Asian Journal of Chemistry; Vol. 24, No. 7 (2012), 3087-3090

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in

Evaluation of Toxicity and Repellency of Essential Oils of Family Rutaceae Against Black Ants (*Lasius niger*) in Pakistan

FERHAT MEHMOOD[1], FARKHANDA MANZOOR[2,*], ZAHEER UD DIN KHAN[1], MAHWISH IMRAN ALI[1], ISLAMULLAH KHAN[3] and SYED MUHAMMAD AKMAL RAHIM[4]

[1]Depertment of Botany, Government College University, Lahore, Pakistan
[2]Depertment of Zoology, Lahore College for Women University, Lahore, Pakistan
[3]Depertment of Chemistry, Government College University, Lahore, Pakistan
[4]Forest Department, Punjab, Pakistan

*Corresponding author: Tel: +92 42 3334204869; E-mail: ferhatmehmood786@yahoo.com

(*Received*: 9 July 2011; *Accepted*: 16 February 2012) AJC-11071

A study was conducted to evaluate the toxicity and repellency of essential oils from leaves of *Skimmia laureola* (DC.), *Murraya paniculata* (Linn) cv. Desi, *Murraya paniculata* (Linn) cv. China and *Murraya koenigii* (Linn.) of family Rutaceae in Pakistan. The oils were tested at three concentrations *i.e.* 1, 5 and 10 %. Common ant species *Lasius niger* (black ant) of Pakistan were selected and subjected to force-feeding at room temperature. Results showed that insecticidal activity of each tested essential oil was significantly different from each other. *Murraya paniculata* (both varieties) was the most effective tested oil (LC_{50} = 6.58 μL) while *Skimmia laureolas* showed least repellent activity (LC_{50} = 10.15 μL).

Key Words: *Lasius niger*, *Murraya paniculata* cv. Desi, *Murraya paniculata* cv. China, *Murraya koenigii*.

INTRODUCTION

Common ant species *Lasius niger* (black ant) of family formicidae were selected for the present study. This ant is a problem for some gardeners because it eats ripe fruits thus causing economic loss. In homes their explorations has lead to a burrowing through mortar and brick[1]. The indiscriminate use of synthetic insecticides has caused environmental contamination to living organisms[2], indicating the new development of products that are not hazardous to the environment. During recent years considerable attention has been paid towards exploration of plant materials in the protection of food commodities from insect infestations. *Tithonia diversifolia* have been reported to possess strong insecticidal activity against different pests[3,4]. Plant derived products namely azadrachtin from *Azadrachta indica*, pyrethrin from *Chrysantthemum cinerariaefolium*, carvone from *Carum carvi* and alkyl isothiocyanate from mustard and horseradish oil have received global attention due to their pesticidal properties and potential to protect several food commodities[5]. Essential oils produced by different plant genera have been reported to be biologically active and are endowed with insecticidal, antimicrobial and bio regulatory properties[6-8]. Pest control by directly or indirectly using natural plant products, including essential oils, is a promising approach[9,10] and as antimicrobial agents[11] and to repel insects or protect stored products[12]. Moreover, essential oils easily biodegrade in the environment[13] and possess little or no toxicity against fishes, birds and mammals[14].

Rutaceae, commonly known as Rue or Citrus family is represented in Pakistan by 11 genera and 27 species, many of which have been naturalized here, being cultivated and hybridized for edible, medicinal and ornamental purposes[15]. Most species are frequently aromatic with glands on the leaves sometimes with thorns. The presence of essential oils in members of family Rutaceae with diverse activities claimed for them and the increasing demand for natural sources of insecticides encouraged us to undertake a comprehensive study of the insecticidal and repellent activities of the essential oils for first time in Pakistan[16].

EXPERIMENTAL

The leaves of *Murraya koenigii* (Curry patta), *Murraya paniculata* (Marwa China), *Murraya paniculata* (Marwa desi) and *Skimmia laureola* (Nair) were collected from natural habitat of Abbotabad, identified at Botany Department Government College University, Lahore.

