period, this study determined that the preseason was the period when no escamoles were found in the nests (January-March 2017), while the season was the escamol production period of the colonies (April-June 2017).

A 0211-V45R-68cx Marathon® electric vacuum pump was used to extract the air from the nests. On one end, the pump was connected to the sampling ports. On the other end, it was connected to a 1L Tedlar® (EPA 1990) bag, with a Thermogreen® LB-2 septum (Supelco®). The bags were previously purged with ultrapure nitrogen (99.9999%) to reduce background contamination. The collected samples were kept in iceboxes. They were transported to the Laboratorio Nacional de la Coordinación para la Innovación y Aplicación de la Ciencia y la Tecnología of the Universidad Autónoma de San Luis Potosí, where they were stored at −20 °C for less than 24 h prior to their analysis.

Analysis of the volatile organic compounds

The air samplings were analyzed using an ultrafast gas chromatograph with a HERACLES II (Alpha MOS, France) electronic nose detector, with two short columns of different polarities, two flame ionization detectors (DB-5-FID 1 and DB-1701-FID 2) and a preconcentration trap that increases sensibility and the chances to obtain a global chemical fingerprint. A vial was used to take a 40-ml air sample from each bag. Subsequently, it was incubated in an autosampler for 900 s, at 40 °C, and with a 500-rpm agitation. One ml of the sample was taken per headspace and was injected to the GC E-nose. The temperature of the injector remained constant at 200 °C. The compounds were separated with a 50 °C temperature program for 30 s, constantly increasing the speed by 10 °C/s, until it reached a maximum of 280 °C, with a constant 1-mL/min hydrogen flux. The separated compounds were detected using the Alpha MOS software of the electronic nose; the VOCs were identified based on the Kovat index, using the C6-C16 standard (Śliwińska *et al.*, 2016).

Statistical analysis

The Alphasoft V.12 software (Alpha MOS, Toulouse, France) was used to analyze the compounds. The identified VOCs were classified per collecting site (nest and foraging site) and production season (preseason or season). The VOCs with high responses and similar chromatographic profiles were considered significative when their representation models had a >20% similarity ratio. A hierarchical cluster analysis was conducted in the Euclidean distance matrices; afterwards, a head map was obtained with the VOCs response of the individual nest samples (Wu *et al.*, 2016). The principal component analysis (PCA) of the InfoStat® V2020 software (Di Rienzo *et al.*, 2020) was used to establish the chromatographic response of the area under the curve (A∩C) of each VOC identified. Synthetic compounds were excluded from the analysis.

Chemical role of the volatile organic compounds

A literature review of the identified VOCs was carried out, in order to understand the semiochemical role recorded for the Himenoptera: Formicidae insects. The metabolites were determined using the MetaboAnalyst v 3.0 software, linked to the following databases: the Human Metabolome Database (HDMB), the PUBCHEM of the information retrival system of the National Center for Biotechnology Information (NCBI), and the Kyoto Encyclopedia of Genes and Genomes (KEGG) (Xia *et al.*, 2015).

