

THE REPELLENT AND ANTIFEEDANT ACTIVITY OF *MYRICA GALE* OIL AGAINST *AEDES AEGYPTI* MOSQUITOES AND ITS ENHANCEMENT BY THE ADDITION OF SALICYLURIC ACID

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SUMMARY

A clinical trial was carried out with *Aedes aegypti* (*Ae. aegypti*) mosquitoes to investigate the repellent and antifeedant activity of oil of *Myrica gale* (*M. gale*) and its enhancement by salicyluric acid. *Myrica gale* was effective as both a repellent and an antifeedant and was in a similar class of efficiency to N, N-diethyl-m-toluamide (DEET), the classical synthetic insect repellent. Salicyluric acid gave partial protection with a 10% solution and complete protection with a 20% solution. Its effects appeared to be mediated by contact 'taste' receptors, since it acted primarily as an antifeedant rather than repelling mosquitoes at a distance. The incorporation of oil of *M. gale* with salicyluric acid in a single formulation (where there is a possibility of synergism) resulted in a superior product in terms of both repellency and antifeedancy. The potential contribution of these compounds to a generation of new, safer and more effective repellents is discussed.

INTRODUCTION

Afrotropical mosquitoes are efficient vectors of disease pathogens, and are implicated in some of the most debilitating and life-threatening diseases of man, including filariasis and malaria. Malaria alone represents 9% of the total disease burden and results in over one million deaths annually, mainly of young children. Mosquito-transmitted diseases remain one of the most serious obstacles for development in Africa; the cost of malaria control alone is estimated at US\$1.8 billion each year. Generalised drug failure forcing governments to adopt more expensive drugs as first-line treatments, together with tolerance and resistance of many mosquito species to a variety of insecticides,¹ has turned attention once more to control of the mosquito itself, particularly through exploitation of its behaviour. For example, protection against endophagic (indoor-feeding) species has been sought in insecticide-treated bednets; in certain areas of Africa these have had a significant impact on child morbidity and mortality (primarily from malaria).²

Protection can also be sought in the use of repellents; appropriate for mosquitoes feeding both indoors and outdoors, during the day and during the night-time. Repellents are substances that act locally or at a

distance, deterring an insect from flying to, landing on or biting human or animal skin. There are two modes of action, either through air-borne molecules as in the vapour of volatile oils or by non-volatile molecules with an antifeedant effect on the skin.

A range of chemical repellents, in various formulations and coming under a number of different trade names, is available commercially. The most widely used compound in insect repellents is DEET, which is the main active constituent in the majority of preparations, in concentrations varying from 10–90%. Since it was first marketed in 1956, DEET has remained the most effective repellent against midges, mosquitoes and other biting pests. These products are effective if applied regularly but their safety has been questioned. Toxicity from casual use appears to be low but there are a number of reports of toxic effects of DEET with long-term use, with the nervous system, immune system and skin being the prime targets for adverse reactions: dermal absorption of DEET has been reported at 3–8% for humans.³ Children appear to be particularly vulnerable, experiencing seizures⁴ and dermatitis⁵ and there is increasing interest in safer alternatives.⁶ For example, oil from the leaves of *Myrica gale* L. (*Myricaceae*), a common shrub of the Scottish Highlands has been shown to possess repellent activity against *Culicoides impunctatus* (*C. impunctatus*), the most abundant species of biting midge in Scotland.⁷

The leaves of *M. gale* have been used as a folklore remedy against fleas and bedbugs on the Isle of Skye. The oil is a pale yellow, highly volatile liquid. It is a complex mixture of terpenes; a number of studies have identified as many as 78 components by gas chromatography. These components vary greatly in their anti-insect activity,⁸ with dilutions as low as 1 × 10⁶ preventing biting and landing by *C. impunctatus*.⁷

Recently, it has been shown that derivatives of salicylic acid, which occurs naturally in yarrow, *Achillea millefolium* (L.) (*Asteraceae*), exert an antifeedant effect on the midge but do not deter landing. The most potent of these derivatives was salicyluric acid.⁹ Since previous studies have also indicated an antifeedant of salicylic acid on mosquitoes,¹⁰ this study aimed to investigate the effects of both *M. gale* and salicyluric acid on the yellow fever

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mosquito, *Ae. aegypti*.