Extraction of essential oils and their analysis: The leaves of plants were separated and subjected to hydro-distillation for about 4 h. The essential oils obtained thus were dried over anhydrous sodium sulphate and stored in dark coloured glass bottle at *ca.* 4 ºC. Chemical composition was determined through GC-MS. GC-MS analyses were performed on a

TABLE-1
PROFILE OF COMPOUNDS IDENTIFIED THROUGH GC/MS ANALYSIS

Name	Area (%)				Mode of identification
	SL*	MK**	MP (d)***	MP (c)****	
Psi-cumene	0.48	5.50	1.57	2.06	a, b
(+)-3-Carene	0.94	-	-	2.95	a, b
D-Limonene	3.06	6.54	0.28	0.48	a, b
Ocimene	1.43		-	-	a, b
β-Linalool	32.32	26.96	-	-	a, b
Columbin	-	6.67	-	1.03	a, b
(-1)-4-Carene	-	-	2.06	13.94	a, b
α–Bisabolene	-	-	1.66	1.38	a, b
Caryophyllene	-	-	12.11	17.99	a, b
Ocimene	-	-	2.04		a, b
α-Cubebene	-	-	27.10	1.38	a, b
γ–Elemene	-	-	7.14	2.19	a, b
Azulene	-	-	2.19	3.16	a, b
Rimantadine	0.24	-	-	-	a, b
Cyclohexene,5,6,di ethenyl,1-methyl	1.03	-	-	-	a, b
1,3-Dimethylcyclopentene	0.58	-	-	-	a, b
1-Methyl-5,6, di methyl cyclohexene	3.89	-	-	-	a, b
α- Terpineol	16.68	-	-	-	a, b
Linalool acetate	23.53	-	-	-	a, b
β-Phillendrene	3.40	-	-	-	a, b
Nerolacetate	9.97	-	-	-	a, b
Mesitylene	1.76	-	-	-	a, b
Germacerene	-	-	-	28.44	a, b
[-]-Zingeberene	-	-	-	13.34	a, b
δ-Selinene	-	-	-	6.25	a, b
3-Methyl-2-butenoicacid,heptadecylester	-	-	-	3.336	a, b
β-Pinene	-	0.48	-	-	a, b
β-Cymene	-	43.07	-	-	a, b
Allene	-	1.87	-	-	a, b
7-Octene-2,one	-	5.18	-	-	a, b
Di-phenylephrine	-	2.19	-	-	a, b
Borane carbonyl	-	4.05	-	-	a, b
Trans-α-Bergamotene	-	-	2.02	-	a, b
Nerolidyl acetate	-	-	1.73	-	a, b
Iso-ledene	-	-	1.51	-	a, b
Nerolidol	-	-	34.07	-	a, b
2-Nonynoic acid	-	-	1.07	-	a, b
Undecanol	-	-	2.23	-	a, b

*SL =*Skimmia laureola;* **MK=*Murraya koenigii;* ***MP(d)=*Murraya paniculata*(desi), ****MP(c)=*Murraya paniculata(china*); a=Retention time; b=MS(GC/MS)

Shimadzu GCMS-QP2010A system given above in EI mode (70 eV) equipped with injector at 250 ºC, using DB-5MS column. Samples were injected at 250 ºC with a split ratio of 50/50. Injection volume was 1 µL and electronic pressure programming was used to maintain a constant flow (0.67 mL/min) of the helium carrier gas. The oven temperature was programmed from 100 ºC (4 min) to 250 ºC at a rate of 2 ºC/min and held at this temperature for 2 min. The mass spectrometer was set to scan the mass range 40 amu to 600 amu with ion source temperature 200 ºC and interface temperature 250 ºC. Analyses were performed in triplicate with a blank run after every analysis. The resulting data was processed using Shimadzu Lab Solution GCMS postrun analysis software. The relative apparent percentage of each compound and of their classes was determined by area normalization method. Comparing the mass fragmentation pattern of the reported data and NIST 147 and NIST 27 libraries identified compounds.

Collection of ants: The ants were collected from Lawns of Lahore College for Women University by putting corrugated cardboard (with syrup) in ground traps, then traps were brought back to Entomological Research Laboratoty, ants were separated and identified with the help of key.

Experimental setup: Laboratory bioassays were conducted to test the repellent effect of above mentioned plants. The method was as adopted by Grace *et al.*[17] with some modifications. For this purpose, three plastic cylinders were taken and holes were inscribed in them through an iron rod. These plastic containers were connected by means of 10 cm long typhon tube. Three different concentrations (1, 5 and 10 %) of each oil were prepared in ethanol as solvent. About 3 cm round filter paper (Whatmann no#1) disks were taken. For each experimental unit three filter paper discs were taken and soaked with water, required oil and (solvent) ethanol. All three filter paper discs were air dried at room temperature prior to force-feeding by ants. In experimental setup, central container served as experimental unit (with oil treated filter paper disc) while container on the sides served as control units *i.e.*, water control and solvent control. Sides containers provided an opportunity to the insects to migrate freely or not, depending upon their response to the repellent effect of oil in the treatment bottle. 15 Ants were placed in the central container that already contained oil treated filter paper disc. All bottles were closed with lids and covered the whole experimental setup with black cloth for the maximum activity of ants. Each essential oil concentration was tested for repellency and toxicity. Observations were made after for every 0.5 h for 5 h.