RESULTS AND DISCUSSION

In the *L. apiculatum* habitat (nests and foraging sites), 48 VOCs were identified (Table I; Figure 1); they were mainly classified as saturated hydrocarbons (17%), aldehydes (17%), alcohols (15%), esters (10%) and, in lower proportions, ethers (6%), ketones (6%), terpenes
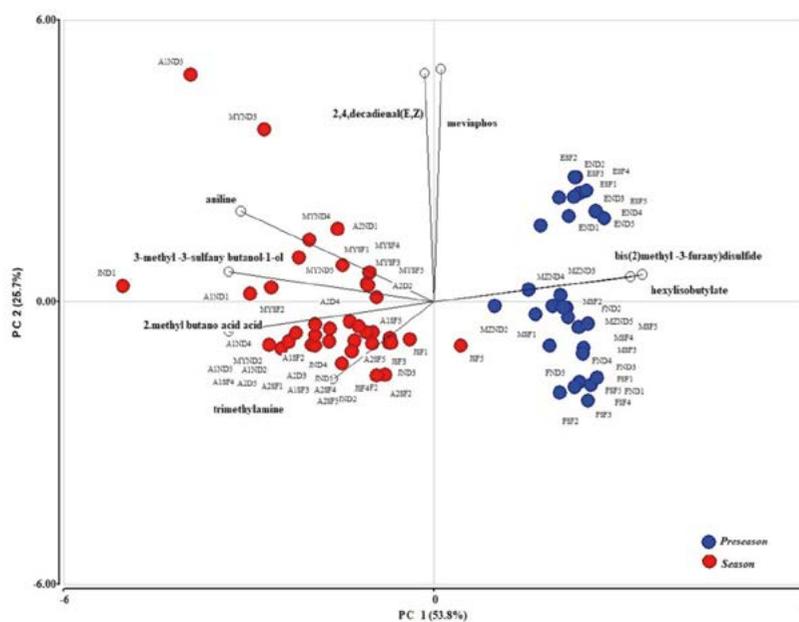

Figure 1. Intensity of the VOCs in *L. apiculatum* habitats (nests and foraging sites), showing the highest variation between escamol seasons (2017).

Table 1. VOCs recorded in the habitat of *L. apiculatum* from January to June 2017, in the UMA El Milagro, Villa González Ortega, Zacatecas. *Indicates agrochemical VOCs.

Retention time	VOCs	Retention time	VOCs
Aldehydes		**Esters**	
15.19-1	Acetaldehyde	54.99-1	Triacetin
24.23-1	2 methyl propanal	58.19-1	methyl undecanoate
26.13-1	Butanal	47.45-1	Hexyl isobutyrate
51.58-1	(E)cinnamaldehyde	34.43-2	methyl hexanoate
42.80-1	Benzeneacetaldehydo	40.48-2	(Z)-octanol
46.76-2	(E, E)2, 4 Nonadienal	**Ethers**	
48.72-2	(Z)-3-Phenyl-2-propanal	20.08-1	disopropyl ether
50.16-2	2,4, decadienal (E, Z)	22.50-1	diethyl ether
Alcohols		45.44-1	2-(2-ethoxiethoxy) ethanol
20.99-1	Propanol	**Amines**	
35.27-1	(E)-2-penten-1-ol	14.70-2	Trimethylamine
37.67-1	3-methyl -2-butane-1-ol	43.60-1	Aniline
39.79-1	(z)-2-hexano-1-ol	**Phenols**	
20.44-2	2-methyl-2-propanol	54.00-1	1,2-benzene dio
39.23-2	3-methyl -3-sulfani butanol-1-ol	38.08-2	1,8-cineole
41.29-2	E-2-None-ol	**Heteromatic compounds**	
Hydrocarbons		25.24-2	2-ethyl furan
18.78-1	Butane	54.35-2	Bis (2) methyl -3-furany) disulfide
31.24-1	2 methyl propane acid acid	**Terpenes**	
15.71-2	2-methyl butane	37.54-2	p-cimene
40.71-1	dimethyl benzene*	43.33-2	Citronellal
52.83-1	1-p-menthen-8-thiol	**Organophosphate esters***	
24.28-2	Heptane	57.44-1	Mevinphos
35.52-2	Decano	62.15-1	Molinate
36.31-2	2-methyl butano acid acid	68.40-1	dicrotophos
Ketones		70.70-2	Monocrotophos
41.55-1	2-methyl-2 cyclopenten 1 one	**Triazine***	
56.21-1	beta-damascenone	70.25-1	Prometon
30.29-2	3-hexanone	60.55-2	Atrazine

(4%), aromatic compounds (4%), amines (4%), phenols (4%), organophosphate esters (8%), and triazines (4%). Out of the total of VOCs identified in the escamolera ant habitat, 36 are semiochemical compounds recorded in insects of the family Formicidae found in plants of the study area. Four of the found compounds (E,E)-2-4-nonadienal, propanol, 2 methyl-2, beta-damascenone) influence the aroma volatility; two compounds are odorants (bis(2-methyl-3-furyl) disulfide; (E,E)-2,4-nonadienal), and six have traces of agrochemicals.

