Repellent effects of the essential oil of *Lavendula angustifolia* against adults of *Hyalomma marginatum rufipes*

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ABSTRACT

The repellent effects of the essential oil of *Lavendula angustifolia* on adults of *Hyalomma marginatum rufipes* was studied at concentrations of 5, 10 and 20 % v/v. A suitable tick climbing bioassay based on the questing behaviour of ticks was used to test for repellency. High percentage repellency (range 70–100) was shown at all concentrations of the essential oil of *L. angustifolia,* although at 5 % v/v it only persisted for the first 40 minutes compared with 120 minutes at other concentrations (10 and 20 % v/v). The repellent strength of *L. angustifolia* compared well ($P > 0.05$) with that of DEET (N,N-diethyl-*m*-toluamide), a commercial reference repellent, for the 2-hour period of the study.

Key words: DEET, essential oil, *Hyalomma marginatum rufipes, Lavendula angustifolia,* tick repellent.

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INTRODUCTION

Ticks and the diseases they transmit to man and animals continue to be a problem particularly in developing countries[1,6]. The current tick control measures rely largely on the use of synthetic acaricides such as amitraz[11,12] and N,N-diethyl-*m*-toluamide (DEET)[5]. However, the persistence of problems resulting from ticks and the diseases they transmit indicate that these tick control methods are at the most partially effective. In addition, the positive effects of these synthetic chemical products are marred by the problems that accompany their widespread use which include the emergence of tick strains resistant to acaricides[14], the accumulation of toxic substances in the environment and in products destined for human consumption[2,7] and the high cost of these chemicals[15] making them inaccessible by small scale farmers. This situation raises the need for alternative tick control methods which pose lesser problems to the environment, animals and man.

Recently, some plant-based products have been evaluated for tick repellent properties[10,16,18]. The results obtained from these studies are quite promising and as a result present plants as possible sources of anti-tick agents. Furthermore, the recent intensification of research on anti-arthropod properties of plant-based products is motivated by the belief by many researchers[3,19] that plant-based anti-arthropod products are biodegradable and may be affordable for use even by small scale farmers. Most recently, Jaenson *et al.*[8] showed that the essential oil of *Lavendula angustifolia* diluted to 30 % in 1,2-Propanediol had 100 % repellencies against the nymphs of *Ixodes ricinus.* In order to gain additional insight on the repellant strengths of the essential oil of *L. angustifolia* against ticks, the present study compared its tick repellant properties against those of DEET using adults of *Hyalomma marganitum rufipes.*

MATERIALS AND METHODS

Ticks, hydrodistillation and climbing repellency bioassay

Colonies of *Hyalomma marganitum rufipes* used in this study were bred on Himalayan rabbits at the Animal Production unit of the Department of Biology, Medunsa campus of the University of Limpopo. Off-host stages of this tick species were kept at 25 ± 1 °C and 75 ± 5 % relative humidity (RH) in glass humidity chambers under natural day and night regimen.

The extraction of the essential oil of *L. angustifolia,* was done by hydrodistillation. *L. angustifolia* plants were obtained from Plant Land Akasia Malanseuns nursery in Pretoria North. Fresh leaves, soft branches and inflorescences weighing 170 g were sliced into smaller pieces and together with 700 mℓ distilled water were introduced into the round-bottom flask and hydrodistilled for 5 hours using a Clevenger-type apparatus. The apparatus was equipped with a thermometer attached on top of the round-bottom flask. A heat-mantle was used to maintain a temperature of 90 °C. The distillate was collected with the essential oil forming a band above water. The essential oil yield was 0.56 mℓ/170 g × 100 = 0.33 % v/w. This procedure was repeated in order to have more essential oil of *L. angustifolia.* Dichloromethane dilutions (concentrations) of the distillate were prepared by making 2 mℓ of solution with 100, 200 and 400 μℓ quantities of the essential oil in varied amounts of dichloromethane. The following concentrations were consequently obtained: 5, 10 and 20 % v/v. To allow for comparison, similar concentrations were prepared for N,N-diethyl-*m*-toluamide (DEET) (DEET Technical, Volcano Agroscience, 95 %), a commercialised tick repellent. DEET is viewed as the standard ingredient of commercial repellents against which the performance of other compounds can be generally evaluated[13,20].