At the end of experiment, number of dead insects was recorded to estimate the toxicity of the oil. Criteria considered for death was loss of any motility in the insect, under dissecting microscope. The dead insects were kept in the separate bottle with distilled water treated filter paper for 24 h to ensure death. For control units blank control and ethanol control units were used. Three replicates of each concentrations were tested. At the end of experiment average number of ants were calculated for each oil and each concentration.

Statistical analysis: The percentage mortality rates were corrected by using Abbott's formula and data were analyzed using a one way ANOVA Test using Excel and SPSS 13.0 (statistical soft ware) on the data.

RESULTS AND DISCUSSION

Yield and physical characteristics of essential oils: The colour of *M. koenigii* essential oil was without any tinge, while that of *M. paniculata* (China), *M. paniculata* (Linn) (Desi) and *S. laureola* was clear yellowish. As far as % yield is concerned, *S. laureola* gave highest yield and *M. paniculata* (China) lowest yield. Results of GC/MS analysis are given in Table-1.

Toxicity and repellent effect: Essential oil of each plant showed toxicity as well as repellent effect against *Lasius niger* (black ant). It was also shown that repellent effect of same concentration of each essential oil is slightly different from each other but significantly different from repellent effect of other concentrations as well as controls respectively. The essential oils with most potent insecticidal activity based on LC_{50} was of *M. paniculata cv.* Desi and *M. koenigii* (6.58 µL) followed by *M. paniculata cv.* China (8.41 µL) and *Skimmia laureola* 10.15 µL. A dose dependant effect was observed with R^2 = 0.997, 0.997, 0.996 and 0.984 of essential oils from *M. koenigii, M. paniculata cv.* Desi and *M. paniculata cv.* China and *S. laureola* respectively. A good percentage of dead and sluggish insects had been observed Table-2 (Figs. 1-3).

All essential oils showed appreciable toxicity and repellent effect against Garden ant, *Lacius niger*, adults. This activity is in agreement with the previous findings[18]. *M. koenigii* and *M. paniculata cv.* Desi essential oils were found to have higher effects against insects followed by *M. paniculata cv.* China and *S. laureola*. Major phytochemicals in the essential oils were monoterpenes and sequiterpenes. *M. koenigii* essential oil was rich in monoterpenes while sequiterpenes dominated in essential oils of *M. paniculata cv.* China and *M. paniculata cv.* Desi. Monoterpenes have acarcidal activity[19]. Nerolidol, the main component of *M. paniculata cv.* Desi is skin penetration

TABLE-2
PROFILE SHOWING PERCENTAGE OF DEAD AND SLUGGISH INSECTS AFTER 5 H OF EXPOSURE TO DIFFERENT ESSENTIAL OILS ON ADULT *Lasius niger* (BLACK ANT) IN COMPARISON WITH ETHANOL AND WATER AS CONTROL

	Oil (%)	Dead (%)	Sluggish (%)
M. paniculata (Desi) leaves	1	13.33	20.00
	5	26.66	46.6
	10	46.6	53.3
M. paniculata (China) leaves	1	0.00	0.00
	5	20.00	26.66
	10	40.00	46.6
M. koenigii leaves	1	13.33	13.33
	5	26.66	26.66
	10	46.6	53.3
S. laureola leaves	1	13.33	26.66
	5	20.00	40.00
	10	33.33	44.00
Water (control)		0.00	0.00
Solvent (control)		0.00	0.00

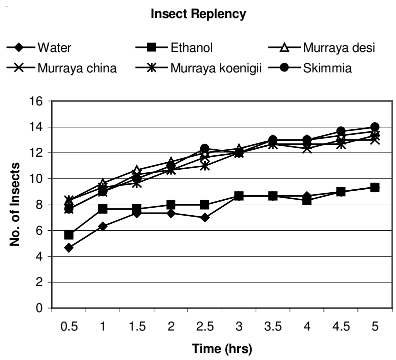

Fig. 1. Time dependant repellent effect of 1 % different essential oils on adult *Lasius niger* (black ant) at various times (h)