The VOCs in the escamolera ant habitat showed a 79.5% seasonal variability (PC1=53.8%, PC2=25.7%) with a 0.95 cophenetic correlation (Figure 1). The hexyl

isobutyrate and the bis(2-methyl-3-furyl) disulfide were the VOCs with the highest intensity variability during the preseason (March). This intensity considerably diminished during the escamol season. During the escamol production season the increase and correlation of the intensity between 2-Methylbutanoic acid, 3-methyl-3-sulfanylbutan-1-ol, aniline, and trimethylamine stood out. A negative correlation between the escamol season and the presence of synthetic VOCs (mevinphos) was recorded.

The VOCs variability in the foraging sites was 89.8% (PC1=61.2%, PC2=28.6%) and was influenced by six VOCs (Table 2; Figure 2), with a 0.99 correlation index. During the exploitation of escamol, a negative correlation was observed between the intensity of hexyl isobutyrate and the bis(2-methyl-3-furyl) disulfide regarding the presence of escamoles, while the intensity of 2-Methylbutanoic acid and 3-methyl-3-sulfanylbutan-1-ol were essential VOCs in the foraging substrates.

The VOCs variability of the nests —determined by the hierarchical cluster analysis with Euclidean distance matrices— resulted in a head map of the VOCs response of the nests with a 68.71 discrimination index (Figure 3). The PCA of the VOCs variability in nests accounts for up to 73% (PC1=45.6%; PC2=28.2%) of the seasonal variability; it included 12 VOCs (Table 3; Figure 4). The escamol season is specially correlated to the intensity increase of 2-methyl propane acid and trimethylamine. Nevertheless, a relevant factor for this analysis is the negative correlation between the escamol production season and the meviphos synthetic compound.

Table 2. Average variability of VOCs from *L. apiculatum* foraging sites during the 2017 January-July preseason and season, in UMA El Milagro, Villa González Ortega, Zacatecas.

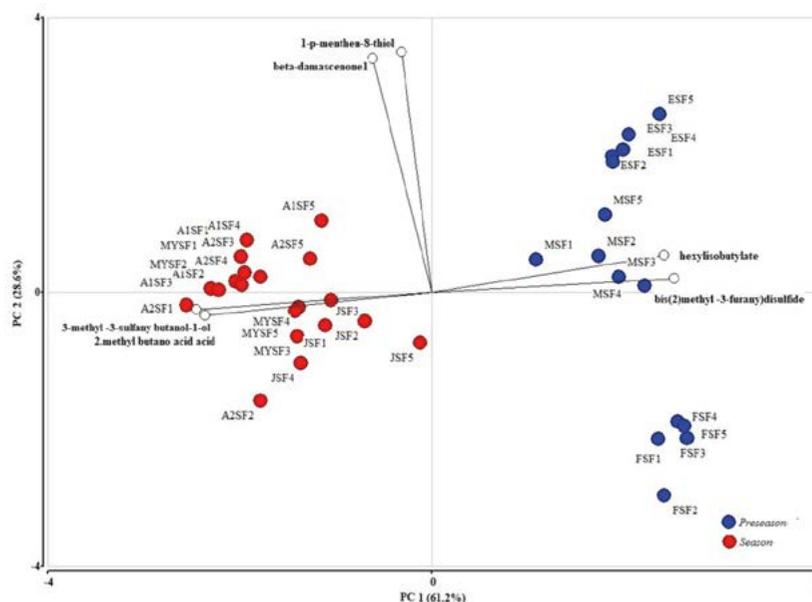| VOC	Preseason A∩C	Season A∩C
Acetaldehyde	43.9	5.5
2-methyl propanoacid	243.9	522
(E)cinnamaldehyde	482	25
E-2-None-ol	495	292
(E,E)2, 4 Nonadienal	1 711	1 066
methyl salicylate	2 232	527
Hexyl isobutyrate	22 539.4	6 064.5
Bis (2) methyl -3-furany disulfide	29 912	9 827.6
1,2-benzene diol	0	48
3-methyl -2-butane-1-ol	41.2	91.1
2-methyl butane acid acid	104	436
2-methyl-2 butand acid	161.2	272.4
(Z)-octanol	226	346.9
methyl hexanoate	329.8	375.1
3-methyl -3-sulfany butanol-1-ol	339	1 142.8
Aniline	3 87.6	831.9
1-p-menthen-8-thiol	1044	1075
Beta-damascenonel	2 857	3 165

