International Journal of Pure and Applied Zoology
Volume 3, Issue 4, pp: 328-333, 2015
http://www.ijpaz.com

ISSN (Print) : 2320-9577
ISSN (Online): 2320-9585
Rishan Publications

Research Article

STUDY ON VOLATILE COMPOUNDS OF FLANK SCENT GLAND AND BEHAVIOURAL ANALYSIS OF SOFT-FURRED FIELD RAT *MILLARDIA MELTADA* (GRAY, 1837)

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Article History: Received 2nd August 2015; Accepted 17th October 2015; Published 31st October 2015

ABSTRACT

The present observation was carried out to study the flank scent gland from sexually mature and reproductively active male and female soft-furred field rat *Millardia meltada*, They were analyzed by gas chromatography coupled mass spectrometry (GC-MS). The pheromone sources of flank scent gland differ between the sexes as well as the nature of volatile varies from source to source. Six peaks were found in higher concentration of male cheek gland. They were identified as Cyclopentanone (I), Hexadecanoicacid (II), Propanoicacid (III), 1-Octane (IV), Tetradecane (V) 3-Heptanone (VI. The cheek gland of female has five major fractions of compounds viz., Difluorohexane (I), Thiophene (II), Hydroxylamine (III), Tetradecane (IV) and 3,Heptanone (V). Odor preference test demonstrated that all the major chemical moieties identified in male flank gland were found to attract both sexes. By contrast the IV compound of male attracted conspecifics of the opposite sex, where compound I of female attracted conspecifics of the opposite sex. The level of attraction also varied from compound to compound. The results conclude that the flank gland of soft-furred field rat contains six major fractions with pheromonal function in maintaining social behavior and reproductive status.

Keywords: flank scent gland, Volatile pheromone compounds, Behavioural analysis, *Millardia meltada*.

INTRODUCTION

Rodents are considered one of the major pests of agricultural crops and stored food grains. Major crop losses caused by field rodents have been estimated in various places in India. Rodents may also adversely affect human health and cause great economic losses. Among rodents, rats are the dominant and highly infectious pests which infest human housing, sewers, animal shelters, day care facilities, warehouses, outdoor recreational areas etc. Furthermore, they serve as a reservoir of several important pathogenic vectors of diseases like plague, leptospirosis, ricketsial pox, rat bite fever and marine typhus fever (Jackson, 1987).

Our experiments further the study of scent marking by increasing the potential ecological relevance of the laboratory testing conditions to rats' selection environment. In particular, we were interested in providing rats with stimulus conditions that might control flank marking. Calhoun (1963) reported that male rats rolled and flank rubbed at the entrance to burrows in the presence of estrous females. Grant and Mackintosh (1963) and Adams (1976) have also noted flank marking of the wall in familiar home cages. To promote flank marking we used a familiar environment in which rats could move between a small, dark burrow and a large illuminated test chamber that contained a number of low objects. The availability of the burrow reduced the contribution of fear motivated locomotion to our results (Montgomery, 1954, 1955). To assess the effects of environmental variables on flank and urine marking by males and females, we manipulated the number and the odor condition of markable objects in the environment.

Olfactory communication is essential to find energy and primary metabolites, avoid toxic substances and withdraw hostile environments. As regards animals, it is also vital for inter-individual communication among their same species, favor reproduction and social life organization. Pheromones are chemical signals, released in the medium, which improve biological process efficiency of congeneric animals, indicating to conspecific

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information of the sex, social and hormonal status of releaser. Anybody secretions are potential routes for pheromonal communication and many mammals have well developed specialized scent glands that they use to deposit scent marks around their environment. All mammals emit chemical cues in their environment via urine, saliva or diverse secretion fluids (Briand *et al*., 2004, Brennan and Keverne, 2004). Specialized scent glands (Adams 1980; Balakrishnan and Alexander, 1985; Kannan and Archunan, 1997, 1998, 1999, 2000a, b, 2001) and faeces (Asha *et al*., 1985) are reported to be the chief sources of mammalian pheromones.

As many as 40 different scent glands are identified in mammals (Balakrishnan and Alexander, 1985), in which the pheromonal communication is well documented in some of the scent glands like preputial glands of mice (Marchlewska-Koj *et al*., 1990) and rat (Kannan and Archunan, 2000a), cheek (Kannan and Archunan, 1999) and clitoral glands (Kannan and Archunan, 2001) of rat, flank glands of musk screw (Balakrishnan and Alexander, 1977) and harderian gland of golden hamster (Bodyak and Surov, 1994). It is generally believed that armpit gland produces pheromonal signals, which are likely to involve in bio-communication. However, the biological significance of the armpit gland is very scanty. The armpit gland is located under the forearm, in between armpit and lateral side of the chest. This gland is embedded in the armpit musculature, having bulging and depression on the surface, and number of storage vesicle (Kannan and Archunan, 2000b). The gland secretes its substances through a number of minute hairs as projections, which are convincingly demonstrated in the laboratory rats (Kannan, 1998) and lesser bandicoot rats (Kannan and Archunan, 2000b). Chemical characterization study has been carried out in a number of species like mouse (Novotny *et al*., 1985), rat (Selvaraj and Archunan 2002a). Hence, the present investigation was carried out to find out the chemical nature of volatile compounds of flank glands and behavioral response soft-furred field rat, *Millardia meltada.*

