Iranian J Publ Health, 2006, Vol. 35, No. 3, pp.7-13

Repellency Effect of Myrtle Essential Oil and DEET against *Phlebotomus papatasi*, under Laboratory Conditions

** MR Yaghoobi-Ershadi [1], AA Akhavan [1], E Jahanifard [1], H Vatandoost [1], Gh Amin [2], L Moosavi [3], AR Zahraei Ramazani [4], H Abdoli [4], MH Arandian [4]*

[1]*Dept. of Medical Entomology and Vector Control, School of Public Health and Institute of Public Health Research, Tehran University of Medical Sciences, Iran*
[2] *Dept. of Pharmacognosy, School of Pharmacy, Tehran University of Medical Sciences, Iran*
[3] *School of Pharmacy, Islamic Azad University, Tehran, Iran*
[4] *Isfahan Health Research and Training Center, Institute of Public Health, Tehran University of Medical Sciences, Isfahan, Iran*

(Received 10 Jan 2006; revised 16 Apr 2006; accepted 7 Jun 2006)

Abstract

Zoonotic cutaneous leishmaniasis (ZCL) is an increasing and important public health problem in Iran. The use of repellents is recommended as one of the important means of personal protection against vectors of ZCL. This paper reports the repellency effect of the plant Myrtle, *Myrtus communis* (Myrtaceae), essential oil for protection against 3-7-day-old unfed females of the sandfly, *Phlebotomus papatas*i Scopoli for the first time in Iran. The tests were carried out under laboratory conditions, using dose-response testing procedure on white rabbits and the results were compared with commonly used repellent, diethyl-3-methylbenzamid (DEET). The modified Wirtz method using K & D apparatus was employed. Effective Dose (EDs) values were estimated from the probit regression line. ED50 was measured as 0.1140 and 0.0006 mg/cm^2 for Myrtle essential oil and DEET, respectively. The laboratory tests showed that both Myrtle essential oil and DEET had repellency effects against *P.papatasi*. In addition, the insecticidal action of Myrtle oil was also observed. We concluded that the two repellents could be used as a mean of personal protection against sand flies.

Key words: Phlebotomus papatasi*,* Myrtus communis*, DEET, Insect repellent, Vector control, Iran*

Introduction

Phlebotomus papatasi Scopoli is the main vector of Zoonotic Cutaneous Leishmaniasis (ZCL) in Iran. The disease is prevalent in 50% of many rural areas in 30 provinces of the country (1).

The control of the vector has received considerable attention in recent years due to the increasing and resurgence of the disease in some non-endemic areas of the country. The use of repellents has been considered as an important means of personal protection against blood feeding insects, as well as for controlling arthropod borne diseases by reducing man-vector contact (2). There are many reports on the repellency effect of natural and synthesized chemical agents on medically important arthropods especially mosquitoes (3-7), comparatively few reports are available with regard to phlebotomine sand fly repellents (8). A major reason for this discrepancy is the relative difficulty of sand fly rearing in sufficient numbers for laboratory repellent tests (9) as well as inadequate information about the components and mode of action of repellents. Although recently WHO proposed that at least five possible modes

***Corresponding author:** Tel: +98 21 8897390-3, Ext 2114, E-mail: yaghoobia@sina. tums. ac. ir

of action for repellents against mosquitoes exist .i.e., inhibit response to an otherwise attractive signal; switch the sensory message from attraction to repulsion; activate a receptor system that controls a competing behavior; activate a noxious odor receptor or activate different receptor types simultaneously causing loss of the specific signal for host finding (10).

The success of the sand fly rearing methods has solved this problem and several studies on the repellent testing, are being pursued (11, 12).

The majority of commercial repellent products contain the chemical DEET (diethyl-3-methylbenzamide, formerly known as diethyl-m-toluamide), which was first synthesized in 1954 (13). Although DEET was used for several years against blood-sucking arthropods and provide 96% protection rate for 6 h against vide variety of mosquito species in tropical environment (14), but due to some report of its allergic and toxic effects the use of this compound is under investigation (15, 16). DEET, in combination with certain other agents, is suspected of causing Gulf War Syndrome (17, 18), although this matter is still controversial. Because of this undesirable side effects of DEET, some researches were actively carried out to find an alternative compound that is safer to use and equally or more effective (19-22).

