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BIOECOLOGY AND CHEMICAL DIVERSITY OF ABDOMINAL GLANDS IN THE IRANIAN SAMSUM ANT *Pachycondyla sennaarensis* (Formicidae: Ponerinae)

Nikbakhtzadeh MR (1), Akbarzadeh K (2), Tirgari S (3)

(1) Department of Medical Parasitology and Entomology, College of Medical Sciences, Tarbiat Modares University, Tehran, Iran; (2) Iranshahr Station of Public Health Research, Iranshahr, Iran; (3) Department of Medical Entomology and Vector Control, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran.

ABSTRACT: The genus *Pachycondyla* is a large group of ants in the Ponerini tribe, known mostly from tropical and subtropical regions. *Pachycondyla sennaarensis*, the so-called Samsum ant in the Middle East, is distributed throughout the African tropics, Arabian Peninsula and Iran, where it is responsible for many cases of insect-induced dermal lesions and systemic reactions in humans. Populations of *P. sennaarensis* were studied in two regions of Iran and some aspects of their biology, ecology and medical importance are herein presented. Colonies of *P. sennaarensis* contain less than 850 workers that live in complicated underground galleries approximately one meter deep. Because of the harsh weather conditions of southern Iran, they can survive only in human disturbed habitats with higher humidity. Neither a real queen (without reproductive division of labor) nor a caste system is found in a *P. sennaarensis* colony. Observations indicated that *P. sennaarensis* is omnivorous, feeding on seeds of various plants, dead ants of other species, the larvae of dipterans and a few other invertebrates. The effect of the *P. sennaarensis* sting is usually mild, resulting in papule formation, erythema and dermal itching. The abdominal gland secretion of *P. sennaarensis* is a complex mixture of saturated and unsaturated hydrocarbons and small amounts of terpenoids, ketones, pyrazines and phenolic compounds that are accompanied by straight-chain hydrocarbons. So far, no case of anaphylaxis has been reported in Iran, a fact probably due to the lack of proteins in *P. sennaarensis* venom. It appears that *P. sennaarensis* populations vary considerably in their toxin composition according to their geographic range, which may ultimately explain symptoms of different severity among local residents.

KEY WORDS: Samsum ant, sting, ants, Formicidae, Ponerinae, *Pachycondyla*.

CONFLICTS OF INTEREST: There is no conflict.

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CORRESPONDENCE TO:
MAHMOOD REZA NIKBAKHTZADEH, Department of Medical Parasitology and Entomology, Tarbiat Modares University, Tehran, PO Box 14115-331, Iran. Fax: +98 21 82884555. Email: nikbakht_m@excite.com.

INTRODUCTION

Ants (superfamily Formicoidea) are probably the most successful of all insect groups, occurring everywhere in terrestrial habitats (1). Ant species are grouped into the single family of Formicidae, which is further divided into 21 subfamilies (2), 296 genera and more than 11000 described species (1). One of the major ant subfamilies is the Ponerinae which is often considered “primitive” because its members show many of the putative ancestral traits (3). These include low queen–worker dimorphism, small colony sizes and predatory behavior, all of which may explain the simple social structuring and simple communicative needs of ponerine ants (4). Ponerine ants are primarily insectivorous and forage outside their nest in order to capture their prey (5).

The ponerine genus *Pachycondyla* Smith 1858, with 200 described species, is a large group of ants in the tribe Ponerini, mostly known from tropics and sub-tropics (6). The generic names *Brachyponera* and *Ectomomyrmex* are synonymous with *Pachycondyla*. The nesting and social biology of *Pachycondyla* is variable (7, 8). This genus lives in colonies of a few dozen to a few thousand workers. Several species of *Pachycondyla* are specialized termite predators or are nectar feeders; however, most species are leaf litter inhabitants that are generalist scavengers or predators who subdue their prey with their venom (9, 10).

