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Field Trials on the Repellent Activity of Four Plant Products against Mainly *Mansonia* Population in Western Ethiopia

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The repellent activity of essential oils of lemon eucalyptus (*Eucalyptus maculata citrodion*), rue (*Ruta chalepensis*), oleoresin of pyrethrum (*Chrysanthemum cinerariaefolium*) and neem (*Azadiracta indica*) have been field tested as 40%, 50% and 75% solutions in coconut oil against populations of mosquitoes consisting mainly of *Mansonia* in Gambella, western Ethiopia. A latin square design was used to randomize the test subjects for possible individual differences for mosquito attraction. Repellency was evaluated as the percentage protection. Deet was included in the study for comparison. All the plant products manifested repellency. At 50% concentration at which the highest repellency was recorded the protection was 91.6%, 87.0%, 96.0%, 97.9% for rue, neem, pyrethrum and deet, respectively. The essential oil of lemon eucalyptus was not tried at this concentration. At a 40% concentration deet, lemon eucalyptus and pyrethrum were significantly ($p < 0.05$) more effective than rue and neem. At a 50% concentration, deet and pyrethrum were significantly better ($p < 0.05$) than rue and neem. At a 75% concentration concentration, deet and lemon eucalypus performed significantly better ($p < 0.05$) than pyrethrum and neem. The difference between pyrethrum and neem was also significant ($p < 0.01$). Copyright © 2003 John Wiley & Sons, Ltd.

INTRODUCTION

Repellents are used as personal protection methods against biting arthropods with the major aim of avoiding nuisance (Trigg and Hill, 1996). By reducing insect bites repellents could increase the productivity of field workers as a result of less distraction by the biting insects. When properly used they are also reported to reduce disease transmission (Gupta and Rutledge, 1994).

Insect repellents are considered useful alternatives where other control measures are either not practical or possible. Repellents properly utilized are an inexpensive means of reducing or preventing arthropod-borne diseases and biting nuisance while acting on a wide range of vectors (Gupta and Rutledge, 1994).

Natural repellents have been in use in different communities for a long time and were the basis for most of the commercial repellents like citronella until diethyl toluamide (deet) came into the picture (Curtis *et al*., 1990). Although deet is the dominant repellent in the market, different communities are reported to use various plants in different forms to protect themselves from insect bites (Curtis *et al*., 1990; Hebbalkar *et al.*, 1992; White, 1973).

However, there are reports that question the safety of deet (Moody, 1989; Tenenbein, 1987). Besides deet is not repellent against all insects (Rojas and Scorza, 1991). Deet is also known to damage plastic and synthetic materials (James and Harwood, 1969).

Hence the search for a safer, better and cheaper repellent is an ongoing effort. Since cost is an important factor, investigation on the use of local plants as repellents is strongly recommended (Curtis *et al*., 1990). India and China are taking the lead on this line; citronella and quwenling (a waste distillate of lemon eucalyptus), both natural products, are available commercially (Curtis *et al*., 1990). A formulation of lemon eucalyptus has been successfully commercialized in the UK (Trigg and Hill, 1996).

This work aimed at evaluating the repellent activity of oils from rue (*Ruta chalepensis*), neem (*Azidirachta indica*), pyrethrum (*Chrysanthemum cineraraiefolium*) and lemon eucalyptus (*Eucalyptus maculata citrodion*). The extracts of neem, pyrethrum and lemon eucalyptus are reported to have repellent activity (Sharma *et al.*, 1993; Curtis *et al*., 1990). Our interest in the scientific investigation of rue was due to an individual's experience and report that rue, *Ruta graveolense*, can serve as a repellent against house flies and also its use as a repellent in historic times (Oruene, 1983). The species of rue found in Ethiopia, *R. chalepensis*, is a very popular plant for its medicinal value in Ethiopia (Abebe and Ayehu, 1993); just as the rue species found in Europe, *R. graveolense*, was known for the same reason (Oruene, 1983). It is cultivated very widely throughout the highlands above 1500 m. In addition to its medicinal value, it is also used to flavour milk, cottage cheese, coffee, tea, and in the preparation of local spices (Gilbert, 1989). Neem is found in the lowland of Ethiopia while pyrethrum is

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common in the country in the highlands. Lemon eucalyptus is confined to two places, at Wondogenet and Alamaya, southern and eastern Ethiopia, respectively.

A field study was conducted to evaluate the repellency of the above mentioned plant products against a mixed population of mosquitoes the majority of which were *Mansonia* species in Western Ethiopia.