Figure 2. VOCs variation in *L. apiculatum* foraging sites per production season.

Table 3. Response intensity of the VOCs with higher variability in *L. apiculatum* nests per season.

Chemical compound	Preseason A∩C	Season A∩C
(E, E)2,4 Nonadienal	2255.25	864.71
(E) cinnamaldehyde	921.293	890.263
2,4, decadienal (E,Z)	1 285	933.302797
Hexyl isobutyrate	15 469.291	3 373. 998
Bis (2) methyl -3-furany disulfide	31 234.229	7 461.019
Trimetilamina	0	100
1,2-benzene diol	35	778.81
2-methyl butane acid	131.909	751
(Z)-octanol	206.553	1 316. 563
3-methyl -3-sulfany butanol-1-ol	470.829	1 166.088
Aniline	586.63	945.96
meviphos	1 181	922

The 48 VOCs detected in the escamolera ant habitat had an important variation in their intensity response during the escamol production preseason and season. Although some compounds had a low intensity response, their semiochemical role can influence the level of response of the organism (Acevedo, 2020).

In this study, the following compounds were identified in the habitat: mainly saturated hydrocarbons, aldehydes, and alcohols, and, in a lower proportion, esters, ethers, ketones, terpenes, aromatic compounds, amines, and phenols. Gordon (2021), Jaffé (2004), Kaspari (2003), and other authors have previously established that the following pheromones