Adverse reactions have been reported in humans after being bitten or stung by ten genera from six subfamilies of such ants (11). Excluding the imported fire ants (*Solenopsis* spp.), *Pachycondyla* is responsible for most cases of anaphylaxis throughout the globe (11). The two most notorious species are *P. chinensis* and *P. sennaarensis*. Allergic and anaphylactic reactions from *P. chinensis* have been reported from China (12), Japan (13), Vietnam (14), New Zealand (15), Korea (16), Taiwan and the United States (13, 17). Similar problems after the stings of *P. sennaarensis* were first reported from the United Arab Emirates (18). *Pachycondyla sennaarensis* (Mayr, 1862), distributed throughout the African tropics, was first described as *Ponera sennaarensis* by Mayr in 1862 (19). It is an aggressive species from the Arabian Peninsula (20, 21). Dejean and Lachaud (22) who studied the species in Zaire, described it as partially feeding on seeds. Iranian Samsum ants were unknown until recently when *Pachycondyla sennaarensis* (Hymenoptera: Formicidae) was reported from southern and southeastern Iran (23, 24). Paknia believes that the evolution of omnivory, including seed consumption, from carnivory

within this group allowed omnivorous taxa to better disperse across the native African boundaries with abundant prey (25). Winged dispersal of female reproductive forms does not seem to play an important role in the spread of *P. sennaarensis* to non-native regions, such as the Arabian Peninsula and Iran (25, 26). It is thus assumed that human activities such as overseas trade, construction and irrigation of planted vegetation are the major means of introduction.

The ecology and the distribution of this species in Iran are not well established. Globally, there are also some variations in the clinical features of these medically important ants. Although abdominal gland secretions have been studied in several species of the genus *Pachycondyla*, no comprehensive study has been yet carried out on *P. sennaarensis* (4, 27). In addition to ecological studies, we have therefore studied abdominal glands of *P. sennaarensis* and herein present these findings in a medical context.

MATERIALS AND METHODS

Field Collection

Foraging workers of four colonies of *P. sennaarensis* were collected in Fajr Park (27.12N, 60.41E) and around Abshekan village (27.19N, 60.46E) from February to May 2007, while the field observations of the marked colonies which were located in Abshekan village (Figure 1) continued until October. Collected specimens were killed by freezing and carried in cold boxes to the laboratory where they were stored at –30°C until 2008, when the workers were dissected for sample preparation. A sample of individuals was retained intact for morphological identification.

Figure 1. A marked colony of *Pachycondyla sennaarensis* in southeastern Iran. The small openings of the colony on the ground are indicated by triangular markers.

Identification

Identification to the species level was carried out by Prof. B. Bolton at Natural History Museum in London and Dr. J. L. Cook at the Sam Huston State University of Texas. All ants collected in the mentioned localities were confirmed as *Pachycondyla sennaarensis* (Hymenoptera: Formicidae, see Figure 2A). Voucher specimens are deposited in the laboratory of Medical Entomology at Tarbiat Modares University.