MATERIALS AND METHODS

The repellent studies were carried out in the laboratories of Professor Estambale, University of Nairobi, under the approval of their research committee. The research had no commercial involvement.

Newly emerged *Ae. aegypti* laboratory-reared (uninfected) mosquitoes were fed on a 10% solution of honey before being starved for two days in preparation for the experiments. The same three human volunteers were used throughout the investigation. One arm was treated with a test substance (1 ml) and the other arm with a control substance, either a cellulose gel or ethanol. Both arms were then placed in cages containing 100 live mosquitoes. The numbers of insects landing and biting on each arm were recorded at zero, two and either four or six hours.

Oil of *M. gale* was obtained by steam distillation of leaves from the plant. The oil was used as a 20% preparation in an inert gel, hydroxy methyl cellulose 'Celacol' (Unilever). DEET (Sigma-Aldrich) was prepared as a 10% solution in ethanol and salicylic acid (2-hydroxyhippuric acid, Sigma-Aldrich) as 10% and 20% solutions, also in ethanol.

RESULTS

Myrica gale

Compared with a control treatment, *M. gale* gave considerable protection against *Ae. aegypti* mosquitoes landing and feeding for at least the first two hours of the experiment, with the protection breaking down between two and six hours. At two hours, although fewer mosquitoes landed on the test arm than the control arm, the difference was not statistically significant, whereas the reduction in the biting rate (as a percentage of those landing) was significantly less on the

arm treated with *M. gale* than the control arm at two hours (Figure 1a).

Compared against the standard mosquito repellent, DEET, *M. gale* performed equally well in preventing biting. There were greater numbers of mosquitoes landing on *M. gale*-treated arms than DEET-treated arms, although this difference was only statistically significant at two hours. Again, the protective effect of both compounds broke down by six hours (Figure 1b).

Salicylic acid

Although just failing to attain statistical significance, 10% salicylic acid gave partial protection from both landing and feeding by *Ae. aegypti*. The inhibition of landing was less marked than that of feeding and feeding inhibition continued until four hours after the initial application of the substance (Figure 2a). A 20% solution of salicylic acid provided complete protection up until four hours post application. No feeding was recorded during this time period and the numbers of mosquitoes landing on the test arms were very low (Figure 2b).

Comparing *M. gale* with salicylic acid, *M. gale* gel (20%) gave its expected protective action, with only low levels of landing and no biting until four hours post-treatment. Salicylic acid (10%), however, had less effect on landing, although still caused significant reductions in feeding (Table 1).

Myrica gale/salicylic acid combined formula

In combination, *M. gale* (20%) and salicylic acid (20%) gave better protection than the single compounds alone, with prevention of landing and feeding extending to four hours and six hours respectively. Their combined activity was equivalent to that recorded for DEET, with no significant differences between the levels of either landing or feeding (Figure 3).

TABLE 1

A comparison of the landing and feeding behaviour of *Ae. aegypti*; test arm treated with 20% *M. gale* in a cellulose gel and control arm with 10% salicylic acid. 100 mosquitoes/cage.

Time	Exp No.	<i>Myrica gale</i> gel			10% salicylic acid		
		No. landing	No. repelled	No. feeding	No. landing	No. repelled	No. feeding
0 h	1	4	4	0	1	1	0
	2	6	6	0	2	2	0
	Total	10	10	0	3	3	0
2 h	1	1	1	0	8	8	0
	2	2	2	0	10	6	4
	Total	3	3	0	18	14	4
4 h	1	5	1	4	10	8	2
	2	4	3	1	8	5	3
	Total	9	4	5	18	13	5
6 h	1	7	3	4	18	12	6
	2	10	4	6	20	12	8
	Total	17	7	10	38	24	14