MATERIALS AND METHODS

Study site. The study was carried out during the month of December 1995 following the mosquito breeding season, which is usually from late September to early January. The investigation was carried out in Pugnudo, 112 km south of Gambella. The locality is near Gilo River, which often floods during the rainy season. As the rainy season ends the water recedes leaving behind swamps and ponds, which provide mosquitoes with suitable breeding places.

Test repellents. Essential oils of lemon eucalyptus and rue were extracted by steam distillation of their leaves; 0.6% and 0.8%–1% yields were obtained for rue and lemon eucalyptus, respectively. Oleoresins of neem and pyrethrum were obtained by solvent extraction of neem seed and pyrethrum flowers using hexane. The seeds and flowers were dried and powdered prior to extraction. Oleoresin contains mainly essential oil and resin. The yields of the oleoresins were 3.7%–4% for pyrethrum and 45.6% for neem. Deet was obtained from McLaughlin Gormley King Company (MGK^R), USA.

Test species. The experiment was performed against a mixed population consisting of about 68% *Mansonia uniformis* and 23.2% *M. nigerrima*, the remainder being *M. fricana*, *Anopheles pharoensis*, *An. tenebrosus* and *Taeniorhnyrchus cristatus*.

Repellent tests. Four candidate repellents were evaluated: rue and lemon eucalyptus as essential oils and pyrethrum and neem as oleoresin. Coconut oil was used as a solvent, because of its better miscibility with the oleoresins than the commonly used ethanol. The test subjects were indigenous *Agnuaks*, with the exception of one person from another ethnic group. They were briefed about the work, gave an informal consent and they were instructed about the application of the repellent and counting the mosquito bites. Prior to the commencement of the study a site was selected where there was no interference from the residents and where there was a high biting rate of mosquitoes. Preliminary human landing collections were also made to determine the peak biting time. A high biting rate was recorded between 1900 to 1930 h; therefore, the experiments were carried out during this time.

A latin square design was used so that every individual received a different treatment every experiment to neutralize possible individual differences to mosquito attraction. Test subjects sat on folding canvas chairs, 1–1.5 m apart. Each test subject received 1 mL of a candidate repellent on each leg. Repellents were applied evenly from knee to ankle. The control subject was treated with coconut oil.

Each mosquito bite on the treated parts of the legs was reported to the investigators who stood behind each test subject. Mosquitoes were chased away with a straw after recording the bite so that one bite was not counted more than once but the mosquito, that was chased away, could return and bite one of the subjects again. Every 2 min legs were examined for biting mosquitoes by the investigators using flashlights in case there were biting mosquitoes that had not been felt by the test subjects. Records were taken with tally counters until the end of the experiment, when they were transferred to record forms. The subjects exchanged seats every 6 min to avoid possible bias in the number of bites due to position in the study site. Candidate repellents were compared with one another at the same concentrations. Different concentrations of the same repellent were also compared. Concentrations of 40%, 50% and 75% were evaluated in most cases but rue and lemon eucalyptus were compared at two concentrations; rue at 40% and 50% and lemon eucalyptus at 40% and 75%, due to a shortage of the essential oils at the time of the study. The results were expressed as percent protection, being the difference between the number of bites received by the control and the treated subject expressed as a percentage of the control score.

Data analysis. A randomized experiment was performed using a latin-square design. Analysis of the data was conducted using the Student's *t*-test.

RESULTS

The daily total percent protections provided by the extracts and the average number of bites received by each treatment per test is presented in Table 1. Lemon eucalyptus essential oil gave the best percent protection of the plant products. At all the concentrations compared, the performance of the plant products in decreasing order was essential oil of lemon eucalyptus, oleoresin of pyrethrum, essential oil of rue and oleoresin of neem. At 40% concentration deet, lemon eucalyptus and pyrethrum were significantly more effective ($p < 0.001$) than rue and neem. At 50% concentration, deet and pyrethrum were significantly better ($p < 0.05$) than rue and neem. At 75% concentration, deet and lemon eucalyptus performed significantly better ($p < 0.05$) than pyrethrum and neem. The highest percent protection for all the plant products and deet was observed at 50% followed by 40% and 75% concentrations.

DISCUSSION

The percentage protection was the preferred method to evaluate repellency rather than the widely used method, time before the first bite, i.e. the time interval between application of a repellent and the first of two bites occurring within 30 consecutive minutes. Although the latter method is the most commonly used, its reliability in some instances like this study could be questionable. Schreck and McGovern (1985) have reported a complete protection time of nearly 5 h with deet

Table 1. Repellent activity of four candidate repellents tested against mosquitoes in Gambella, Ethiopia