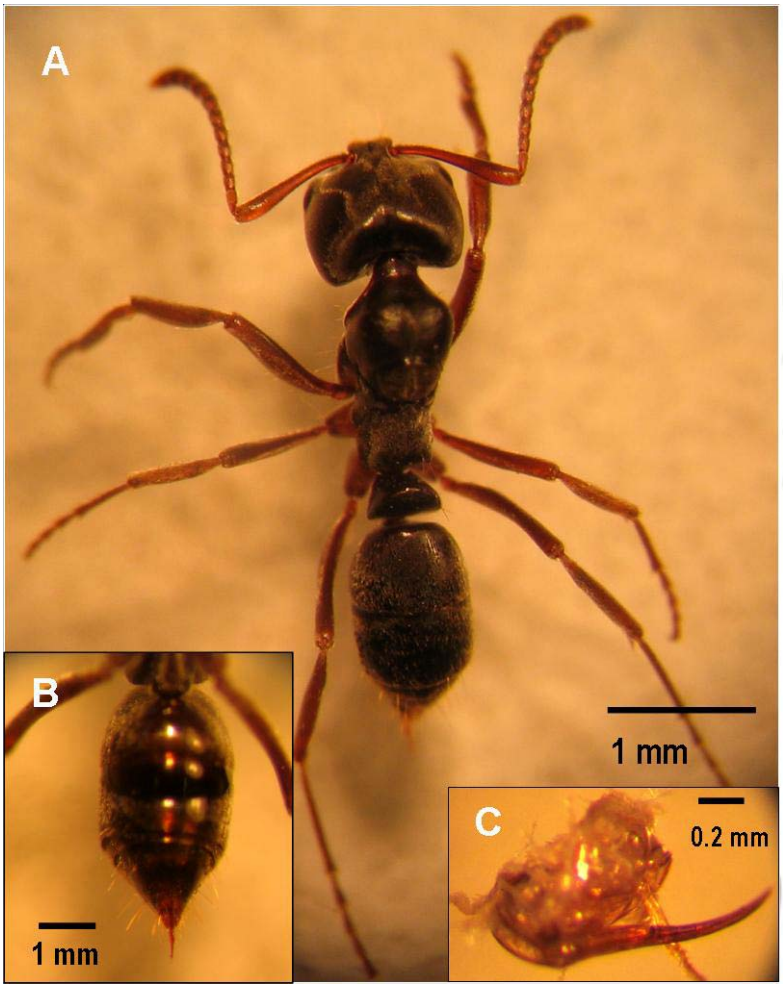

Figure 2. The Iranian Samsum ant *P. sennaarensis*: (**A**) worker, (**B**) the posterior abdominal sting and (**C**) the sting apparatus attached to the abdominal gland structure.

Monitoring of Ant Behavior

The ability of fertilized workers to found colonies was monitored under field conditions using 20 open Plexiglas cylinders (60 cm height × 30 cm diameter), which had been fixed at the soil depth of 20 cm, at an alfalfa farm in Abshekan village. The farm was irrigated twice a week by flooding. One fertilized ant worker was introduced into each cylinder which was covered at top with a piece of polyester net to prevent escape. Cylinders were observed daily for 40 days.

Clinical Features

Symptoms, induced by the Samsum ant, *Pachycondyla sennaarensis*, were observed on hands of the authors (n = 3) at the TMU Lab. The forearm was exposed to the ants to be repeatedly stung after which the clinical trend of the symptoms was

recorded for 72 hours. Basic emergency medical care was available and volunteers were monitored under direct supervision of medical staff.

Preparation of Extract from Abdominal Glands and Chemical Analysis

Workers (n = 7) were thawed in a refrigerator and then their abdominal gland structure, attached to the sting apparatus (Figures 2B and 2C), was pinched out in distilled water with the aid of a dissecting microscope (Stemi SR®, Zeiss, Germany). Dissected venom glands were placed in 0.5 mL microtubes and disrupted by ultrasonic waves (UP 400S®, Hielscher, Germany) for three minutes in a cycle mode of 0.5 second, 50 kHz in either 250 µL hexane (chromatography grade, Merck, Germany) or 50 µL of a 9:1 solution of acetonitrile and trifluoroacetic acid. The resulting mixtures were shaken for one minute and membranes were removed by centrifugation for five minutes at 3000 r/m. The hexane-based extract was concentrated by nitrogen flow and examined by GC-MS on a Varian Star 3800® instrument linked to a Varian Saturn 2000® Mass Selective Detector, set to monitor *m/z* 40-350 at 70 eV with a scanning speed of 1 scan/sec. The samples were analyzed using a fused silica capillary column (50 m × 0.25 mm ID, 0.12 µm FT) coated with CP-Sil8cb (5% phenyl, 95% polydimethylsiloxane). The oven temperature was programmed to rise from 50°C to 300°C at 15°C/minute. The carrier gas was helium at 1.2 mL/min. The injector port temperature was held at 200°C. The peak area of each component in the chromatogram was determined and then the percentage of each substance in the gland calculated by the Saturn® GC/MS Workstation computer package Saturn view® version 5.2.1, 1989-1998 (Varian Associates, Inc., USA).

High-performance liquid chromatography (HPLC) was performed using a Waters 6000A® pump and Waters R401® (USA) differential refractometer detector. Ten microliters of the acetonitrile/trifluoroacetic acid extract was injected into a C_{18} reserved phase column (5 µm, particle size; 220 × 2.1-mm column; Vydac) and separation was monitored at a flow rate of 20 µL/minute (28).

Chemical Identification

Identification was primarily accomplished by using spectra from the NIST library (NIST Mass Spectrometry, 2007). Precise identification was based on comparison of GC retention time and mass spectra with either authentic or the laboratory-

synthesized compound. Authentic trimethyl pyrazine, 2,5-piperazinedione, phenol-2,4-bis (1,1 dimethylethyl), alkenes and methylated hydrocarbons were obtained from Sigma-Aldrich Chemie GmbH (Germany). Acetonitrile and trifluoroacetic acid were purchased from Merck (Germany). A commercial product consisting of pentadecan-2-one (Lancaster Synthesis Ltd., UK) was used as standard. A sample of 2,6,10-trimethylundecan-2,9-dien-4-one was synthesized through the Grignard reaction (29). Authentic samples of aliphatic alkanes were obtained from Accu Standard (USA).