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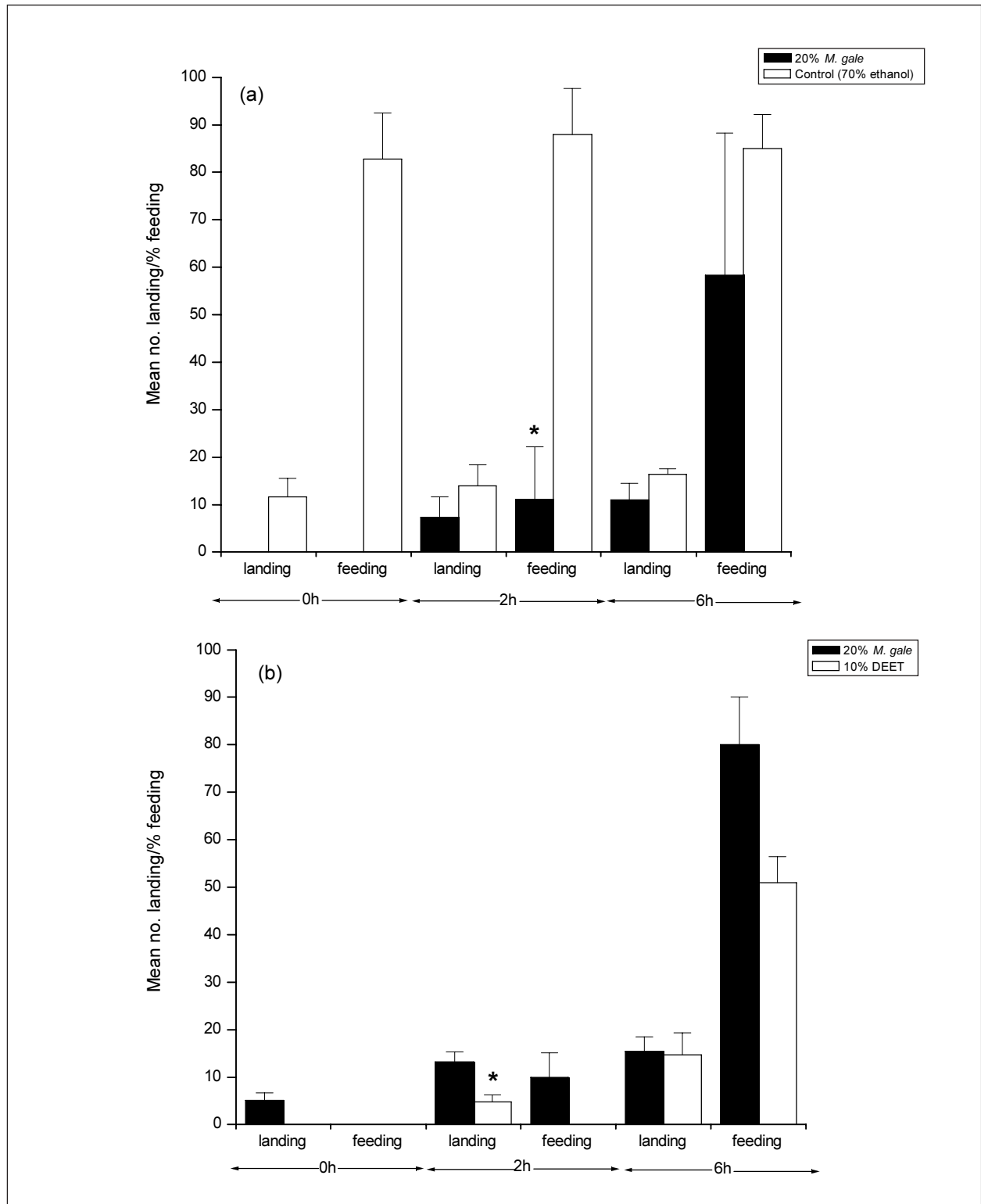


FIGURE 1

Landing and feeding of *Ae. aegypti* mosquitoes on (a) test (20% *M. gale*) and control (70% ethanol) arms and (b) test (20% *M. gale*) and control (10% DEET) arms. Landing shown as the mean numbers landing (\pm SE) and feeding as the numbers feeding as a percentage (mean \pm SE) of those landing.