The medicinal plants have received close attention because of their acceptability, availability, and low cost (23, 24). *Myrtus communis* is a native evergreen shrub distributed in south, north, and central parts of Iran (25, 26). Myrtle is a medicinal plant that has been used for complaints of incipient phthisis, bronchitis, cystitis and pyelitis, traditionally for cerebral affections, dyspepsia and scorpion bite (27) and as a disinfectant (28). This plant was used as an insect repellent against stored-product pests (29) but there is no published data on its repellency effects on the sand flies.

The objectives of present study were to evaluate Myrtle, *Myrtus communis* (Myrtaceae), essential oil for repellency against *Phlebotomus papatasi* and its comparative efficacy with DEET, a common repellent used in personal protection against blood feeding insects, under laboratory condition.

Materials and Methods

Sand flies The colony of *P.papatasi* used in this study originated in Badrood rural district, an endemic focus of ZCL, Natanz county, central Iran. This colony has been maintained at the sand fly Insectary of School of Public Health, Tehran University of Medical Sciences, Iran. *P.papatasi* has been initiated using Killick-Kendrick and Killick- Kendrick (11) and was reared using Modi & Tesh methods (12). The sand flies were reared at 26±2 °C and 85± 5% relative humidity (RH) under photoperiod (L:D) 14:10. Female sand flies were tested 3-7 d after eclosion. The insects were hold in the test cages without water or food 9-10 h prior to experiments.

Chemicals DEET was purchased from Merck, Germany (8.17033.1000 DIETHYLTOLUAMIDE USP, Batch S36954, Assay 98.8%, Density: 0.998 gr/cm^3) which is widely used in commercially available preparation (30). The essential oil of Myrtle was extracted from the dried leaves, collected from its natural habitat in Manjil, north of Iran, using hydrodistillation method (31). Collected specimens were identified and deposited in the herbarium of Faculty of Pharmacy (TEH), Tehran University of Medical Sciences under Voucher Number 6648-TEH. To prepare corresponding concentrations, the compounds were diluted by absolute ethanol.

Test procedure The test system was similar to the white rabbit method as described by Wirtz et al. (32) and using modified K& D (33) apparatus. In summary, white rabbits, 6-9 m old, were used in all experiments. The animals were anesthetized with Ketamin hydrochloride (1ml/kg) and Acepromazine (1 ml/kg). The belly of the animal was shaved before testing. Three sets of 12 cm^2 test areas were outlined on the rabbit's belly using a cardboard template and marker pen. These areas were treated with 50 µl of the repellents in absolute ethanol. To prevent

any interference in each test only one dose of each repellent was applied. Absolute ethanol was also used as control. The treated areas were allowed to dry for 5 min and then the quadrangle holes in the bottom of K & D apparatus aligned with treated areas. Each test cage contained 4-7 flies, altogether 12-21 for each apparatus. For each dose only one rabbit was used. Probing counts were recorded at 1 min intervals for duration of 5 min and the results pooled for statistical analysis. The tests were then repeated at different doses at various interval occasions. To obtain an acceptable estimate of ED50 and ED90, the treated areas on the rabbit's belly were swabbed with Isopropanol pads. The tests were usually conducted several times to reduce the heterogeneity of populations of sand flies. Fly mortality was also recorded 24 h after recovery period.

Statistical analysis Data were subjected to the Finney probit 1971 (34) and from the regression line, ED50, ED90, confidence limits and slope values were measured.