RESULTS

Bioecology of *P. sennaarensis*

Pachycondyla sennaarensis build complicated underground galleries of about 1 m depth with small openings (0.5 cm diameter) which include little circumferential compact soil. They establish their colony in humid microclimates, e.g. parks and greens, near irrigation channels, below concrete surfaces and in human and animal premises. Openings of a colony are circular (3-5 mm in diameter) and visible at the infested room corners. No colony was detected in dry sandy soils. None of the newly mated workers, which were placed in a Plexiglas cylinder, could establish a new colony. Fertilized wingless workers were observed on the periphery of colonies. These workers are 1 mm longer than the others and can be easily identified by observing the base of the loose wings on their thorax. The number of workers in a colony never exceeded 850. Our surveillance indicated that winged ants appear in early spring and autumn. Foraging is randomly carried out by workers with no tracing model to the food resource. Field observations showed that *P. sennaarensis* were omnivorous, feed on seeds of various plants, corpses of other ants, larvae of dipterans, a few species of Isoptera and some isopods such as *Porcellio* spp.

Ant Sting and Clinical Manifestations

A short while after being stung by a Samsum ant, small papules appeared (Figure 3A) whose diameters grew to about 0.5 cm in 3 to 5 minutes. In cases of multiple-stings, papules became conjoined to form a nodule. Fine nodules were usually replaced by erythema (Figure 3B) and distinct inflammation in less than 15 minutes, but no pustule was observed. No visible symptom remained after 5 hours; however local itching that had commenced in the first minutes lasted for 24 hours.