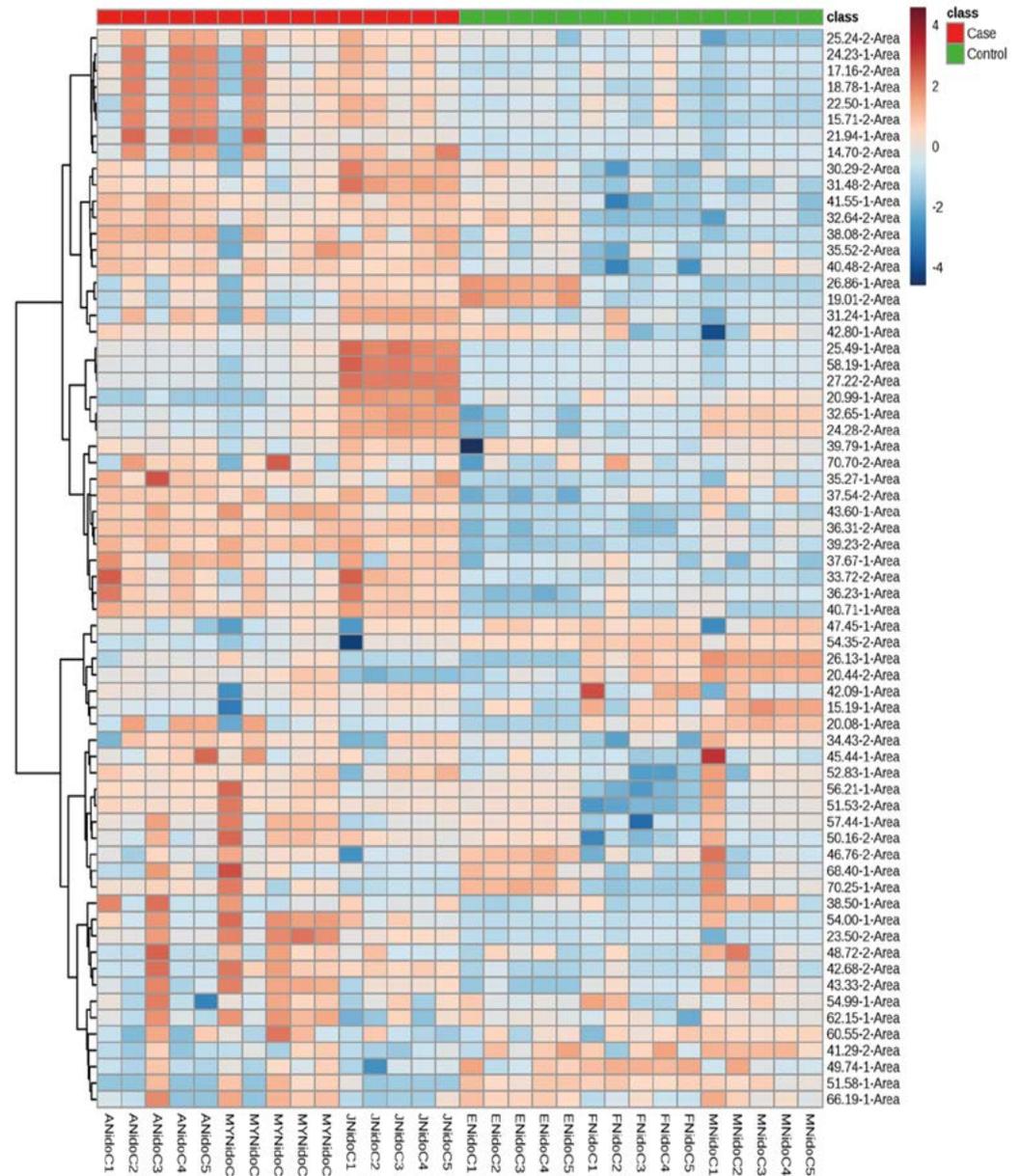
Figure 3. VOCS head maps in the air samplings obtained from the *L. apiculatum* nests during the production season (green: preseason; red: escamol season). The intensity of each VOC is represented in the horizontal axis. Each square shows the individual response of each compound of the samples.

regulate most of the life of ants: acetates, aldehydes, alcohols, alkanes, and ketones (Cantúa-Ayala *et al.*, 2019; Cruz-Labana *et al.*, 2023). Paterson and Adams (2022) have determined that alkanes are the main components of the tracking, warning, and sexual pheromones of family Formicidae. In addition, they play a fundamental role in the antimicrobial activity, creating toxins that repel predators. Alagappan *et al.* (2021) found that alkanes account for 50%, of the VOCs in the body of ants, followed by alcohols and, in lower proportions, aldehydes (0.8%) and the amides in the carboxylic acid (0.2%). In this study, the VOCs with
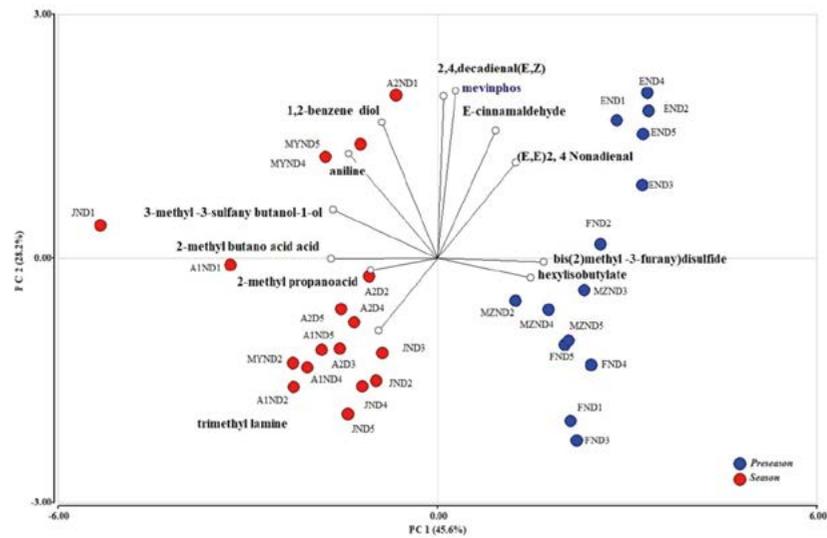
Figure 4. Variation of the response intensity of the VOCs found in *L. apiculatum* nests per escamol production season, from January to June 2017.