Results

From 100 gram of the Myrtle-dried leaves, 1.9 ml essential oil was obtained. The density of Myrtle oil was calculated as 0.8897gr/$cm^{3.}$ Table1 shows the results of 118 tests conducted to estimate the median effective dose (EDs) of the two experimental repellents. The tests were carried out on 275 and 273 *P.papatasi* for DEET and Myrtle oil, correspondingly. ED50 and ED 90 were calculated as 0.1140 and 0.6711 mg/cm^2 for Myrtle essential oil, respectively. The figures for DEET were 0.0006 and 0.0111 mg/cm^2, in that order. The ranking of the repellents was based on the point estimates of the ED50. The observed data and calculated dose-response curves for these repellents are shown in Fig. 1 and 2. Significant difference was observed between ED50 of DEET and Myrtle essential oil (P<0.05). The insecticidal action of Myrtle oil was also observed during the study. Table 2 shows the mortality rate of the sand flies exposed to Myrtle oil after 24 h recovery period. The mortality after exposing to repellents was only observed when sand flies exposed to high doses of Myrtle oil. The highest mortality rate was 62.2% at dosages of 1 mg/cm^2.

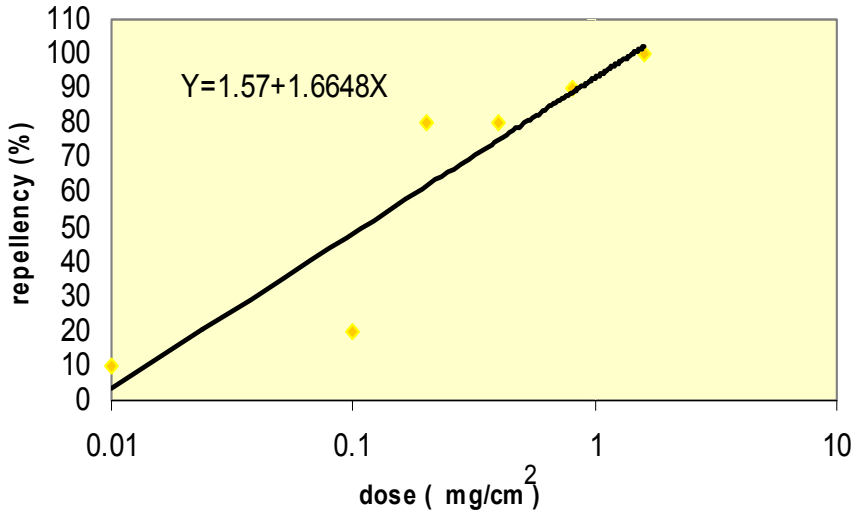

Fig. 1: Dose-response curve for Myrtle essential oil against *P.papatasi* tested with white rabbits. Dosages are plotted on the logarithmic scale, Sand fly Insectary, SPH, TUMS, 2005

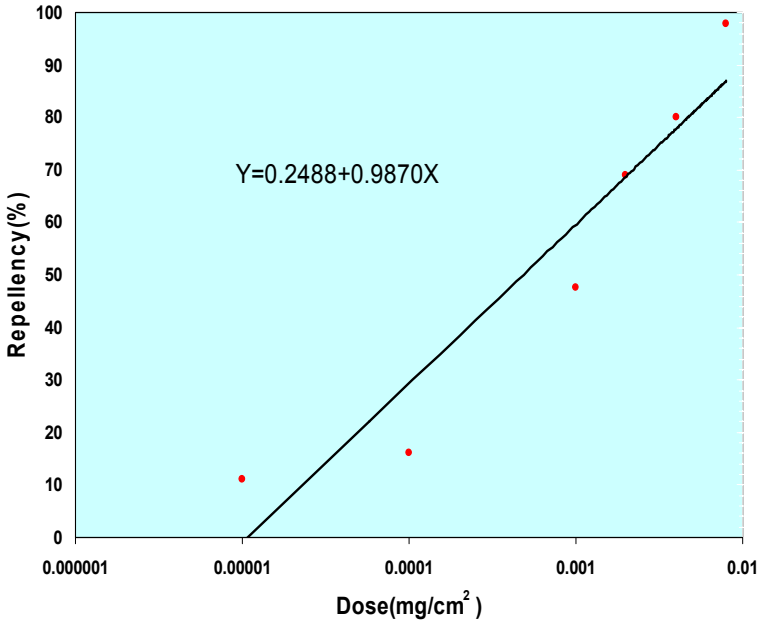

Fig. 2: Dose-response curve for DEET against *P.papatasi* tested with white rabbits. Dosages are plotted on the logarithmic scale, Sand fly Insectary, SPH, TUMS, 2005.