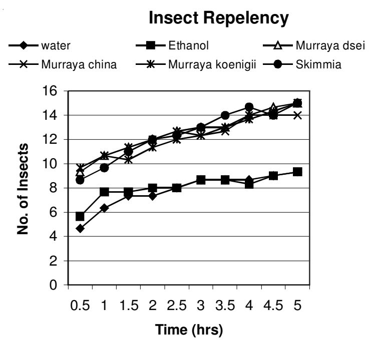

Fig. 2. Time dependant repellent effect of 5 % different essential oils on adult *Lasius niger* (black ant) at various times (h)

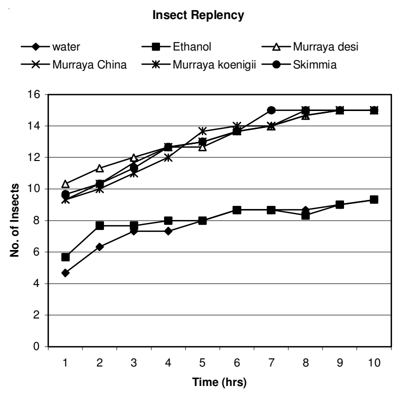

Fig. 3. Time dependant repellent effect of 10 % of different essential oils on adult *Lasius niger* (Black ant) at various times (h)

enhancer[20]. *M. paniculata cv.* China essential oil possess Germacerene, which has antimicrobial and insecticidal properties[21], while caryophllyne has psycomodulatory effect[22]. β-Linalool from *M. koenigi* is monoterpene alcohol and possess insecticidal property[23], α-terpineol is highly aromatic[24], while linalool acetate is mildly toxic to fish and extremely toxic to Daphnia[25,26].

The toxic and repellent efficacy of essential oils may be attributed to an individual or a combined effect of the compounds or chemical groups given above. The mechanism of action of the antimicrobial activity of terpenoids and essential oils[27,28] is not fully understood but may be involved in membrane disruption by the lipophilic compounds. Variation in the toxicity and repellency of the essential oils tested in this study may be attributed to the difference in the targets on the insects for action of the compounds, differences in the active principles present in essential oils, qualitatively and/or quantitatively. Different compounds/active principles of essential oils may have different targets to exert toxicity and repellency on insects. The known target sites on parasites are solely proteins and include ion channels, enzymes, structural proteins, transport molecules, *etc.*,[29-32].

In conclusion, in spite of differences in the biology of bacteria, fungi, protozoa, helminths and insects, there are some common targets among them, which can also be utilized by the compounds having insecticidal activity. These may include inhibition of enzymes, complexing with proteins, polysaccharide, formation of ion channels, *etc*. This effect may result in disturbing the normal biochemical and physiological processes leading to starvation, structural changes, neuromuscular disturbances and other effects on insects. In fact, most of these are the known target sites for commonly used insecticides[32,33]. Moreover due to aromatic nature and strong odour, essential oils had exhibited repellent effect more than the toxicity. The vapourized essential oil got easy entrance through the tracheal system of the insects, thus choking them and causing irritation at the same time, hence compelled the insect to flee from the treatment bottle (having essential oil), towards the control bottles (having filter paper discs treated with either water or ethanol). The excellent repellent effect of essential oil is indicative of their promising commercial prospects as insect repellent if prepared in some formulation. Their repellent effect can further be tested by experimentation on non-human mammals prior to be recommended for human use. Toxicity, although was less than the repellent effect but very encouraging but as data indicates, higher concentrations can improve toxicity due to dose dependant effect. The LC_{50} values of essential oils in this study are compareable to those of extracts of *M. paniculata* observed in early studies[34,35]. Essential oils from leaves of *M. paniculata cv.* China have shown better toxicity than that of essential oil of *Z. armatum* studied earlier[36,37].