For (a) *: significant reduction in feeding on arms treated with *M. gale* than control arms at 2 h ($t = 5.10$, $P < 0.001$). For (b) *: significant reduction in numbers landing on arms treated with DEET than arms treated with *M. gale* at 2 h ($t = 4.76$, $P < 0.05$).

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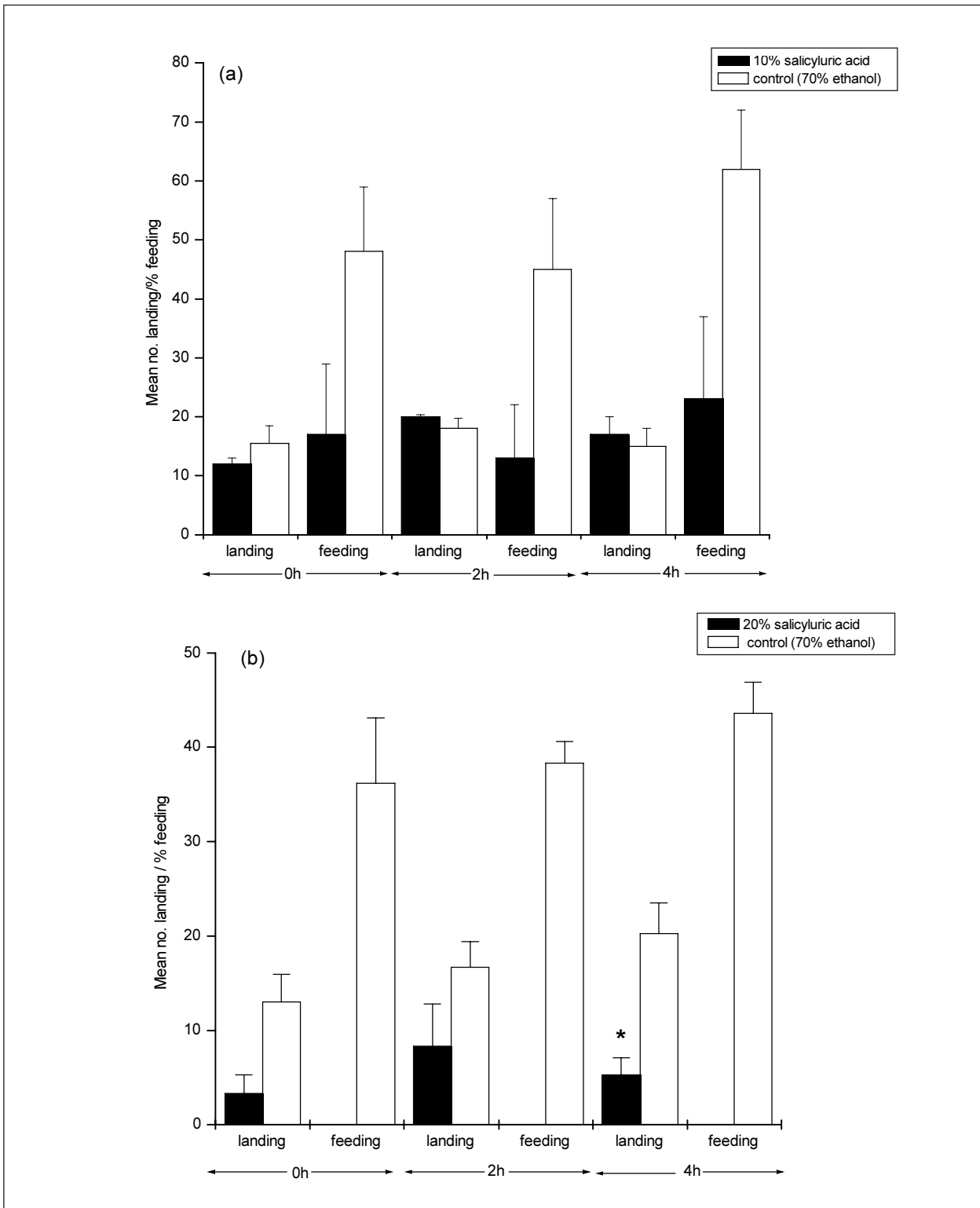