Table 1: Effectiveness of DEET and Myrtle essential oil against *Phlebotomus papatasi* tested on white rabbits, Sand fly Insectary, Tehran, 2005.

Repellents	No. flies	ED50 (mg/cm^2)	95% C.L* (mg/cm^2)	ED90 (mg/cm^2)	95% C.L* (mg/cm^2)
DEET	275	0.0006	0.0001-0.0017	0. 0111	0.0031-0.4338
Myrtle	273	0.1140	0.0472-0.1975	0.6711	0.3663-2.3019

*Mean dosages are significantly different (P<0.05) from each other if 95% confidence limits (C.L) do not overlap.ED50: effective dose cause 50% of prohibiting of bites

Table 2: Mortality rate (%) of *P.papatasi* exposed to Myrtle essential oil after 24 hours recovery period

Concentration	No. sand flies	Abbot correction mortality (%)
0.01 mg/cm^2	46	0
0.1mg/cm^2	41	0
0.2 mg/cm^2	45	4.4
0.4 mg/cm^2	30	4.4
0.8 mg/cm^2	45	50
1.6 mg/cm^2	45	62.2

Discussion

In this study, the repellency effect of the Myrtle essential oil against *P.papatasi* was studied under laboratory condition and compared with DEET for the first time in Iran and the world as well. DEET was found to be more effective as a repellent than Myrtle essential oil against the sand fly. It is postulated that essential oil of the

plant may contain different components and their mode of action should be studied furthermore. It should be mentioned that different methods have been used in the evaluation of the repellents. Due to the lack of a standard method, the results of different investigation are not completely comparable. However, regarding the available published data, the strain of *P.papatasi* used in this study was more sensitive to DEET than *Lu.longipalpis* (35) but by comparison with the other strains of *P.papatasi* is controversial. ED50 of DEET against other strains of *P.papatasi* has been reported 0.0022 mg/cm^2 and 0.21 ng/m^2. (36, 37). The less effectiveness of Myrtle essential oil in comparison with DEET is similar to some other botanic compounds (37). *P.papatasi* seems to be more sensitive to repellents than certain tsetse flies, mosquitoes, and reduviid bugs (38- 40).

In this study, the insecticidal property of Myrtle essential oil was also observed. This action was demonstrated when Pulse beetle, *Callosobruchus chinensis*, was exposed to Myrtle extract (29). This property against *Culex pipiens* was also observed (41). The proper use of repellents would appear to be an especially effective method to prevent biting and sand fly borne diseases.

It was concluded that the two repellents could be used as one of the means of personal protection against *P.papatasi*. For recommendation of both repellents in the field, a comprehensive study on the durability and repellency effect is undergoing in laboratory and field conditions.

Acknowledgments

The authors wish to thank the staff of the herbarium of School of Pharmacy particularly Mr. Badarkhani, for his kind assistance in plant collection. The sincere thanks are also extended to leishmaniasis staff of Isfahan Health Research and Training Center for their assistance in sand fly collection and colony initiation. Thanks are also due to Ms Saidi for maintaining and breeding of *P.papatasi* at the Sand fly Insectary of School of Public Health, Tehran University of Medical Sciences, Iran. This project received financial support from vice chancellorship for research affairs of TUMS (Project No 132.10466-83.12.26).

