J Vect Borne Dis 42, September 2005, pp. 95–99

Larvicidal and mosquito repellent activities of Pine (*Pinus longifolia*, Family: Pinaceae) oil

M.A. Ansari[a], P.K. Mittal[b], R.K. Razdan[a] & U. Sreehari[a]

Malaria Research Centre (ICMR), [a]20-Madhuban, [b]2-Nanak Enclave, Delhi, India

Background & objectives: Various plant-based products are safe and biodegradable alternatives to synthetic chemicals for use against mosquitoes. Oil of *Pinus longifolia* is traditionally used for protection against mosquitoes in some rural areas but there is no documented report of its use against mosquitoes. The present study was undertaken to scientifically evaluate the activity of Pine oil against mosquitoes.

Methods: The oil was procured from the market and its contents were chemically analysed. Larvicidal activity of oil was tested in laboratory bioassays, while repellent action was studied during whole night bait collections in field by direct application on the skin and after its impregnation on mats.

Results: Results showed varying degree of larvicidal activity of Pine oil against mosquitoes with LC_{50} values ranging between 82 and 112 ppm. The Pine oil had strong repellent action against mosquitoes as it provided 100% protection against *Anopheles culicifacies* for 11 h and 97% protection against *Culex quinquefasciatus* for nine hours respectively. Electrically heated mats prepared from Pine oil provided, 94 and 88% protection against *An. culicifacies* and *Cx. quinquefasciatus* for 10 and seven hours respectively.

Interpretaion & conclusion: Pine oil is effective against mosquito larvae at very higher doses which are not of any practical utility. However, Pine oil showed strong repellent action against *An. culicifacies* (malaria vector) and *Cx. quinquefasciatus* (pest mosquito). Thus its use could be popularised as mosquito repellent.

Key words *Ae. aegypti* – *An. culicifacies* – *Cx. quinquefasciatus* – larvicide – mosquitoes – Pine oil – repellent

Many plant-based products are widely used for their insecticidal/repellent properties for control of mosquitoes/protection from mosquito bites[1,2]. In recent years interest in plant-based products has been revived because of the development of resistance, cross-resistance and possible toxicity hazards associated with synthetic insecticides and their rising cost. Phytochemicals obtained from the huge diversity of plant species are important source for safe and biodegradable chemicals, which can be screened for mosquito repellent and insecticidal activities and tested for mammalian toxicity[3]. A large number of plant products have been reported to have mosquito larvicidal[4-11] and/or repellent activity against adult mosquitoes[12-15]. Protection against mosquito bites was reported for the genus *Azadirachta indica*[15-17]; *Cymbopogan*[12, 18]; *Mentha*[4]; *Eucalyptus maculata citriodon*[19,20]; *Tagetus*[21]; and *Lantana camara* flowers[13]. Citronella and lemon eucalyptus provide the active ingredient of commercial repellents sold under several brand names. *Pinus longifolia* (Family: Pinaceae) commonly known as Pine, yield oil which is traditionally used for the protection from mosquito bites. It is also used as a herbal medicine in some rural areas in India. In addition to oil, resins of the Pine have been used as a mounting medium for the preservation of insects. Since no

scientific study had been reported on insecticidal and repellent actions of Pine oil, the present study was carried out.

Material & Methods

The Pine oil used in the present study was procured from the market. The chemical constituents of the oil were analysed at the Indian Agricultural Research Institute (IARI), New Delhi, India and are given below:

Ingredient	% composition
K–pinene	0.45
B–pinene	0.70
1:B Cineole	0.79
Caryophyllene	2.94
K–terpineole	12.89
Eugenyl acetate	1.76
Eugenol	3.14
Isoeugenol	4.93
Camphor	1.26
Unidentified	13.93

Larvicidal activity: The larvicidal activity of Pine oil was determined against three major urban mosquito vectors — *An. stephensi, Cx. quinquefasciatus* and *Ae. aegypti* after making serial dilutions — 5, 2, 1, 0.5, 0.25 and 0.125% in acetone. Later 1 ml of the dilution was made up to 250 ml with distilled water to obtain a final concentration ranging between 200 and 6.25 ppm. Four replicates were used in the bioassays against III instar larvae of the three species along with concurrent controls. These larvae were obtained from the cyclic colonies of these mosquitoes maintained in the insectary at Malaria Research Centre, Delhi. Mortality was recorded after 24 h and percent-corrected mortality was determined using Abbott's formula[22]. LC_{50} and LC_{90} were calculated using probit analysis as decribed by Finney[23].