the highest presence in the habitat were saturated hydrocarbons and aldehydes, which helps to recognize a VOCs profile for this ant species.

Traces of VOCs used in agrochemicals were also detected in the *L. apiculatum* habitat (Carrera-Sánchez *et al.*, 2019). The source of these VOCs can be found in the agricultural areas inside or nearby the study area. Particularly, the intensity of the meviphos in the nests has a negative correlation (0.95) with escamol production. This compound is widely used in pesticides, as an acetylcholinesterase inhibitor (NCBI, 2023). Consequently, further studies should be carried out regarding the effect of agrochemicals in the reproductive biology of edible insects such as the escamolera ant and their impact on the escamol production in semiarid regions, given the lack of records about their influence in scientific literature.

On the one hand, in the foraging sites the intensisty of hexyl isobutyrate and the bis(2-methyl-3-furyl) disulfide diminished during the escamol production season. On the other hand, 1-p-Menthen-8-thiol, beta-damascedone, 3-methyl-2-butene-1-ol, and 2-methyl-2 butene acid reached high peaks of intensity noticeable in the environment.

Meanwhile, the intensity of bis(2-methyl-3-furyl) disulfide and hexyl isobutilate in the nests recorded higher peaks than the foraging sites. The intensity considerably diminished in the nests during the escamol season. Trimethylamine was an exclusive compound in the nests during the escamol season. Another characteristic was the increase of the intensity of 2-Methylbutanoic acid, 3-methyl-3-sulfanylbutan-1-ol, aniline, and 1,2-benzene diol. These compounds had the highest variation in the nest during the escamol production season. These compounds should be evaluated to determine their role in the reproductive biology of *L. apiculatum* and its influence in the escamol production.

Other VOCs have been reported in the inter-specific communication of ants. Their presence and intensity in the air of the nests and foraging sites can be related to the specific flora of their habitat, nesting substrates, and extrafloral nectaries. A wide variety of

plants depend on ants to spread their seeds and to pollinate them. However, other type of plants produces various types of compounds to protect themselves (Delnevo *et al.*, 2020). For instance, (E)-2-none-ol volatil compound, a characteristic element of pricklypears (*Opuntia* sp.) (HMDB41498) can be associated to the inclusion of pricklypears in the diet of the escamolera ant and its presence in the nesting substrate (Lara-Juárez *et al.*, 2015). Meanwhile, (E) cinnamaldehyde attracts ants to pollinize plants (*Cytinus* spp.) (Vega *et al.* 2014). For its part, the 2-methylbutanoic acid compound is produced by plants of the family Salicaceae to reduce its palatability in the *Spodoptera eridania* larvae (NCBI: Manuwoto *et al.*, 1985).

Meanwhile, the intensity of acetaldehyde in the habitat is interesting, because this compound has been related to the foraging activity of ants. The *escamol* season includes part of the winter, where the sources of food are scarce and the temperatures are low (Kaspari, 2003). Consequently, *L. apiculatum* must adapt to weather variations when they forage seeking sources of food. The intensity and concentration of foraging pheromones depend on the source of food (Cruz-Labana *et al.*, 2023). Therefore, the semiochemical role of the VOCs found in the external environment (*i.e.*, the foraging sites of *L. apiculatum*) are determinant in the *escamol* production.

