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NEEM OIL AS A SAND FLY (DIPTERA: PSYCHODIDAE) REPELLENT

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ABSTRACT. The repellent action of neem oil was evaluated against sand flies under laboratory and field conditions. Concentrations of 2% neem oil mixed in coconut or mustard oil provided 100% protection against *Phlebotomus argentipes* throughout the night under field conditions; against *Phlebotomus papatasi* it repelled sand flies for about 7 h in the laboratory. Neem oil is an indigenous product and a low-cost alternative for personal protection against sand fly bites.

Phlebotomine sand flies are the vectors of leishmaniases (World Health Organization 1984) and phlebotomus fever (Goverdhan et al. 1976). The repellents developed against sand flies (Schmidt and Schmidt 1969, Buescher et al. 1982, Fossati and Maroli 1986, Rojas and Scorza 1991) are expensive and do not offer 100% protection. In search of alternatives, we tested the repellent action of neem oil, which is cheap and indigenous to India.

The neem tree, *Azadirachta indica* A. Juss (Meliaceae) is known for its insecticidal properties (Schmutterer 1990). The alkaloids of the neem tree have been investigated as insect antifeedants (Ladd et al. 1978). We report the first results of the repellent action of neem oil against *Phlebotomus papatasi* (Scopoli), the vector of Oriental sore, and *Phlebotomus argentipes* Annandale and Brunetti, the vector of kala-azar.

Neem oil used in the study is marketed by Unjha Ayurvedic Pharmacy, Unjha, Gujarat. It is extracted locally from neem seeds throughout the country and marketed at a price of U.S. $1.00–3.00/liter. In our earlier studies with neem oil, we demonstrated that 2% neem oil mixed in coconut oil provided complete protection from the bites of anopheline mosquitoes (Sharma et al. 1993). Bihar is endemic for malaria with a high density of anopheline mosquitoes. Although the village selected for repellent study on sand flies is endemic for kala-azar and not malaria, it was considered strategically important to test the efficacy of 2% neem oil and also to observe the efficacy of a higher dosage (5%) for protection from sand fly bites to select a dosage that would protect from both the vectors of malaria and sand fly-borne pathogens. Therefore, we selected 2 and 5% neem oil in coconut and mustard oils for laboratory and field evaluation.

Laboratory studies on *P. papatasi* were carried out at 25–29°C and 60–70% RH. Known numbers of laboratory-bred, 2-day-old unfed sand flies were released into 3 wood-framed cages (30 × 30 × 30 cm) (Christophers et al. 1926) that were slightly modified for repellent testing, which used neem oil mixtures and concurrent controls (with diluent oil and without oil). About 1 ml of a neem oil mixture in coconut or mustard oil was applied to the forearm of a volunteer and the treated arm was introduced into a cage containing sand flies. One volunteer applied only coconut or mustard oil and the other exposed his arm without any oil as a control. One arm in each cage was exposed for 40 min every hour from 2000 h until 0600 h. Sand flies probing the skin were collected and held separately. Each test was replicated 6 times.

Field observations on *P. argentipes* were carried out in November 1992 in Govindpur village in the Patna District of Bihar. The density of *P. argentipes* is highest in November in Patna (Dhanda et al. 1983). Test volunteers applied 2 and 5% neem oil and volunteers with mustard oil only and without oil served as controls. About 2–3 ml of oil was applied on exposed arms, legs and the face, and all 4 volunteers were in the same room, which had mud-plastered walls, a thatched roof and no ventilators. Observations on sand fly biting were made from 1800 to 0600 h. Sand flies landing or feeding on volunteers were collected at hourly intervals with suction tubes and kept in separate tubes for counting and sexing. Observations were made for 5 nights. In the experimental room the per man-hour density of resting adults of *P. argentipes* during the 5 nights varied from 150 to 350, and the room temperature ranged from 21.0 to 25.5°C and the RH was 72–79%.

Under both laboratory and field conditions, the test and control volunteers were rotated to avoid any bias resulting from the host. The volunteers were exposed as bait to sand flies 5 min after application of the oil. The time from when the first sand fly probed until 0600 h was taken as the protection time.

Under laboratory conditions (Table 1), concentrations of 2 and 5% neem oil provided 100% protection from bites of *P. papatasi* up to a mean time of 7 h 20 min ± 34 min and 7 h 54 min ± 25 min, respectively. Because of the delayed biting on the controls, the protection time provided by coconut and mustard oil (protection time of

Table 1. Neem oil as repellent to *Phlebotomus papatasi* under laboratory conditions; based on 6 nights of observations.