References

1. Yaghoobi-Ershadi MR, Zahraei-Ramazani AR, Akhavan AA, Jalali-Zand AR, Abdoli H, Nadim A (2005). Rodent control operations against Zoonotic Cutaneous Leishmaniasis in rural Iran. *Ann Saudi Med*, 25(4): 309-12.
2. Curtis CF (1992). Personal protection methods against vectors of disease. *Med Vet Entomol*, 80: 543-53.
3. Rutlege LC, Sofield RK, Moussa MA (1978). A bibliography of diethyl toulamide. *Bull Entomol Soc Am,* 24: 431-39.
4. McGovern TP, Schreck CE, Jackson J (1980). Mosquito repellents: cyclohexanoic carboxamides as a repellent for *Aedes aegypti*, *Anopheles quadrimaculatus* and *Anopheles albimanus. Mosq News*, 40: 394-98.
5. Wirtz RA, Turrentine JD, Rutledge JR, Rutledge LC (1980). Mosquito area repellents: laboratory testing of candidate materials against *Aedes aegypti*. *Mosq News*, 40: 432-39.
6. Kalyanasundaram M (1982). A preliminary report on the synthesis and testing of mosquito repellent. *Ind J Med Res*, 76: 190-95.
7. Curtis CF, Lines JD, Baolin L, Renz A (1989). Natural and synthetic repellents. In: Appropriate Technology in Vector Control. Ed, by CF Curtis. CRC Press, Boca Raton, Florida, pp.75-92.
8. Schreck CE, Kline DL, Chaniotis BN, Wilkinson N, McGovern TP, Weidhass DE (1982). Evaluation of personal protection methods against Phlebotomine sand flies including vectors of leishmaniasis in Panama. *Am J Trop Med Hyg*, 31: 1046-53.

9. Sherlock IA, Sherlock VA (1972). Practical methods for the creation of phlebotomine in the laboratory. *Rev Brasil Biol*, 32: 209-17.
10. World Health Organization (2000).Global collaboration for development of pesticides for public health (GCDPP), repellents and toxicants for personal protection.WHO/CDS/WHOPES/GCDPP/2000.5.
11. Killick-Kendrick M, Killick-Kendrick R (1991). The Initial establishments of sand fly colonies. *Parasitologia,* 33(suppl.1): 315-20.
12. Modi GB, Tesh RB (1983). A simple technique for mass rearing *Lutzomyia longipalpis* and *Phlebotomus papatasi* in the laboratory. *J Med Entomol*, 20(5): 568-69.
13. McCabe ET, Barthel WF, Gertler SI, Halls SA (1954). Insect repellents, III.N, N-diethylamides. *J Org Chem*, 19: 493-98.
14. Debboun M, Stricknman DA, Klun JA (2005). Repellents and the military: our first line of defense. *J Am Mosq Control Assoc*, 21 (4): 4-6.
15. Anonymous (1988). Are insect repellents safe? *Lancet*, 2: 610-11.
16. Gupta RK, Rutledge LC (1994). Role of repellents in vector control and disease prevention. *Am J Trop Med Hyg*, 50(suppl.): 82-6.
17. Abou-Donia MB, Wilmarth KR, Jensen KF, Oehme FW, Kut TL (1996). Neurotoxicity resulting from coexposure to pyridostimine bromide, DEET, and permethrine-implications of Gulf War chemical exposures. *J Toxicol Environ Hlth*, 48: 35-6.
18. Haley RW,Kurt TL (1997). Self-reported exposures to neurotoxic chemical combinations in the Gulf War. *J Am Mosq Control Assoc*, 227: 231-37.
19. Robert LL, Hallam A, Seeley DC, Roberts LW, Wirtz RA (1991). Comparative sensitivity of four *Anopheles* (Diptera: Culicidae) to five repellents. *J Med Entomol*, 14: 417-20.
20. Schreck CE, Leonhardt BA (1991). Efficacy assessment of quwenling, a mosquito repellent from China. *J Am Mosq Control Assoc*, 7:433-36.
21. Dua VK, Gupta NC, Pandey AC, Sharma VP (1996). Repellency of *Lantana camara* (Verbenaceae) flowers against *Aedes* mosquitoes. *J Am Mosq Control Assoc*, 12: 406-408.
22. Walker TW, Robert LL, Copeland RA, Githeko AK, Wirtz RA, Githure JI, Klein TA (1996). Field evaluation of arthropod repellents, DEET and a piperidine compound, AI3-37220, against *Anopheles funestus* and *Anopheles arabiensis* in Western Kenya. *J Am Mosq Control Assoc*, 12:172-76.
23. Barnard DR (2000). Repellents and toxicants for personal protection. Available at:*WHO/CDS/WHOPES/GCDPP/2000*. 5,49pp.
24. Bodeker G (2004). Introduction. In: *Traditional Medicinal plants and Malaria*. Ed, Hardman R. CRC Press publishing, United Kingdom.
25. Zargar A (1996). *Medicinal plants*. 6th ed. Tehran University Press. Tehran.
26. Rechinger KH (1966). *Myrtaceae in Flora Iranica* (No. 34:1). Ed, KH Rechinger, Akademische Druck-u. Verlagsanstalt, Graz.
27. Prajaparti ND, Kumar U (2005). *Agro's Dictionary of Medicinal plants*, Agrobios, India.
28. Amin Gh (2005). Popular medicinal plants of Iran. Vice Chancellorships for Research affairs, Tehran University of Medical Sciences, Tehran.
29. Al Lawati HT, Azam KM, Deadman ML (2002). Insecticidal and repellent properties of subtropical plant extracts against Pulse Beetle. *Callosobruchus Chinensis*. *Agr Sci,* 7(1):37-45.