REFERENCES

1. B. Holldobler and E.O. Wilson, Journey to the Ants. The Belknap Press of Harvard University Press, Cambridge, Massachusetts (1994).
2. R.B. Raizada, M.K. Srivastava, R.A. Kaushal and R.P. Singh, *Food Chem. Toxicol*., **39**, 477 (2001).
3. M. Abdollahi, A. Ranjbar, S. Shadnia, S. Nikfar and A. Rezaie, *Med. Sci. Monit.*, **10**, 141 (2004).
4. C.O. Adedire and J.O. Akinney, *Ann. Appl. Biol.*, **144**, 185 (2004).
5. C.G. Athanassiou, D.K. Kontodimas, N.G. Kavallieratos and M.A. Veroniki, **98**, 1733 (2005).
6. N.K. Dubey, R. Kumar and P. Tipathi, *Curr. Sci. (India)*, **86**, 37 (2004).
7. A.H. Holley and H. Patel, *Int. J. Food Microbiol.*, **22**, 273 (2005).
8. M.B. Isman, *Annu. Rev. Entomol.*, **51**, 45 (2006).
9. F. Batisha, S.P.S. Hamminder, K.K. Ravinder and K. Shalinder, *Forest Ecol. Manage.*, **256**, 2166 (2008).
10. F.S. Bakkali, D. Averbeck, S. Averbeck and M. Idaomar, *Food Chem. Toxicol.*, **46**, 446 (2008).
11. H.J.D. Dorman and S.G. Deans, *J. Appl. Microbiol.*, **88**, 308 (2000).
12. M.B. Isman and C.M. Machial, Naturally Occurring Bioactive Compounds, Elsevier, Amsterdam (2006).
13. R.K. Chalannavar, H. Baijnath and B. Odhav, *African J. Biotechnol.*, **10**, 2741 (2011).
14. R. Singh, O. Koul, P.J. Rup and J. Jindal, *Inter. J. Trop. Insect Sci*., **29**, 93 (2009).
15. G. Singh, Plant Systematics: An Integrated Approach. Enfield, New Hampshire: Science Publishers, pp. 438-440 (2004).
16. H. Nakata, Y. Hirakawa, M. Kawazo, T. Nakabo, K. Arizono, S.I. Abe, T. Kitano. H. Shimada, I. Wat and X. Ding, *China. Environ. Pollut.*, **133**, 415 (2005).
17. J.K. Grace, B.S. Goodell, W.E. Jones, V. Chandhoke and J. Jellsion, *Proc. Hawaiian Entomol. Soc.*, **31**, 249 (1992).
18. N.P. Manandhar, Plants and People of Nepal. Timber Press Inc., USA (2002).
19. S. Perrucci, G. Macchioni, P.L. Cioni, G. Flamini and I. Morelli, *J. Nat. Prod.*, **58**, 1261 (1995).
20. K. Moser, K. Kriwet, A. Naik, N. Kalia and R.H. Guy, *Eur. J. Pharm. Biopharm.*, **52**, 103 (2001).
21. F. Deguerry, L. Pastore, S. Wu, A. Clark, J. Chappell and M. Schalk, *Arch. Biochem. Biophys.*, **454**, 123 (2006).
22. J. Gertsch, M. Leonti, S. Raduner, I. Racz, J.Z. Chen, X.Q. Xie, K.H. Altmann, M. Karsak and A. Zimmer, *Proc. Natl. Acad. Sci. (USA)*, **105**, 9099 (2008).
23. S.S. Yao, W.F. Guo, Y. Lu and Y.X. Jiang, *J. Agri. Food Chem.*, **53**, 105 (2005).
24. C.L. Chang, I.K. Cho and Q.X. Li, *J. Econ. Entomol.*, **102**, 203 (2009).
25. Grade R.U.N.E.P, SIDS Initial Assessment Report SIDS, SIAM 14, For Linalool Acetate, pp. 41-42 (2002).
26. Bogers M.U.N.E.P, SIDS Initial Assessment Report. 14, For Linalool acetate, pp. 41-42 (2002).
27. B. Suresh, S. Sriram, S.A. Dhanaraj, K. Elango and K. Chinnaswamy, *J. Ethnopharmacol.*, **55**, 151 (1997).
28. J.A. Amaral, A. Ekins, S.R. Richards and R. Knowles, *Appl. Environ. Microbiol.*, **64**, 520 (1998).
29. E. Lacey, *Int. J. Parasitol.*, **18**, 885 (1998).
30. T.G. Geary, R.D. Klein, L. Vanover, J.W. Bowman and D.P. Thompson, *J. Parasitol.*, **78**, 215 (1992).
31. R.J. Martin, *Pharmacol. Therap.*, **58**, 13 (1993).
32. P. Kohler, *Int. J. Parasitol.*, **31**, 336 (2001).
33. L. Mottier, L. Alvarez, L. Ceballos and C. Lanusse, *Exp. Parasitol.*, **113**, 49 (2006).
34. J.U. Mollah and W. Islam, *Pak. Entomol*., **30**, 61 (2008).
35. P.U. Rani and P. Devanand, *Res. J. Seed Sci.*, **4**, 1 (2011).
36. S. Dubey, A. Kumar and S.C. Tripathi, *Annals of Botany*, **4**, 457 (1990).
37. E.P. Siskos, *J. Econo. Entomol.*, **100**, 1215 (2007).