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A Comparison of Pheromonal Compounds in the Mandibular Gland of Tropical Fire Ant, *Oecophylla smaragdina* and Thorn Black Ant, *Lusios niger*

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Research Article

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ABSTRACT

The present investigation was carried out to differentiate the pheromonal compounds of mandibular gland of two different ant species namely tropical fire ant, *Oecophylla smaragdina* and thorn black ant, *Lusios niger* from Sivkasi town. The results indicated that there is a distinct difference between the two species in their aromatic and hydrocarbons compounds through GC-MS analysis. The major compounds in the *O. smaragdina* was found to be mainly hydrocarbon (58%) and aromatic (48%) while comparing with *L. niger* it has hydrocarbons (71%) and aromatic (29%). The mandibular gland extracts were tested for the pheromonal compounds in the field and we obtained a significant observation of attraction of ants towards the head extracts.

INTRODUCTION

The ants encountering the trail receive the chemical message through chemoreceptors located in their antennae. Pheromones evaporate easily, thus ensuring an upward movement from the trail to the receiving ant's antennae. Chemical communication is important in the social organization of ants. The use of pheromones as transmitters of information allows for efficient functioning of colonies made up of thousands of individuals. These pheromones nest mates to food sources, alarm, defense, and other actions. These functions correspond to the huge variety of exocrine glands found in ants. Pheromones with different functions originate from different glands, although one gland may have entirely different functions in separate species. The chemical nature of glandular secretions is indeed often species-specific. This fertile complexity of glands and pheromones ensures smooth social organization in the ant societies [1].

Ant foraging and food gathering provides an excellent opportunity for the researchers to directly observe a form of animal communication. The focal point of this activity is the ability of foraging insects to “lay down” chemical trails. Ants, the most widely studied of the foraging insects, use pheromone trails to lead colony members to food sources and new nest locations [2]. The secretion of Dufour gland is generally made up of mixtures of long-chain alkanes and alkenes, some terpene hydrocarbons, and oxygenated alkyl compounds, such as alcohols, aldehydes, ketones and esters The gland’s secretion is known to mark substances for long-lasting trunk routes.

Pheromones are defined as the substances secreted by an animal to influence the behaviour of other animals of the same species [3]. They are generally synthesized within specialized exocrine glands and secreted directly. Ants secrete the trail pheromone from an exocrine gland located in the posterior abdomen and the trail is established as the pheromone flows down the sting [4].

Trail pheromone mixtures usually contain multiple pheromones that are derived from different glandular source. Trail pheromones of ants can be employed with great efficiency for multiple purposes. The odour trail pheromone of Solenopsis invicta Buren functions as an alarm pheromone, as well as an effective communicator of food location and an organizer of mass foraging [5].

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Solenopsis geminate are found commonly around urban areas and are attracted to electric fields, and can cause chewing damage to PVC coatings of electrical wiring They also build ugly mounds in lawns, steal seeds from seedbeds, bite holes in fabrics and feed on a range of household foods [6].

MATERIALS AND METHODS

Worker ants of Tropical fire ant, *Oecophylla smaragdina* and Thorn black ant, *Lusios niger* were collected at Viswanatham village, Sivakasi, Tamil Nadu, India. The ants were collected from the domestic places of houses and placed in Ziploc bags and then put in a freezer for 1 h to immobilize the ants.

Oecophylla smaragdina

Lusios niger

The head of 100 individual ants of each species were placed in glass vials with Teflon-lined stoppers containing 2.0 mL of dichloromethane. To prevent cross contamination, all equipments used in the collection of the ant heads was thoroughly cleaned before use on different species. Decapitation and storing ant heads in solvent is a standard method for extraction of the mandibular gland secret ions of ants when they cannot be analysed immediately [7]. Then they are given for GC-MS analysis to find the compounds mandibular pheromone compounds **(Tables 1 and 2)**.

Further the separation of protein compounds from the head extracts of the two different species of ants, they are taken along with the 15% SDS PAGE preparation and electrophorized.

RESULTS AND DISCUSSION

During the analysis of tropical fire ant *Oecophylla smaragdina*, different peaks were found to be present in the chromatogram, the important pheromonal compounds is heptadiene and the other compounds include trimethyl Benzoxazepine thione, dihydro dimethyl Di-n-decylsulfone, tert-Butyl methylphenoxy dimethylsilane, Tris tert-butyldimethyl silyloxyasane, Alpha-chlorocinnamic acid, 2-Propenoic acid, chlorophenyl, Octadecyne, Tetradecyne Acetamido dioxo dihydrobenzofurazan. From the result it was inferred that the nature of compound was found to be present in head extract of tropical ant (*Oecophylla smaragdina*) was mainly hydrocarbon (58%) and aromatic (48%).

In the thorn black ant (*Lusios niger*) the compounds found to be Trimethyl-1-sila-4-thiacyclopentane, Dimethyl-quinazolinone, Dihydroindole-ol-one, dibromo - dimethyl. From the result it was inferred that the nature of compound was found to be present in head extract of thorn black ant (*Lusios niger*) was mainly hydrocarbons (71%) and aromatic (29%).

Table 1. Chemical compounds identified from the GC-MS analysis of head extract from tropical fire ant (*Oecophylla smaragdina*).