The VOCs identified in this study also have been recorded in the intra-specific communication of the physiological processes of the insects. For instance, decano is the main element of the warning pheromone of *Camponotus obscuripes*, *Lasius fuliginosus, L. latreille*, and *Fomica rufa* ants (Hölldobler and Wilson, 1990; Mizunami *et al.*, 2010), while 3-methyl-3-sulfanylbutan-1-ol is an element of the volatile warning pheromone of the northern giant hornet (*Vespa mandarinia*) (Ono *et al.*, 2003). The VOCs related to defensive chemical secretions prevent the theft of eggs, larvae, or food, as well as other threats to the nests (Hölldobler and Wilson, 1990; Lara-Juárez *et al.*, 2015). For instance, hexyl isobutirate is a compound produced by the males and females of two species of bedbugs (*Lygus lineolaris* and *L. elisus*), as a defense mechanism against predators; however, it is also used as sexual pheromones (Byers *et al.*, 2013).

Escamoles are harvested only once per year and requires specific conditions in the wild habitats. It also requires a previous mating that, in the Altiplano Potosino, takes place from March to April (Lara-Juarez *et al.*, 2015). However, the semiochemicals involved in the reproduction of the escamolera ant are unkown.

In this study, a variation in the intensity of the 2-methylbutanoic acid was recorded; this compound can be found in the mandibular gland of the *Cephalotes alfaroi* and *C. cristatus* ants (Wood *et al.*, 2011). The importance of this gland lies in the initiation pheromones, which, in some insect species, produces the queen substance that inhibits the reproduction of worker ants. However, Fujiwara-Tsujii *et al.* (2006) and Alagappan *et al.* (2021) have reported the lack of information about VOCs in the reproduction pheromones.

Compounds such as bis(2-methyl-3-furyl) disulfide considerably diminished their intensity during the escamol season (Buttery *et al.*, 1984). This compound has been associated with the smell of roasted beef, which has a 1.0% odor detection threshold. Meanwhile, its taste has been described as sulfuruous and meaty, with traces of onion and roastbeef and a taste detection threshold with soupy and tasty characteristics, an a slightly metallic

flavor. It does not have known allergenes. In high concentrations, it can be associated with a rancid or rotten meat smell (Wang *et al.*, 2020). In its synthetic form, it is an aromatic compound used to improve the natural, fresh, and rich sensation of milk. In this study, the decrease of the intensity of this compound in the habitat was determinant for *escamol* production. Further research would determine its effects on the aromatic characteristics and taste of escamoles. (E,E)-2,4-nonadienal is another compound related to the smell of food; it has been reported as an aromatic substance that can be found in butter and in the taste and fragance of caviar (HMDB: 31685). Its intensity increases during the escamol production season. These VOCs are probably related to the characteristic smell and flavor of the escamol, which Holldöbler and Wilson (1990) have compared to the smell and flavor of rancid butter. As a result of its distinctive flavor, escamoles are popularly known as the Mexican caviar.

Finally, thrimethylamine was only found in the nests during the *escamol* season; however, it was not detected in the foraging sites. This compound is involved in the growth of the eggs of *Daphnia magna* (Lass *et al.*, 2001). Meanwhile, the size of the eggs of the escamolera ant caste may be influenced by the presence of trimethylamine.

CONCLUSIONS

This study identified 48 VOCs in the *L. apiculatum* habitat, during the preseason-season. The variability in the intensity of the VOCs found in the nests and foraging sites enabled their charactyerization during the escamol production season. This study recorded, for the first time, the semiochemical profiles related to the reproductive behavior of the escamolera ant. This species has the potential to contribute to food security. However, further research is required to evaluate the role of VOCs and the variability of their concentrations in the physiological behavior of ants. VOCs can influence the preservation and exploitation of this edible insect. Finally, the agrochemical impact of VOCs on the production of escamoles should be acknowledged.

ACKOWLEDGEMENTS

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