Mosquito repellent activity: Experiments were carried out in the village of Dehra in Dhaulana PHC, District Ghaziabad, Uttar Pradesh against *An. culicifacies* and *Cx. quinquefasciatus* in field conditions. The population of the village is about 8000 distributed in 1400 houses. Pure oil (1 ml) without dilution was applied on exposed parts of hands, legs, necks and faces of the human volunteers "bait" in the evening. Informed and free consent was obtained from the volunteers for the present experiment. Volunteers were selected randomly and health conditions were monitored prior to the evaluation. Those who showed allergic symptoms to the oil were excluded from the study. The volunteers were allowed to sit or relax on a string beds laid at five metre apart in a row throughout the night. Untreated (control) baits were also allowed to rest in similar manner at spacing of five metre. Mosquitoes landing on treated and untreated volunteers were collected throughout the night by trained insect collectors and were identified with the help of a hand lens and confirmed in the laboratory. The insect collectors and baits were interchanged to prevent bias. Percent protection was calculated according to standard procedures described earlier[12].

$$\% \text{ protection} = \frac{\text{Control} - \text{Treated (Experimental)}}{\text{Control}} \times 100$$

For comparative evaluation of repellent action, Citronella (Lemon grass) oil, a known herbal repellent product obtained from IARI, New Delhi was used. The protection time (the time between start of experiment till the first mosquito collection on bait) was determined for each night and average of 15 nights was calculated as "average protection time".

Mats: Pine oil mats were prepared from 5% Pine oil in acetone supplied by M/s. Knight Queen, Delhi. The repellent activity of Pine oil mats was tested for eight nights using mosquito-landing collection on human baits throughout the night. The tests were performed on *An. culicifacies, An. subpictus, An. annularis* and *Cx. quinquefasciatus*. The mats were vapourised using electrically heated machine in well-ventilated rooms having human volunteers as bait. The mats were heated throughout the night between 1900 and 0600 hrs after sunset till early morning and the mosquito

landing on human bait was collected throughout the night. The number of mosquitoes collected during each hour was recorded. Commercially available Knight Queen "Mat" made with allethrin was used as control. Percent protection and average protection time were calculated according to standard procedures described earlier.

Results

Data of the larvicidal activity of Pine oil against three species of mosquitoes are presented in Table 1. In terms of lethal concentrations for 50% mortality (LC_{50}) Pine oil appeared to be most effective against *Ae. aegypti* (LC_{50}—82.1 ppm) followed by *Cx. quinquefasciatus* (LC_{50}—85.7 ppm) and *An. stephensi* (LC_{50} —112.6 ppm).

Table 1. Larvicidal activity of Pine oil against different mosquitoes

Concentration (ppm)	Percent larval mortality		
	An. stephensi	*Cx. quinque-fasciatus*	*Ae. aegypti*
200	84	88	96
100	38	50	50
50	6	24	24
25	8	10	10
12.5	2	4	4
6.25	0	0	0
Control	0	0	0
LC_{50}	112.6	85.7	82.1
LC_{90}	329.5	283.4	252

Table 2 shows the repellent activity of Pine oil against mosquito bites. Both, Pine and Citronella oils provided 100% protection for 11 h against *An. culicifacies.* Against *Cx. quinquefasciatus* Pine and citronella oils provided 97.4 and 98.5% protection with an average protection time for nine hours. Table 3 shows the mosquito repellent activity of heated mats prepared from Pine oil. These mats provided 94.1% protection as compared to "Knight Queen" mats against bites of *An. culicifacies* with an average protection time of 10.3 h and 92% protection against *Cx. quinquefasciatus* with an average protection time of 8.2 h. Hourly data on mosquito landing indicate that Pine oil mats produced six hours absolute protection against *An. culicifacies*, three hours absolute protection against *Cx. quinquefasciatus* and 11 h against *An. subpictus* and *An. annularis*. Pine oil mats produced 93.7% protection against all the mosquitoes as compared to "Knight Queen" mats.