Concentration	Treatment	Mean no. of bites (SE)	Average protection (%)†	Ratio to deet	*p* value*
40%	Neem	64 (11.3)	74[a]	0.79	0.006
	Rue	44 (9.4)	78[a]	0.84	0.001
	Pyrethrum	20 (3.9)	90[b]	0.96	0.000
	L.eucalyptus	17 (3.2)	91[b]	0.98	0.000
	Deet	15 (4.1)	93[b]	1.00	0.000
	Control	230 (36.1)			
50%	Neem	37 (8.6)	87[a]	0.88	0.001
	Rue	26 (8.1)	92[a]	0.93	0.001
	Pyrethrum	14 (5.5)	96[b]	0.98	0.001
	Deet	6 (2.6)	98[b]	1.00	0.000
	Control	323 (55.7)			
75%	Neem	74 (12.8)	56[a]	0.60	0.024
	Pyrethrum	21 (4.7)	84[b]	0.90	0.001
	L.eucalyptus	10 (2.2)	92[c]	0.99	0.000
	Deet	8 (2.6)	93[c]	1.00	0.000
	Control	151 (55.2)			

* *p* value from comparison of mean values of each treatment with mean of control (*t*-test).
† Same letters at each concentration are not significantly different.

(25% concentration) against populations in the field consisting of 90% *M. titillans*; performing the best among six repellents evaluated. Das *et al.* (1988) have also reported deet as the best repellent against *M. uniformis* and *M. indiana* with a protection time of 4.4 h at 20% concentration and concluded deet could be considered as a repellent of choice for personal protection against *Mansonia*. If time until the first bite, as defined above was used as a method of evaluation for this work, the results and conclusions made by the above mentioned investigators would have gone against the present result. Because in all treatments deet treated subjects received 6–15 bites within the first 30 min which would have ruled it out as an effective repellent. However, based on the percent protection, i.e. percentage calculated as

$$\left(\frac{\text{Number of bites on control} - \text{bites on treatment}}{\text{Bites on control}}\right) \times 100$$

the present result consolidates that of Schreck and McGovern (1985) and Das *et al.* (1988). Deet gave the best percent protection at all concentrations (Table 1).

Since the percent protection only gives information about the repellent deposit when freshly applied, protection time should also be determined as persistence is important to a repellent user. Schreck and McGovern (1985) have described a method of determining both the percent protection and the complete protection time. This method could help to avoid prematurely rejecting promising repellents when the time before the first bite is used alone.

Another interesting point in this investigation is that no increment in the percent protection was observed when the concentration of the candidate repellents and deet was increased from 50% to 75% concentration; rather a decrease in the percent protection was seen (Table 1). One possible explanation could be due to the variation of species in the mosquito population during the tests at the different concentrations, although the same time and place were used throughout the experiments, the assumption being that there would be no variation in the species composition. Therefore, mosquito collection was not made at each test. Only two collections were made to sample the species composition: at the end of the 50% and 75% concentration trials. In the first collection 56.4% of the mosquitoes were *M. uniformis* and 35.2% were *M. nigerrima.* In the second collection 83.2% were *M. uniformis* and 8.1% were *M. nigerriama*. Variation of the species composition during the tests might affect the result of the percent protection by increasing or decreasing the number of susceptible or tolerant mosquito species during the tests.

Although pyrethrum is known as a repellent, it was reported that it was unsuccessful as a skin formulation (Curtis *et al*., 1990). In this study, however, the result is encouraging giving a percent protection of 87.8–96.0 in a crude form of hexane extract. Lemon eucalyptus oil has been found unsuccessful against *Aedes aegpti* in laboratory tests based on protection time (Curtis *et al*., 1990). However, the waste distillate, called quwenling, after the extraction of the oil was found to be effective against mosquitoes in China (Curtis *et al*., 1990). In subsequent tests in the USA quwenling was reported to have a shorter duration of effectiveness than deet (Schreck and Leonhardt, 1991). In the present study, lemon eucalyptus oil gave a percent protection equivalent to that of deet at identical concentrations. Similar results have recently been reported by a new repellent (trade name 'Moisiguard Natural') developed from eucalyptus oil with the principal active component p-menthane-3,8-diol (Trigg and Hill, 1996) which is also the chief active component of quwenling (Curtis *et al*., 1990). This new repellent has been found almost as effective as deet against *An. gambiae* and *An. funestus* in the field in Tanzania (Trigg, 1996) and against other arthropods in the laboratory (Trigg and Hill, 1996). Neem's performance should not be overlooked owing to the fact that it was tested as oleoresin. According to Sharma *et al.* (1993), neem oil produced by mechanical crushing provided complete

protection for 12 h from bites of anopheline mosquitoes in the field at 2% concentration. No exact comparison could be made between Sharma's findings and this study due to the difference between the methods of obtaining neem oil, which could result in different concentrations of the active components in the oil and the different mosquito species against which the oils were tested.

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