



Malaria Research Lead Programme

**Testing Plant Samples for Mosquito Repellency
Time Lag Trials
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The work described in this report is being carried out in the Durban laboratories of the Malaria Research Programme of the Medical Research Council and was commissioned for CSIR.

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Objective

This study was conducted over a three hour period to determine if a specific plant extract had a repellent effect when testing using three different solvents and a cream.

Materials and Methods

Repellency tests had been conducted following similar guidelines to that of the standard WHO protocol and using the target species, *Anopheles arabiensis* (WHO, 1996).

The rodent *Mastomys coucha* was the test animal used for the screening of the samples for repellency activity. Ethical approval for the use of *Mastomys* in these trials was sought from the MRC's Ethics Committee for Research on Animals.

Each adult *Mastomys* had been weighed individually, and injected intraperitoneally with sodium pentobarbital at a rate of 1ml per 2.25 kg. The anaesthetized rodents were shaven on the ventral surface and the product was applied.

Paper cups (500ml) were modified by replacing the base of the cup with mosquito netting held in place with a rubber band and covering the mouth of the cup with transparent plastic film.

The trial comprised of four tests namely, one sample, a repeat test, and one negative and one positive control.

Thirty unfed 4-day old *Anopheles arabiensis* females was introduced into a cup and held in contact with exposed skin of each rodent. Mosquito activity was observed through the transparent plastic film. After a period of two minutes the number of mosquitoes probing had been recorded. To ensure the extracts were indicative of constant repellency, time lag trials had been conducted hourly for a period of three hours. A new cup of mosquitoes were used for each test. The mosquitoes exposed to the

product had been monitored one hour post exposure to determine if the product had induced a knockdown effect.

The rodent was returned to the animal facility and allowed to recover from the effects of the anesthetic. Each rodent was monitored for 7 days for adverse reactions to the sample.

Results

Table one: Results of repellency trials

Sample Solution/Cream	Solvent/ Formulation type	Test	% Repelled		
			2 min	1st hour	2nd hour
One (20%) Water extraction Gen 130-05A	Distilled water	1	73.33	53.33	20.00
		2	40.00	20.00	17.00
Two Cream Gen 130-05C	Aqueous cream	1	60.00	17.00	10.00
		2	17.00	20.00	10.00
Three (20%) Organic extraction Gen 130-05B	Acetone-ethanol (1:1)	1	66.67	40.00	30.00
		2	80.00	26.67	23.33
Negative	Distilled water	1	33.00	23.00	23.00
Negative	Untreated	1	17.00	30.00	17.00
Negative	Acetone-ethanol (1:1)	1	23.00	13.33	17.00
Positive	DEET	1	100.00	100.00	100.00
Positive	DEET	1	100.00	100.00	100.00
Positive	DEET	1	100.00	100.00	100.00

Discussion

Trials were conducted in an insectary under controlled conditions. The CSIR had provided the MRC laboratories with three samples of the same extract.

As per the instructions, each of the three samples had been re-constituted in either distilled water, aqueous cream or a combination of acetone and ethanol (1:1)

To ensure validity of results, a negative control (solvent only) and positive control (DEET) was included in each trial.

The negative controls tested were distilled water, acetone-ethanol mixture and an untreated area on the rodent. The results of all the negative control tests have shown that the test species were greatly attracted to the rodent thus proving that the solvent had no repellent effect.

The result of the positive control testing has shown a very high repellent effect of 100% repellency over a period of 2 minutes and had maintained its repellent stability over a period of three hours.

The results of testing (Table 1) have shown that only one sample had indicated significant repellent activity during the initial two minute exposure. The organic extract Gen 130-05B, had shown an average of 73% repellency however a gradual decrease in repellent stability was observed resulting in an average of 27% repellency by the second hour of testing. This trend of decreasing repellency has observed with all the samples tested and none of the extracts had satisfied the criteria of a promising repellent which was 100% repellency in accordance with DEET. The results have shown that the repellent effect of the organic sample had been very short lived and would require frequent re-application should it be considered as a possible repellent.

Knockdown monitoring indicating temporary paralysis of the mosquito over a period of one hour post exposure had shown that the extract had no effect on the mosquitoes.

References

WHO,CTD/WHOPES/IC/96.1 Protocols for laboratory and field evaluation of Insecticides and Repellents