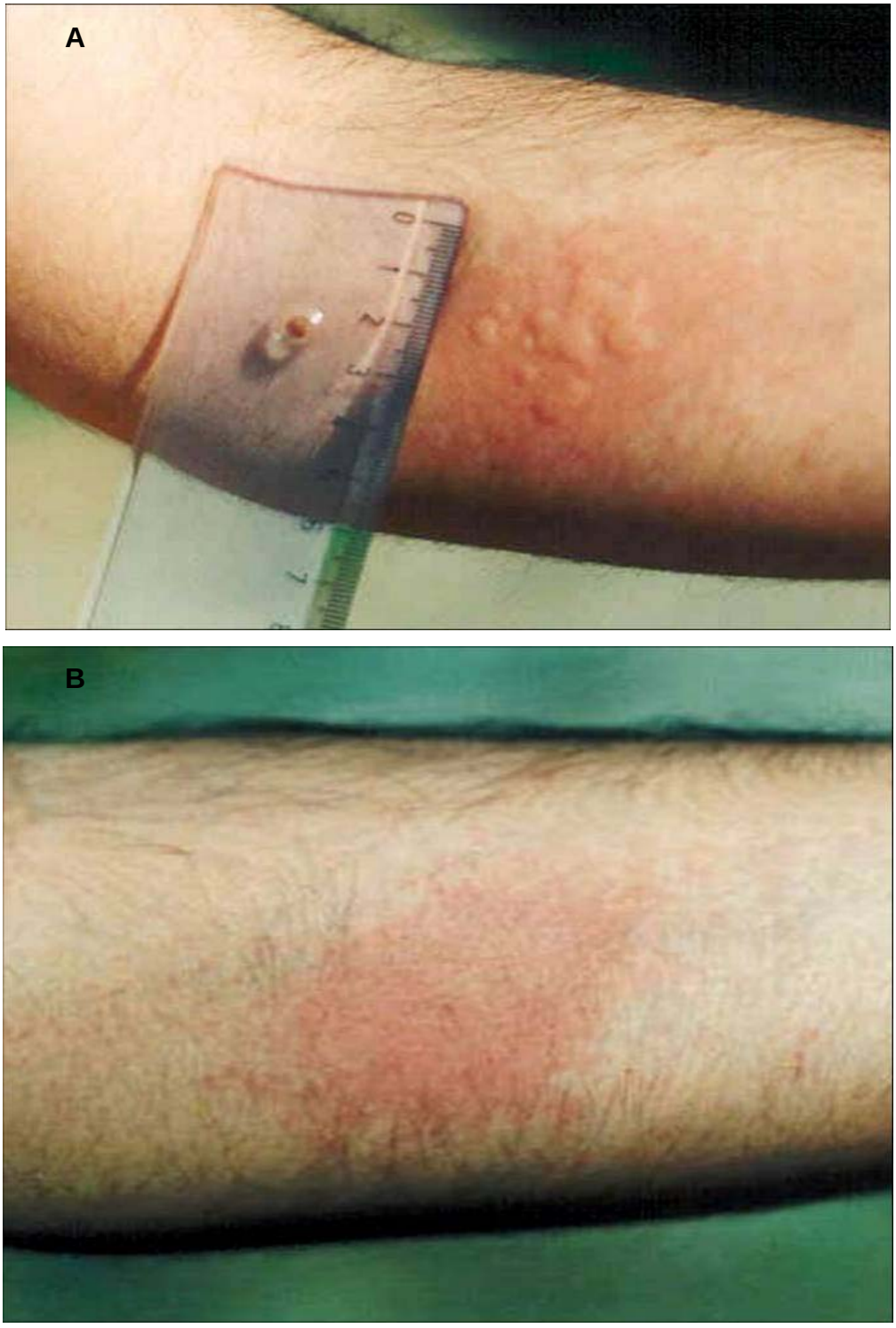

Figure 3. Clinical symptoms after *P. sennaarensis* sting: (**A**) papules are the first symptoms and are followed by (**B**) erythema in less than 15 minutes.

Remarkable Components of Abdominal Glands

Abdominal gland secretions of *P. sennaarensis* are mainly a complex mixture of linear and methyl-branched hydrocarbons. The alkane series starts as undecane and continues to heptacosane. Heptadecane, nonadecane, pentadecane and lower concentrations of hexadecene, hexadecane and undecane constitute the highest volume of the glandular components (Table 1).

Abdominal secretions also include small amounts of terpenoids, mostly identified as 2,6,10-trimethylundecan-2,9-dien-4-one ($C_{14}H_{22}O$), 2,5-piperazinedione and trimethyl pyrazine ($C_7H_{10}N_2$), which are all accompanied by straight-chain hydrocarbons. EI mass spectra of pyrazine (Figure 4A) revealed major fragments at *m/z* 42 and 81 with the molecular ion (M^+) at *m/z* 122. Mass spectra of $C_{14}H_{22}O$ (Figure 4B) indicated characteristic fragments at *m/z* 84 and 124 with M^+ at *m/z* 206. Base peaks of 2,5-piperazinedione appeared correspondingly at *m/z* 30, 114, 71 and 28 (Figure 4C).

Interestingly, a minute amount of another structure of $C_{14}H_{22}O$ was also eluted in some of our collected specimens with mass spectral (EI mode) fragments at *m/z* 191 and 57 with the molecular ion (M^+) at *m/z* 206 (Figure 4D), which are characteristics of phenol-2,4-bis (1,1 dimethylethyl). Confirmation was made via a comparison to the retention time and mass spectra of the injected authentic compound. The gland secretions also contained the methyl ketone, pentadecan-2-one, ($C_{15}H_{30}O$) with the mass spectra characteristic fragments at *m/z* 58, 59, 43 and M^+ at *m/z* 226 (Figure 4E). The spectrum and retention times of the authentic material that we obtained were compared and proven to be the same as that of the ant. There was also a compound with base peaks at *m/z* 55, 82, 96 and 69 and a molecular mass of 278 (Figure 4F). Identification of this compound could not be confirmed, but mass spectra were consistent with a formula $C_{20}H_{38}$. Although they appeared in trace volumes, 2-tridecyl acetate, dodecyl butyrate and 2- methyl hexadecanal (Table 1) were among the other interesting compounds that could be detected in the abdominal gland secretions of *P. sennaarensis*. Surprisingly, no peptide elution was monitored through a series of HPLC experiments.

Table 1. Volume of the abdominal gland reservoir in *Pachycondyla sennaarensis* workers and composition of its chemical components based on GC-MS analyses (n = 7)[*]