Compound Name	Molecular Formula	Molecular Weight	Retention Time	Peak Area (%)	Nature of Compound
Alpha-chlorocinnamic acid	C_9H_7CLO	182.60	6.45	5.31	Aromatic
2-Propenoic acid, 2-chloro-3-phenyl-	$C_{12}H_{13}CLO_3$	240.68	6.45	5.31	Aromatic
1,4 Heptadiene 3,3,6 trimethyl	$C_{10}H_{18}$	138.05	13.84	7.65	Hydro carbons
Octadecyne	$C_{18}H_{34}$	250.46	13.84	7.65	Hydro carbons
4-Tetradecyne	$C_{14}H_{26}$	194.35	13.84	7.65	Hydrocarbons
Benz[b]-1,4-oxazepine-4(5H)-thione, 2,3-dihydro-2,8-dimethyl	$C_{11}H_{13}NOS$	207.20	17.26	5.99	Aromatic
5-Acetamido-4,7-dioxo-4,7-dihydrobenzofurazan	$C_8H_6N_4O_4$	222.15	17.26	5.99	Aromatic
Di-n-decylsulfone	$C_{20}H_{42}O_2S$	346.61	18.13	18.65	Hydro carbons
Hexahydropyridine, 1-methyl-4-[4,5-dihydroxyphenyl]	$C_{12}H_{17}NO_2$	207.26	18.13	18.65	Hydro carbons
tert-Butyl(5-isopropyl-2-methylphenoxy)dimethylsilane	$C_{16}H_{28}OSi$	264.47	18.96	31.59	Aromatic
Cyclotrisiloxane, hexamethyl	-	-	18.96	31.59	Aromatic
5-Acetamido-4,7-dioxo-4,7-dihydrobenzofurazan	$C_8H_5N_3O_4$	207.14	18.96	31.59	Aromatic
Cyclohexa-2,5-diene-1,4-dione, 2-methyl-5-(4-morpholinyl)	-	-	19.75	30.82	Hydrocarbons
Cyclotrisiloxane, hexamethyl-	$C_6H_{18}O_3Si_3$	222.46	19.75	30.82	Hydrocarbons

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Table 2. Chemical compounds identified from the GC-MS analysis of head extract from thorn black ant (*Lusios niger*).

Compound Name	Molecular Formula	Molecular Weight	Retention time	Peak Area (%)	Nature of Compound
Dimethyl sulfoxide	C_2H_6OS	78.13	2.34	71.05	Hydro carbons
1,1,3-Trimethyl-1-sila-4-thiacyclopentane	$C_6H_{14}SSi$	146.32	10.67	29.95	Aromatic
2,6-Dimethyl-4[3H]-quinazolinone	$C_{10}H_{10}N_2O$	174.19	10.67	29.95	Aromatic
2,3-Dihydroindole-4-ol-2-one, 5,7-dibromo-3,3-dimethyl-	$C_{10}H_9Br_2NO_2$	334.99	10.67	29.95	Aromatic

Separation of Chemical Compounds by SDS-PAGE

SDS profile of the chemical compounds of the colonies of ant species *Oecophylla smaragdina* and *Lusios niger* head extract revealed that the presence of many protein compounds in the sample with the molecular masses 28-32 kDa respectively as shown in the **Figure 1**.

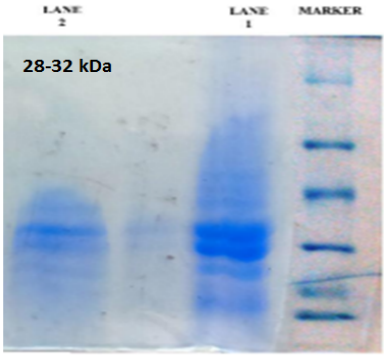

Figure 1. Protein profile of ant head extracts separated by 15% of SDS PAGE (Lane 1: 5 µl of *Oecophylla smaragdina*; Lane 2 5 µl of *Lusios niger* Lane 3: marker).

The SDS - PAGE protein profiling experiment were carried out by using the head extracts of worker colonies of ant species namely *Oecophylla smaragdina* and *Lusios niger*. The formation of bands in the gel shows the presence of different compounds made up of protein compounds with the different molecular weight.

The previous analysis of Wood and Chong [8] in the mandibular gland secretion of *Crematogaster mimosae* and *C. nigriceps* using a chromatographic column packed with a liquid phase covering a solid support showed 3-octanone and 3-octanol to make up 95% of the volatiles detected.

Pheromones are released mainly from exocrine glands as liquids that evaporate into the surrounding air and form a cloud of vapour about the signaling animal. The chemical nature and distance through which a pheromone may transmit a message is a function of the volatility of the compound, its stability in air, its rate of diffusion, olfactory efficiency of the receiver, and wind current. The *Lusios niger* Dufour gland or mandibular glands that distinguished it chemically from examples from the large tribe of Ponerini, but *E. ruidum* belongs to the increasing number of ponerines that produce diterpenes Methyl 6-methylsalicylate, which is present, is a known alarm pheromone in the South American species *G. pleurodon* [9], *Lusios niger* GC-MS analysis present in dimethyl sulphoxide. Where it is found in the mandibular glands, but it has also been identified as a trail pheromone in the venom gland of the myrmicine ants *Tetramorium impurum*.

CONCLUSIONS

Further studies have to be taken to separate, isolate and characterize a particular pheromonal compound for different species of ants using MALDI-TOF. That particular compound can be synthesized and can be used as an attractant or repellant in replace of chemical methods of ant control.

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