MATERIAL AND METHODS

Sexually mature and reproductively active adult male and female soft-furred field rats were collected from nearby local area and housed individually in polypropylene cages (40 X 25 X 15) cm with 2.0 cm of husk as bedding material. The bedding material was changed twice a week. Rat was feed with Sai Durga Feeds and Foods (Bangalore) and water was provided *ad libitum*. Animals were maintained on a 14L:10D photoperiodic schedule in a climate controlled environment with a temperature of 25±3ºC. Lights were from 06.00 and 18.00 hours.

Twenty-five females (estrus stage) and 25 male rats were sacrificed by cervical dislocation under mild diethyl ether anesthesia. After autopsy, flank glands were dissected out under a dissection microscope and placed in double distilled solvent mixture (n-hexane and dichloromethane 1:1 ratio v/v) and ground well separately for about 10 minutes with glass homogeniser under ice-cold condition. The supernatant was filtered through silica gel (50–60 µ mesh size) and collected in a glass vial and sealed with air tight screw type cap made up of glass (Borosil). The sample vials were stored at –20ºC until they were used for analysis. Gas chromatography coupled mass spectrometry (GC-MS) analysis was made in a Shimadzu QP5000 (Japan) instrument under computer control at 70 eV. Ammonia was used as reagent gas at 95eV (Kannan, 1998) performed chemical ionization. The identified compounds were fractionated by the method of Pause et al. (1997). The extracted samples (20 ml) were distilled for 5–10 minutes at room temperature (30ºC) under a vacuum of 0.2 torr. The distillate was condensed by cooling with liquid nitrogen and concentrated to 2 ml. The volatiles form the distilled fractions were subjected to gas chromatography for cross checking and confirmation of compounds in each fraction (Pause *et al.,* 1997).

Assuming the importance of compounds in pheromone activity, the odor preference test was conducted in the Y-maze apparatus with the modified procedure of Ferkin and Seamon (1987). The Y-maze apparatus was made up of tin sheet, which consists of a central arm and two choice arms. The lateral sides were closed with glass plates where as top portion was covered with wire meshes. This apparatus had facility to provide food and water *ad libitum*. The size of central arm was about 80 cm length and 15 cm width. The remaining two choice arms were 75 cm length and 15 cm width each. The glandular extract slide was placed on the extreme right arm and the solvent slide (control) was placed on the extreme left arm during behavioral analysis. The position of the odor (left or right choice arms) was alternated. Three individuals (either male or female) were randomly taken from a pool of rats kept separately for each sex and used for each behavioral analysis. Test animals were released in the central arm and their behavior was assessed for 15 minutes with the identified compounds (experimental) and the solvent mixture was used as control. Each animal was tested thrice against individual compound, making a total of nine observations on each set. The time taken for visiting each compound was recorded.

RESULT

In the present study, Cyclopentanone (I), Hexadecanoicacid (II), Propanoicacid (III), 1-Octane (IV), Tetradecane (V) and 3-Heptanone (VI) compounds were found in male flank gland secretions (the results are summarized in Figure 1 and Table 1. It was very interesting to note that all the six volatile fractions of male flank gland significantly attract female rats. Similar chemical identification was performed in female flank gland (Figure 2 and Table 2), which showed that it had five major volatile compounds namely Difluorohexane (I), Thiophene (II), Hydroxylamine (III), Tetradecane (IV) and 3-Heptanone (V). Two identified compounds of male flank gland namely; Tetradecane and 3-Heptanone effectively attracted the individuals of both sexes belonging to the same species. Similar study was carried out in female, which revealed that the male and female responders spent greater time towards all the identified compounds except compound III of female flank.