FIGURE 2

Landing and feeding of *Ae. aegypti* mosquitoes on (a) test (10% salicylic acid) and control (70% ethanol) arms and (b) test (20% salicylic acid) and control (70% ethanol) arms. Landing shown as the mean numbers landing (\pm SE) and feeding as the numbers feeding as a percentage (mean \pm SE) of those landing.

For (b) *: significant reduction in numbers landing on arms treated with 20% salicylic acid than control arms at 4 h ($t=4.13$, $P<0.05$).

CONCLUSIONS AND DISCUSSION

The present data demonstrate that *M. gale* crosses the species barrier, being an effective repellent against insects from two different families of biting insects (i.e. biting midges (*Ceratopogonidae*)⁷ and in the present investigation, mosquitoes (*Culicidae*)).

With *Ae. aegypti*, *M. gale* gave almost complete protection from biting for up to two hours post-treatment and in addition, the number of mosquitoes landing was reduced compared to control (solvent only) arms. DEET also gave substantial protection for two hours, with the effect breaking down by four hours. *M. gale* and DEET were comparable in their inhibition of mosquito feeding, although in general, DEET was slightly superior in inhibiting mosquito landing. Hence, it can be concluded that *M. gale* is effective as both a repellent (discouraging mosquitoes to land on a subject) and as an antifeedant (preventing blood-feeding), and that it is in a similar class of efficiency as DEET.

Salicyluric acid also appears to cross the species barrier, although its activity against *Ae. aegypti* was less than that against *Culicoides* biting midges.⁹ In the latter study, 10% salicyluric acid had a significant antifeedant effect, although it did not repel midges at a distance and it was

hypothesised that the antifeedant activity may result primarily from contact (mediated primarily through receptors located on the mouthparts of *C. impunctatus* (Blackwell, unpublished data)), rather than detection by olfactory receptors (mediated primarily through the antennae¹¹). The results were similar with *Ae. aegypti*, with the antifeedant activity of salicyluric acid being far greater than the repellent activity. A 10% solution of salicyluric acid provided only partial protection against the mosquitoes, although far more encouraging data were obtained with a 20% solution and this is an area that merits further investigation.

The practical use for salicyluric acid (having mainly an antifeedant effect) may be in combination with volatile repellents and indeed, the failure of salicyluric acid to prevent landing was entirely reversed by the addition of oil of *M. gale*. Salicyluric acid's effect on feeding was not affected by the addition of *M. gale* and in fact, there may have been an additive effect in the overall antifeedant effect.

The ideal repellent should exert an initial 'anti-landing' effect by providing an air barrier of volatile molecules and a secondary surface barrier of a different type. Insects which penetrate the air barrier of volatile