Component number[1]	Compound	Proportion (%, mean ± SD)
1	2,5-piperazinedione	0.9 ± 0.3
2	Trimethyl pyrazine	1.1 ± 0.2
3	Undecane	**4.6 ± 0.85**
4	Phenol-2,4-bis (1,1 dimethylethyl)[2]	0.1 ± 0.1
5	2,6,10-trimethylundecan-2,9-dien-4-one	0.3 ± 0.07
6	Pentadecane	**14 ± 2.3**
7	4,11-dimethyltetradecane	1 ± 0.6
8	12-methylpentadecane	3.2 ± 1.9
9	Hexadecene[3]	**8.3 ± 4.7**
10	Hexadecane	**9.1 ± 2.8**
11	2-tridecyl acetate	trace
12	Heptadecane	**26 ± 6.4**
13	Pentadecan-2-one	1.2 ± 0.01
14	Octadecene	3.6 ± 1.3
15	Octadecane	3.4 ± 0.7
16	Dodecyl butyrate	trace
17	2-methylhexadecanal	0.1 ± 0.1
18	Nonadecane	**19.6 ± 7.2**
19[4]	RT: 20.1 min, M^+ 278, *m/z* 55 (100[5]), 82 (74), 96 (60), 69 (57), 41 (55), 83 (54), 81 (52)[6]	trace
20	Heneicosene	trace
21	Heneicosane	2.7 ± 2.33
22	2,6,10,14 tetramethyl hexadecane	0.2 ± 0.1
23	Heptacosane	0.6 ± 0.3
Volume of abdominal gland reservoir (µL)		2.7 ± 0.6

[*]Average values higher than 5% are written in **bold**; when the value is underlined not all the individuals within the four studied colonies contained the compound in detectable amounts.
[1]Components are arranged according to their elution time.
[2]Could be only detected in the specimens collected in Abshekan village (27.19N, 60.46E).
[3]Present as two isomers in some specimens.
[4]This secretion consisted of a probable terpene that could not be identified, so its approximate retention time and the principal mass spectral ions are given.
[5]Recorded intensity.
[6]Other *m/z* represented a weak ion.