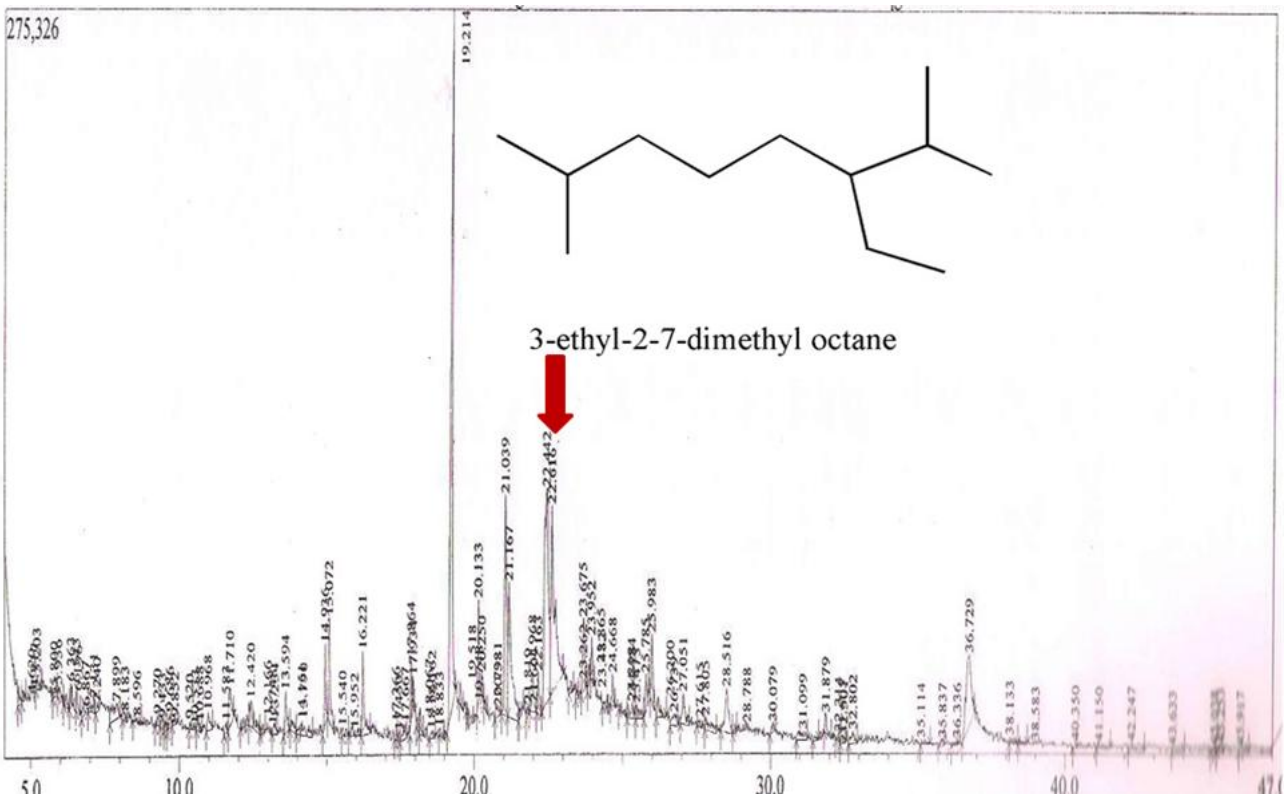

Figure 1. GC-Profile of volatile compounds in the flank gland of male *Millardia meltada.*

Table 1. Volatile compounds identified in the flank gland of male *Millardia meltada.*

S.No.	Retention time	Compound name	Molecular formula	Molecular weight
1	16.900	Cyclopentanone	C_5H_8O	84
2	19.144	Hexadecanoicacid	$C_{16}H_{32}O_2$	256
3	21.611	Propanoicacid	C_8H16O_2	144
4	22.550	1-Octane	C_8H_{16}	112
5	25.926	Tetradecane	$C_{14}H_{30}$	198
6	29.915	3-Heptanone	$C_8H_{16}O$	128