30. United States Environmental Protection Agency (1980). *Registrants with pesticide products containing the active ingredient N, N-diethyl-m-toluamide and other isomers*. Washington .D.C.
31. Tuberoso CIG, Barra A, Angioni A, Sarritzu E, Pirisi FM (2006). Chemical composition of volatiles in Sardinian Myrtle (*Myrtus communis* L.) alcoholic extracts and essential oils. *J Agric Food Chem*, 54: 1420-26.
32. Wirtz RA, Rowton ED, Hallam JA, Perkins PV, Rutledge LC (1986). Laboratory testing of repellents against the sand fly *Phlebotomus papatasi* (Diptera: Psychodidae). *J Med Entomol*, 23(1): 64-7.
33. Klun JA, Debboun M (2000). A new module for quantitative evaluation of repellent efficacy using human subjects. *J Med Entomol*, 37(1): 177-81.
34. Finney DJ (1971). *Probit analysis*, 3rd ed. Cambridge University Press.
35. Buescher MD, Rutledge LC, Wirtz RA, Glackin KB, Moussa MA (1982). Laboratory tests of repellents against *Lutzomyia longipalpis* (Diptera: Psychodidae). *J Med Entomol*, 19 (2):176-80.
36. Kalyansundram M, Srinivasan R, Subramanian S, Panicker KN (1994). Relative potency of DEPA as a repellent against the sandfly *Phlebotomus papatasi*. *Med Vet Entomol*, 8: 68-70.
37. Pitasawat B, Choochote W, Tuetun B, Tippawangkosol P, Kanjanapothi D, Jitpakdi A, Riyong D (2003). Repellency of aromatic *Curcuma aromatica* under laboratory and field conditions. *J Vect Ecolo*, 28(2):234-40.
38. Wirtz RA, Roberts LW, Hallam JA, Macken LM, Roberts DR, Buescher MD, Rutledge LC (1985). Laboratory testing of repellents against the tsetse *Glossina morsitans* (Diptera: Glossinidae). *J Med Entomol*, 22 (3):271-75.
39. Buescher MD,Rutledge LC, Wirtz RA, Nelson JH (1985). Laboratory repellent tests against *Rhodnius prolixus* (Heteroptera: Reduviidae). *J Med Entomol*, 22: 49-53.
40. Oshaghi MA, Ghalandari R, Vatandoost H, Shayeghi M, Kamali-nejad M, Tourabi-Khaledi H, Abolhassani M, Hashemzadeh M (2003). Repellent effect of extracts and essential oils of *Citrus limon* (Rutaceae) and *Melissa officinalis* (Labiata) against man malaria vector, *Anopheles stephensi* (Diptera: Culicidae). *Iranian J Pub Hlth*, 32: 47-52.
41. Trabousli AF, Taouli K, El-Haj S, Bessiere JM, Rammal S (2002). Insecticidal properties of essential plant oils against the mosquito *Culex pipiens* molestus (Diptera: Culicidae). *Pest Manag Sci*, 58: 491-95.