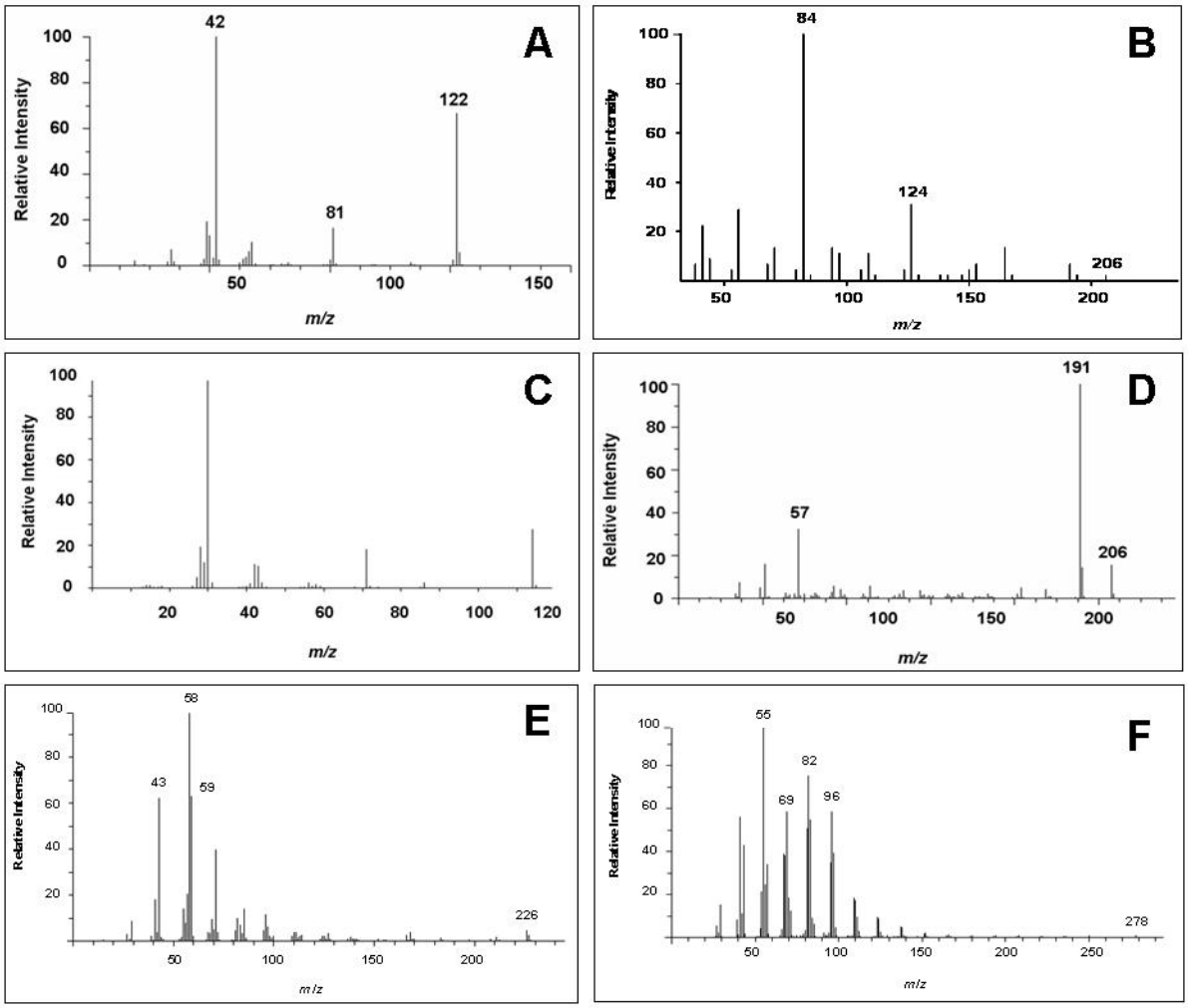

Figure 4. Mass spectra (70 eV) of (**A**) trimethyl pyrazine with base peaks at *m/z* 42 and 81; (**B**) 2,6,10-trimethylundecan-2,9-dien-4-one with fragments at *m/z* 84 and 124; (**C**) 2,5-piperazinedione with characteristic fragments at *m/z* 30, 114, 71 and 28; (**D**) phenol-2,4-bis (1,1 dimethylethyl) with mass spectral fragments at *m/z* 191 and 57; (**E**) pentadecan-2-one with the spectral fragments at *m/z* 58, 59 and 43, and (**F**) the unknown compound with the probable chemical formula $C_{20}H_{38}$. Electron ionization provides base peaks at *m/z* 55, 82, 96 and 69 and the M^+ at *m/z* 278.

DISCUSSION

Pachycondyla sennaarensis is a savannah and forest species in Africa (30), which requires high humidity, but in Iran it is more associated with humans in urban and rural localities. Because of the harsh weather conditions of southern Iran, it can survive only in well-irrigated agricultural ecosystems and human dwellings that provide protection from excessive water loss. *Pachycondyla* species are usually

considered specialist predators (31). McGlynn reported 147 species of ants that have been collected in non-native habitats in various parts of the world (32).

Although Iranian Samsum ants share some characteristics of invasive species (25), it is widely believed that they should be still considered a tramp species. Unlike invasive species, tramp species do not spread rapidly or dominate new environments but are tied to human activities. Such species prefer disturbed habitats in close association with humans. The incidence of stings by these ants is likely to increase with the accelerating rate of urbanization. Urban development and sprawl disrupt natural ecosystems, bringing humans in contact with those species that thrive in disturbed habitats. In addition, increased trade and travel provide invasion routes for exotic ants (11).

Anaphylaxis has been attributed to both of the medically important species, *P. sennaarensis* and *P. chinensis* (13, 18). Despite reports on the severe clinical symptoms, e.g. systemic allergic reactions, anaphylactic shocks, asthma and even death, in response to stings and bites from other ant species (11), the effects of the Iranian populations of *P. sennaarensis* are usually mild, i.e. at the worst case, forming papules and dermal itching due to multiple stinging. It should be noted that clinical manifestations due to ant stings are affected by the quantity of venom injected, the location of the stung area in terms of skin thickness, proximity to a vessel, its relation to extremities, head and neck or mucosa and finally the immune status of the patient. The efficacy of the sting as the defense weapon of ants is based on the toxic properties of the secretion produced by their venom gland. It is believed that the ability to produce pain is, in defensive terms, the most important aspect of these venoms (33). Fire ant venom contains piperidines and pyrazines, which cause a burning sensation in stung individuals, and a small percentage (< 1% volume of the venom secretion) of proteins, which can cause anaphylaxis in those stung (34). Peptides are also responsible for inducing pain and tissue damage (27). We believe that lack of the systemic reactions after stings of the Iranian populations of *P. sennaarensis* is due to the absence of protein components in their venom secretions.