The climbing repellency bioassay used in this study, was a modification of that described by Carroll[4]. However, the modification was substantial enough necessitating a full re-description. The bioassay is based on the climbing behaviour of ticks. Except for the genus *Amblyomma,* ticks naturally climb up vegetation to quest for a host[17]. A glass vial (height of 7.2 cm and diameter of 2.3 cm) filled with polystyrene was firmly inserted on the centre of the glass beaker. The polystyrene provided support to the vertically inserted glass rod (length 22.1 cm) and also served as a platform on which ticks were placed. Additional polystyrene was used to support the glass vial, preventing it from falling in the glass beaker (see Fig. 1). Water was poured into the beaker to completely surround the glass vial and to almost reach its height. This was done to discourage ticks from crawling away from

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the polystyrene platform and to stabilise humidity[4]. The top 5 cm of the glass rod was covered with Whatman number 1 filter paper (2.5 × 5 cm) on which the plant extract or DEET was released. The second filter paper of the similar kind and size was fixed just below the top one to serve as neutral filter paper. No essential oil of *L. angustifolia* or DEET was released on this filter paper. The neutral filter paper provided an alternative questing place comparable with the treatment filter paper. Similarly, a control was set up save that only an appropriate solvent was released on the top filter paper (Fig. 1). The solvent in both the treatment and control filter papers was allowed to evaporate for 15 minutes before the start of the experiment.

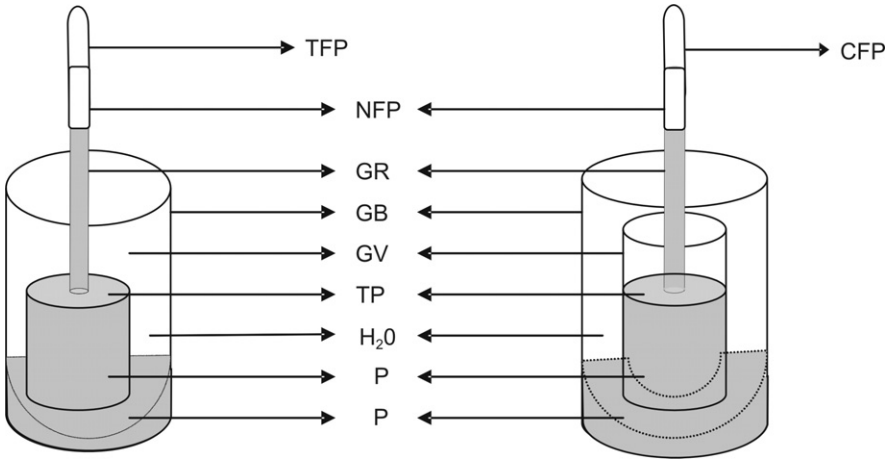

Fig. 1: **Apparatus used to test for repellency of the essential oil of *Lavendula angustifolia* against adults of *Hyalomma marginatum rufipes*.** CFP, control filter paper; TFP, treatment filter paper; NFP, neutral filter paper; GR, glass rod; GB, glass beaker; GV, glass vial; TP, tick platform; H_2O, water; P, polystyrene.

Experimental procedure

Ten unsexed *H. m. rufipes* ticks (3–5 weeks old) were placed on a platform of the treatment apparatus and subsequently the same was done on the control apparatus. Prior to the start of the experiment the ticks were allowed 15-minute acclimatisation period, following which their position on the glass rod was noted at 10-minute intervals for 60 minutes and thereafter at 30-minute intervals until the 120th minute. After each 10-minute interval ticks on the glass rods were moved back to the polystyrene platform.

Ticks on the treatment or control filter paper were considered not repelled while ticks not found on the treatment or control filter paper (*i.e.* those found on the neutral filter paper or glass rod) were considered repelled. Ticks that dropped into surrounding water were dried using Kimberly-Clark paper towel and replaced onto the platform using forceps. Five replications were done for each concentration of the essential oil and DEET. The effective concentration to repel 50 % (EC_{50}) of the ticks was calculated using Probit analysis a free software package (US Environmental Protection Agency; http://www.epa.gov/nerleerd/nerleerd/stat2.htm)[21]. Percentage repellency was calculated using the formula[9]:

Percentage repellency =
100 – [mean number of ticks on test]/[Mean number of ticks on control] × 100.