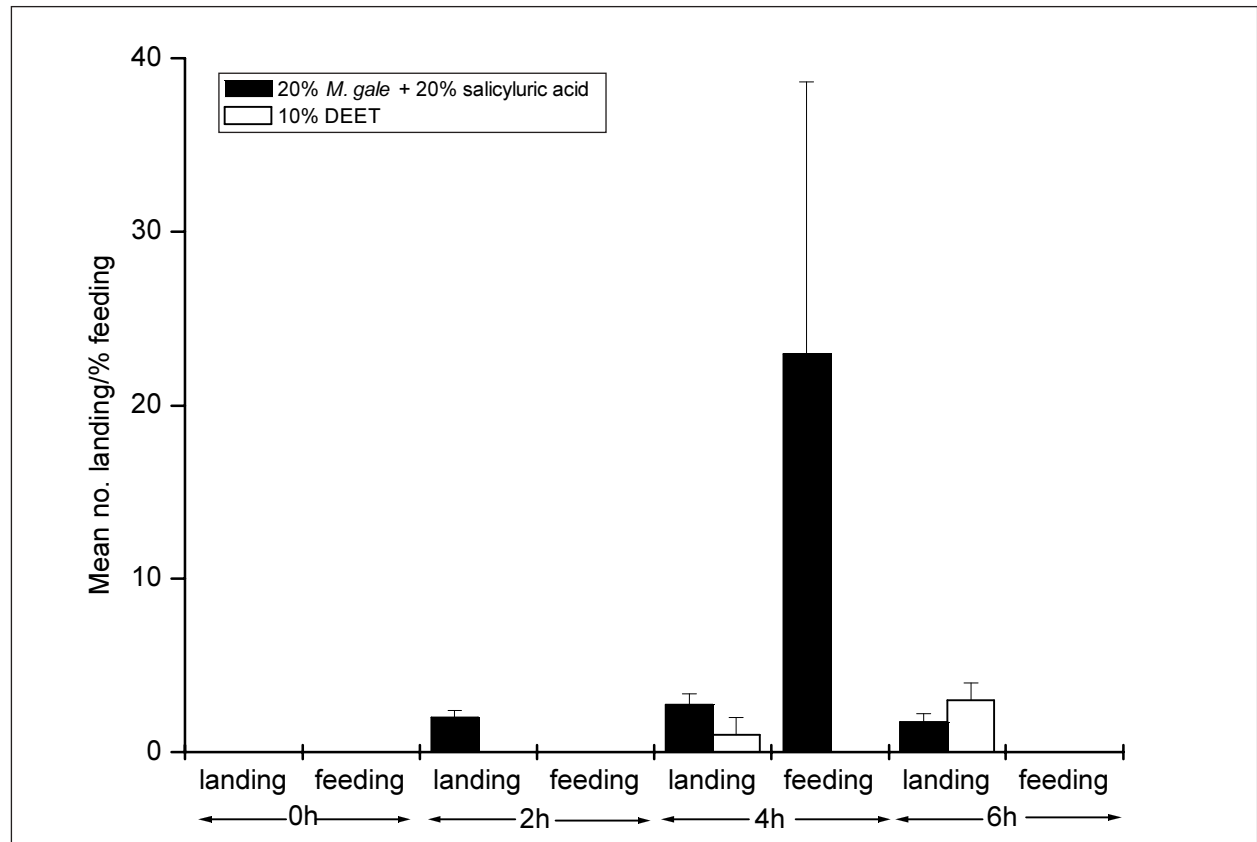


FIGURE 3

Landing and feeding of *Ae. aegypti* mosquitoes on (a) test (20% *M. gale* + 20% salicyluric acid) and control (10% DEET) arms. Landing shown as the mean numbers landing (\pm SE) and feeding as the numbers feeding as a percentage (mean \pm SE) of those landing.

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molecules are further deterred if they meet with a novel repellent which is relatively non-volatile but possesses an antifeedant effect. The antifeedant effect will be of particular importance in reducing disease transmission where the insect is acting as a vector of pathogens of medical or veterinary importance. Salicylic acid is of specific interest since it is formed in the mammalian liver during detoxification of acetylsalicylic acid¹² and together with its congeners is largely of low toxicity to mammals. The overall message of this communication is that by combining a non-volatile compound with a volatile substance, it is possible to block the insect attack both in the air and at the surface. Such enhanced mixtures may provide a guide to the development of improved preventatives.

ACKNOWLEDGEMENTS

The authors are grateful to the Royal College of Physicians of Edinburgh for funding the Nairobi aspect of this work. In addition, we would particularly like to thank the three laboratory technicians who volunteered to take part in the research.

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