The non-peptide venom constituents of *P. sennaarensis* were not known until this study. These components are not only species-specific, but different among geographical populations of a single species. Piperidines have been twice detected in *Pachycondyla* spp. In the first case 2,5-piperazinedione was separated from a multi-protein matrix in the venom of *Pachycondyla apicalis* and in the second, 2,6-

dimethyl piperidine was isolated from *Pachyconcondyla tarsata* (4, 35). Pyrazines, which are frequently found in formicid ants, are formed from amino acids. This group of chemicals has been repeatedly reported from the venom of ponerine and myrmecine species. For example, small amounts of 2,5-dimethylpyrazine and 2,5-dimethyl-3-ethylpyrazine were found in the venom gland of *Pachycondyla obscuricornis* (4).

Venom gland components of only 19 out of 200 described species of the genus *Pachycondyla* have been so far examined. Peptides and proteins were found in 15 species; however, in some cases no explicit reference was made to the involved compounds (27). Proteins were identified from the venom secretions of *P. villosa* (36), *P. insularis* and *P. tridentata* (37), *P. apicalis* (35) and *P. tarsata* (4). Orivel and Dejean (27) reported that *Pachycondyla* venom contained mostly histolytic and neurotoxic peptides. The occurrence of peptides was reported in the venom secretions of *P. sennaarensis* and *P. obscuricornis* (27), but it could not be verified by subsequent studies of other workers (our unpublished data from HPLC and in reference 4). Such differences may reflect natural variation among geographic populations of *P. sennaarensis*. This might also provide an explanation for the different severity of symptoms among stung persons on the two sides of the Persian Gulf. The venom gland components of *P. chinensis* are only partially described and appear to contain amines, formic acid, histamines, hyaluronidase, phospholipases and terpenes (13).

Another remarkable chemical detected in our study was 2,6,10-trimethylundecan-2,9-dien-4-one, which along with its other structurally similar sesquiterpenoids was once reported to originate from female cuckoo bees, *Nomada lathburiana* (29). It allegedly plays a repellent or defensive role, although no supporting test was carried out. The phenolic compound Phenol-2,4-bis (1,1 dimethylethyl) has been reported in the mycelia of *Tuber borchii* (Tuberales) while no record exists on its occurrence in insects (38). Extremely low amounts of this compound could be detected in the colonies of Abshekan village, but was totally absent among the Fajr Park specimens which had indicated a considerable titer of 2,6,10-trimethylundecan-2,9-dien-4-one.

Pentadecan-2-one has been repeatedly found in the abdominal glands of ants. Of 15 examined species of the genus *Myrmecocystus*, ten were found to bear pentadecan-2-one in the Dufour glands of workers (39). Similarly, variable volumes of pentadecan-2-one were reported in different species within *Cataglyphis bicolor* group

(40). 2-Alkanones are also among the typical products of a number of *Lasius* (41, 42) and *Acanthomyops* species (43). We also detected small amounts of an unknown compound with the probable chemical formula $C_{20}H_{38}$, although we were unable to precisely determine its structure. Such a formula was found in *P. obscuricornis* and *P. apicalis* and characterized as a ditepene (4). The occurrence of diterpenes in combination with hydrocarbons appears to be common in members of the tribe Ponerini.

A very low titer of 2-tridecyl acetate was once found in the formicine ant, *Cataglyphis viaticus*, while dodecyl butyrate was isolated from *Cataglyphis bombycinus* (40). No explanation has ever been provided on the probable function of these compounds in the studied ant species.

It is still not known why only the two species of *P. chinensis* and *P. sennaarensis* are of public health importance, while the substances required to induce symptoms are found in the venom glands of a much broader spectrum of species (4, 27). Two reasons for the paucity of sting reaction reports attributed to the other species can be hypothesized. First, most stings were not sufficiently severe for victims to seek medical attention. Second, the causative agent in cases of severe reactions may have been misidentified by victims and medical practitioners as another ant or arthropod species. Morgan et al. examined *Pachycondyla apicalis* and found a mixture of five proteins. They believe that compounds such as pyrazines and piperazinedione in the venom secretions of *P. apicalis* are made from amino acids (4). Pyrazines of the abdominal secretions of *P. sennaarensis* and *P. obscuricornis* could not be formed from amino acids because no peptide or protein was detected in their venom gland. Plant sources may explain the origin of such compounds (4).

After many years of studying stinging ants, many questions on the biology, ecology, behavior, defense and medical importance of these species have remained. Our working group is currently working on different geographic populations of *P. sennaarensis* in the hope of finding answers to some of these emerging questions.

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