Discussion

The present study has shown that Pine oil has larvicidal as well as repellent activity against various spe-

Table 2. Efficacy of Pine oil as mosquito repellent on human volunteers

Repellent oil	*Cx. quinquefasciatus*				*An. culicifacies*			
	No. of mosquitoes collected on bait		% protection Mean ± SE	Av. protection time	No. of mosquitoes collected on bait		% protection	Av. protection time
	E	C			E	C		
Pinus (Pine oil)	9	270	97.4 ± 1.7	9	0	23	100	11
Citronella (Lemon grass oil)	4	270	98.5 ± 1.4	9.6	0	23	100	11

E—Experimental bait (treated with repellent oil); C—Control bait (untreated).

Table 3. Efficacy of Pine oil mats for protection against mosquitoes

Species	Time of collection (hrs)																							
	1900-2000		2000-2100		2100-2200		2200-2300		2300-2400		2400-0100		0100-0200		0200-0300		0300-0400		0400-0500		0500-0600		Total		% protection	Av. protection time
	E	C	E	C	E	C	E	C	E	C	E	C	E	C	E	C	E	C	E	C	E	C	E	C		
An. culicifacies	0	0	0	3	0	0	0	2	0	2	0	2	1	2	0	3	0	1	0	1	0	1	1	17	94.1	10.3
An. annularis	0	0	0	0	0	2	0	3	0	4	0	2	0	1	0	0	0	1	0	0	0	0	0	13	100	11
An. subpictus	0	0	0	0	0	1	0	5	0	1	0	0	0	0	0	1	0	1	0	0	0	0	0	9	100	11
Total anopheline	0	0	0	3	0	3	0	10	0	7	0	4	1	3	0	4	1	2	0	1	0	0	1	37	97.3	10.3
Culex	0	8	0	8	0	10	1	12	0	7	0	3	1	3	2	4	1	8	0	9	0	3	6	75	92	8.2
Total mosquitoes	0	8	0	11	0	13	1	22	0	14	0	7	2	6	2	8	1	10	0	10	0	3	7	112	93.7	7.6

E—Pine oil mats; C—Knight Queen mats; Data in the table is average of eight night collections.

cies of mosquitoes. Though Pine oil has shown the potential against mosquito larvae, it would not be practical for use as a larvicide in non-potable water in large breeding habitats as it requires very high doses to be effective, however, it might be used as larvicide selectively in small breeding places such as in domestic and peri-domestic containers, desert coolers, etc., where water is stagnant. The Pine oil has strong repellent action against mosquitoes in general and particularly against *An. culicifacies*, which is responsible for about 70% of the malaria transmission in the northern rural plain area of India. The oil is already used as a mosquito repellent in some rural areas in India and this is the first report, which has measured the repellent action against mosquitoes particularly against *An. culicifacies*. Further studies are needed on the repellent efficacy of oil against different species of mosquitoes. Recently, strong repellent actions of *Azadirachta indica, Cymbopogan martini martini* var *sofia, C. citratus, C. nardus, Dalbergia sisoo* and *Mentha piperita* have been reported against *An. stephensi* and other species of mosquitoes[12, 15–17]. These findings have re-emphasised the need to explore the possibility of using herbal-based repellents[3] as supplementary and complimentary measures for malaria control. This will reduce the chemical burden on the environment.

The Pine oil has more or less the same repellent action as that was observed with aromatic oil from *Citronella* (Lemon grass), which is used as insect repellent in some commercial preparations. Therefore, its use as a repellent may be promoted. However, pilot studies are indicated to evaluate the epidemiological impact and cost-effectiveness of the natural oils, which are reported to be effective in mosquito control or provide protection against mosquito bites.

References

1. Jacobson M, Crosby DG. *Naturally occurring insecticides.* New York : Marcel Dekker Inc 1971; p. 585.
2. Sukumar K, Perich MJ, Boobar LR. Botanical derivatives in mosquito control: a review. *J Am Mosq Contr Assoc* 1991; *7*(2): 210–37.
3. Mittal PK, Subbarao SK. Prospects of using herbal products in mosquito control. *ICMR Bull* 2003; *33*(1): 1–10.
4. Ansari MA, Vasudevan P, Tandon M, Razdan RK. Larvicidal and mosquito repellent action of peppermint

(*Mentha piperita*) oil. *Bioresource Technol* 1999; *71:* 267–71.