Observation time (h)	No. of sand flies fed on volunteers					
	2% neem oil (in coconut oil)	Coconut oil (control)	No oil (control)	5% neem oil (in mustard oil)	Mustard oil (control)	No oil (control)
2000–2100	0	0	3	0	0	6
2101–2200	0	0	4	0	0	3
2201–2300	0	0	5	0	2	9
2301–2400	0	4	3	0	2	6
0001–0100	0	8	0	0	7	0
0101–0200	0	6	2	0	7	0
0201–0300	3	2	1	0	4	0
0301–0400	6	0	0	8	0	0
0401–0500	2	0	0	6	0	0
0501–0600	0	0	0	1	0	0
Sand flies fed (%)	11 (13.9)	20 (60.6)	18 (100)	15 (23.4)	22 (66.7)	24 (100)
Unfed ♀ sand flies released	79	33	18	64	33	24
Percent protection	86.1	39.4	0	76.6	33.3	0
Mean protection time (hours and minutes)	7 h 20 min ± 34 min	4 h ± 20 min	1 h 48 min ± 21 min	7 h 54 min ± 25 min	3 h 48 min ± 23 min	1 h 39 min ± 24 min

diluent − protection time of control) was 2 h 32 min and 2 h 9 min, respectively. The addition of 2 and 5% neem oil to the diluents further increased the protection time by 3 h and 4 h 6 min, respectively. Thus the actual protection provided by 2 and 5% neem oil was 5 h 32 min and 6 h 15 min, respectively, indicating that 5% neem oil proved to be a better repellent than 2% neem oil. A one-way analysis of variance was done on protection time provided by 3 treatment groups having 6 replicates of 2 and 5% neem oil each and controls. The value of F (variance ratio) was 39.31 for 2% neem oil and controls and 61.60 for 5% neem oil and controls. The critical value

Table 2. Neem oil as a repellent against *Phlebotomus argentipes* in kala-azar endemic village in Bihar; based on 5 nights of collection.

Observation time (h)	No. sand flies landing/feeding on volunteers							
	2% neem oil (in mustard oil)		5% neem oil (in mustard oil)		Mustard oil (control)		No oil (control)	
	♂	♀	♂	♀	♂	♀	♂	♀
1800–1900	0	0	0	0	0	0	0	0
1901–2000	0	0	0	0	0	0	0	0
2001–2100	0	0	3	0	2	0	13	0
2101–2200	0	0	3	0	2	0	40	4
2201–2300	0	0	3	0	2	0	14	2
2301–2400	0	0	4	0	4	0	31	13
0001–0100	0	0	0	0	0	0	15	7
0101–0200	0	0	3	0	0	0	7	5
0201–0300	0	0	0	0	0	0	7	3
0301–0400	0	0	0	0	0	0	0	1
0401–0500	0	0	0	0	0	0	0	0
0501–0600	0	0	0	0	0	0	0	0
Total	0	0	16	0	10	0	127	35

for 2 df (source of variation among 3 treatments) and 15 df (source of variation within 6 treatments, 6 replicates) at the 1% level is 6.36, indicating a highly significant difference in protection time of test and control treatments.

The results of experiments with *P. argentipes* (Table 2) showed 100% protection from bites of female sand flies when using 2 or 5% neem oil. However, on volunteers with 5% neem oil and mustard oil, 16 and 10 male sand flies landed, respectively. Mustard oil also showed 100% repellent activity against the females of *P. argentipes*. However, further laboratory and field studies with greater sand fly biting pressure need to be conducted to show the effectiveness of mustard oil as a repellent.

The overall results indicate that neem oil is a more effective repellent against *P. argentipes* than against *P. papatasi*. Recently, essential lemon oil tried against *Lutzomyia youngi* Feliciangeli and Murillo in Venezuela (Rojas and Scorza 1991) was reported to give better protection than DEET and citronella (70% protection vs. 63 and 33% for the latter 2, respectively). Thus neem oil offers to be a more promising repellent against sand flies.

Phlebotomus argentipes is the only proven vector of kala-azar in India. The failure of insecticides to contain transmission is shown by the 19,217 cases of kala-azar in 1988, which increased to more than 62,000 cases in 1992 (Directorate of Health Services, Patna, Bihar). Resistance to DDT in *Phlebotomus papatasi* has been reported from Bihar (Joshi et al. 1979). Increasing dependence on spraying alone may make spraying DDT less and less effective. Also in areas where insecticides are not sprayed for administrative and financial reasons, there is no effective method for protection against sand fly bites. Information on night biting activity of *P. papatasi* is not known from Bihar. Because of the observed peak biting hours of *P. argentipes* in Bihar (2100–0400 h), the application of 2% neem oil during the peak biting period can be recommended as prophylaxis against the bites of sand flies (*P. argentipes* and *P. papatasi*) and mosquitoes.

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