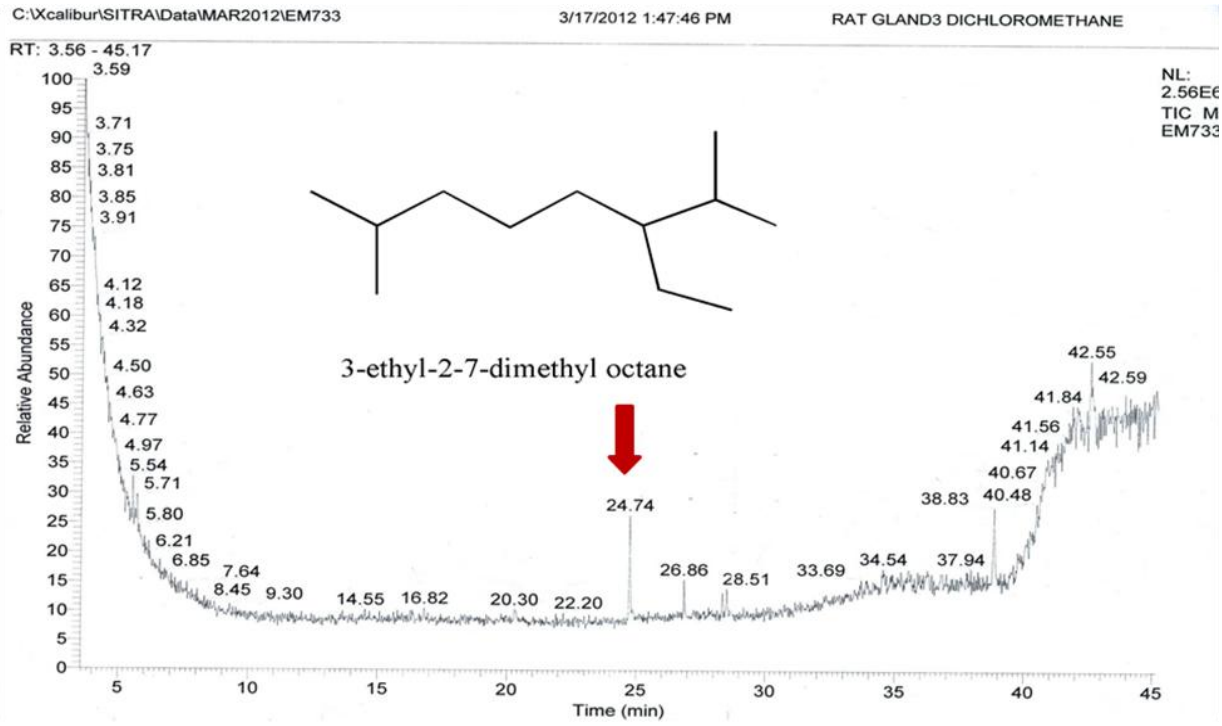

Figure 2. GC-Profile of volatile compounds in the flank gland of female *Millardia meltada.*

Table 2. Volatile compounds identified in the flank gland of female *Millardia meltada.*

S.No.	Retention time	Compound name	Molecular formula	Molecular weight
1	16.34	Difluorohexane	$C_6H_{12}F_2$	170
2	20.97	Thiophene	C_5H_8O	84
3	22.56	Hydroxylamine	$C_{10}H_{23}NO$	173
4	26.91	Tetradecane	$C_{14}H_{30}$	198
5	29.91	3,Heptanone	$C_8H_{16}O$	128

DISCUSSION

In the present investigation, six major volatile compounds are identified as major constituents in male and five major volatile compounds are identified female flank glands extract. Our experiments demonstrate that male and female rats flank marked an entryway more frequently than a glass window. Flank marking, a phenomenon previously reported only anecdotally (Adams, 1976; Calhoun, 1963; Grant and Mackintosh, 1963), is a relatively infrequent, but topographically distinct behavior whereby a rat presses its side against a vertical structure. The occurrence of flank marking suggests a behavioral function for the sebaceous glands that abound in the flank region of rats (Ebling, 1963). Despite the prediction of the sexual dimorphism hypothesis that females will mark less than males or not at all (Rails, 1971; Thiessen and Rice, 1976), the expected pattern of results has not emerged for most forms of scent marking by rats. For example, in our study a larger proportion of males than females flank marked; however, support for the hypothesis must be qualified because the rate of flank marking was comparable for actively marking males and females. With one exception (Brown, 1978), studies have obtained objectmarking results inconsistent with the hypothesis.

An informal observation that rats scratched the flank region with the hind paw, which may stimulate the sebaceous glands, and which appeared less vigorous than routine scratching, supports this conjecture. In female rats, the chemical identification was performed during estrus period only because it is found to be the period of releasing chemical signals and their perception. Similar observation was made in white and tailed deer's vaginal fluid and urine (Jemiolo *et al*., 1995) and in house mouse's urine (Andreolini *et al*., 1987).

Several reports are available regarding the significance of pheromone trap in insect pest management (Cork *et al*., 2003). The insect pheromone traps are commercially

available to reduce the insect pest menace in agricultural crops. However, control of rodent population is not effective to our expectations, as there are always problems. Moreover, the study on the usage of mammalian pheromone in rodent pest management is very limited. Nevertheless, the recent study in our laboratory showed that scent gland extracts are capable of improving poison bait acceptance and increase the rate of mortality in *Rattus norvegicus* (albino rat) (Selvaraj and Archunan, 2002b). Developing pheromonal trap would be the best method of rodent pest management program. Hence, identifying the rodent pheromones would definitely provide a strong foundation to introduce a novel and more achievable technique for rodent pest management programs.

CONCLUSION

From this investigation, it could be concluded that the possibility of specific olfactory signals can be produced by more than one gland. Further, it suggests that the odor produced by more than one gland act together to manifest the specific pheromonal function. The present findings clearly emphasize that in addition to other scent glands, the flank gland also faithfully involved in conveying social signals between the individuals.

ACKNOWLEDGEMENTS

The first author thanks to the UGC for the award of Rajiv Gandhi National Fellowship for this work. The authors are thankful to the Principal and Head of the Department of Zoology, Government Arts College (Autonomous), Kumbakonam for the facilities provided to carry out this work.

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