5. Ansari MA, Razdan RK, Tandan Mamta, Vasudevan P. Larvicidal and repellent actions of *Dalbergia sisoo* Roxb. (F. Leguminosae) oil against mosquitoes. *Bioresource Technol* 2000; *73*(3): 207.
6. Green M, Singer JM, Sutherland DJ, Hibben CR. Larvicidal activity of *Tagetus minuta* (Marigold) towards *Aedes aegypti*. *J Am Mosq Contr Assoc* 1991; *7:* 282–6.
7. Mittal PK, Adak T, Sharma VP. Bio-efficacy of six neem (*Azadirachta indica*) products against mosquito larvae. *Pest Res J* 1995; *7*(1): 35–8.
8. Zebitz CPW. Effect of some crude and *Azadirachtin* enriched neem (*Azadirachta indica*) seed kernel extracts on larvae of *Aedes aegypti*. *Entomol Exp Appl* 1984; *35*: 11–6.
9. Perich MJ, Wells C, Bertsch W, Tredway KE. Toxicity of extracts from three *Tagetes* against adults and larvae of yellow fever mosquito and *Anopheles stephensi* (Diptera: Culicidae). *J Med Entomol* 1994; *31*(6): 833–7.
10. Macedo ME, Consoli RA, Grandi TS, dosAnjos AM, deOliveira AB, Mendes NM, Queiroz RO, Zani CL. Screening of Asteraceae (Compositae) plant extracts for larvicidal activity against *Aedes fluviatilis* (Diptera: Culicidae). *Mem Inst Oswaldo Cruz* 1997; *92*(4): 565–70.
11. Pathak N, Mittal PK, Singh OP, Sagar Vidya, Vasudevan P. Larvicidal action of essential oils from plants against the vector mosquitoes *Anopheles stephensi* (Liston), *Culex quinquefasciatus* (Say) and *Aedes aegypti* (L). *Internatl Pest Contr* 2000; *42*(2): 53–5.
12. Ansari MA, Razdan RK. Relative efficacy of various oils in repelling mosquitoes. *Indian J Malariol* 1995; *32:* 104–11.
13. Dua VK, Gupta NC, Pandey AC, Sharma VP. Repellency of *Lantana camara* (Verbenaceae) flowers against *Aedes* mosquitoes. *J Am Mosq Contr Assoc* 1996; *12*(3): 406–8.
14. Moore SA, Lenglet A, Hill N. Field evaluation of three plants based insect repellents against malaria vectors in VACA Diez Province, the Bolivian Amazon. *J Am Mosq Contr Assoc* 2002; *18*(2): 107–10.
15. Sharma VP, Ansari MA, Razdan RK. Mosquito repellent action of neem (*Azadirachta indica*) oil. *J Am Mosq Contr Assoc* 1993; *9*(3): 359–60.
16. Sharma VP, Nagpal BN, Srivastava Aruna. Effectiveness of neem oil mats in repelling mosquitoes. *Trans R Soc Trop Med Hyg* 1993; *87:* 626.
17. Sharma VP, Ansari MA. Personal protection from mosquitoes (Diptera: Culicidae) by burning neem oil in kerosene. *J Med Entomol* 1994; *31*(3): 505–7.
18. Das MK, Ansari MA. Evaluation of repellent action of *Cymbopogan martinii martinii* Stapf var *sofia* oil against *Anopheles sundaicus* in tribal villages of Car Nicobar Island, Andaman Nicobar Islands, India. *J Vect Borne Dis* 2003; *42*(3–4): 100–4.
19. Trigg JK. Evaluation of a eucalyptus based repellent against *Anopheles* spp in Tanzania. *J Am Mosq Contr Assoc* 1996; *12*: 243–6.
20. Schreck CE, Leonhardt BA. Efficacy assessment of Quwenling, a mosquito repellent from China. *J Am Mosq Contr Assoc* 1991; *7*(3): 433–6.
21. Tyagi BK, Ramnath T, Shahi AK. Evaluation of repellency effect of *Tagetus minuta* (Family: Compositae) against the vector mosquitoes *Anopheles stephensi* (Liston), *Culex quinquefasciatus* (Say) and *Aedes aegypti* (L). *Internatl Pest Contr* 1994; *39*(6): 184–5.
22. Abbott WS. A method for computing the effectiveness of the insecticide. *J Econ Entomol* 1925; *18:* 265–7.
23. Finney DJ. Probit analysis. III edn. London: Cambridge University Press 1971; p. 1–333.

Corresponding author: Dr. M.A. Ansari, Dy. Director (SG), Malaria Research Centre (ICMR), 20 Madhuban, Delhi–110 092.
e-mail: ansari@ndf.vsnl.net.in