Mann-Whitney *U*-tests (two-tailed) (using Analyse-it for Microsoft Excel© 1997–2000; Analyse-it Software: http://www.analyse-it.com/order.asp) were used to determine the significance of the differences between treatment and control. Student's *t*-tests were used to calculate the significance of the differences between the repellent effects of DEET and essential oil of *L. angustifolia.*

RESULTS

The results obtained in this study are summarised in Tables 1 and 2. Figure 2 shows that ticks avoided filter paper treated with essential oil of *L. angustifolia.* They settled on the lower neutral filter paper. This is unlike ticks that quested at the top of the control glass rods on the control filter paper. Similar, observations were made for DEET.

High percentage repellency (70 – 100 %) against adults of *H. m. rufipes* was recorded in all 3 concentrations (*i.e.* 5, 10 and 20 % v/v) of *L. angustifolia* used in this study, although for 5 % v/v concentration it only persisted for 40 minutes compared to 120 minutes of the other (10 and 20 % v/v) concentrations (see Tables 1, 2). In general the repellent strength of the essential oil of *L. angustifolia* against adults of *H. m. rufipes* was similar ($P > 0.05$) to that of DEET against the same tick species. EC_{50} could not be calculated at the 10th, 20th and 30th minutes probably because data did not contain at least two concentrations for which the percenge responding is between 0 and 100 % for both DEET and *L. angustifolia.* From the 40th minute to the 120th minute, the EC_{50} generally increased with increasing time for both *L. angustifolia* and DEET.

DISCUSSION AND CONCLUSIONS

The present study describes the repellent properties of the essential oil of *L. angustifolia* diluted in dichloromethane against adults of *H. m. rufipes* ticks. As

Table 1: **Mean percentage repellency of *Lavendula angustifolia* essential oil against *Hyalomma marginatum rufipes*.**

Conc. (v/v %)		**Time** (min)							
		10	**20**	**30**	**40**	**50**	**60**	**90**	**120**
5	Percentage repellency (PR)	96.3	100	97.9	73.2	68.2	50	36.7	30
	Range of PR (Lowest–Highest)	75–100	100	88.9–100	33.3–90	55.5–77.8	40–70	0–70	10–60
	P-value (Treatment and Control)	*	*	*	*	*	*	**	**
10	Percentage repellency (PR)	85.7	92.3	97.7	93.2	89.1	89.1	80.9	85.1
	Range of PR (Lowest–Highest)	57.1–100	71.4–100	87.5–100	80–100	77.8–100	60–100	75–80	66.7–100
	P-value (Treatment and Control)	*	*	*	*	*	*	*	*
20	Percentage repellency (PR)	100	100	100	97.9	100	95.9	92	83.3
	Range of PR (Lowest–Highest)	100	100	100	90–100	100	90–100	80–100	60–100
	P-value (Treatment and Control)	*	*	*	*	*	*	*	*
	$EC_{50(\%)}$	–	–	–	2.508	3.540	3.189	6.491	6.699

*Significant difference between, $P < 0.05$; **no significant difference, $P > 0.05$; – not determined.

Table 2: **Mean percentage repellency of DEET against adults of *Hyalomma marginatum rufipes.***

Conc. (v/v %)		Time (min)							
		10	**20**	**30**	**40**	**50**	**60**	**90**	**120**
5	Percentage repellency (PR)	100	97.4	82.5	85.4	63.6	58.7	48.9	31.3
	Range of PR (lowest–highest)	100	87.5–100	71.4–90	66.7–90	50–75	50–75	10–80	12.5–70
	P-value (treatment and control)	*	*	*	*	*	*	**	**
10	Percentage repellency (PR)	100	100	100	86.1	86.5	86.1	86.1	68.6
	Range of PR (lowest–highest)	100	100	100	66.7–100	66.7–100	60–100	66.7–80	50–100
	P-value (treatment and control)	*	*	*	*	*	*	*	*
20	Percentage repellency (PR)	100	100	100	100	97.2	94.3	85.7	71.4
	Range of PR (lowest–highest)	100	100	100	100	87.5–100	77.8–100	66.7–100	40–90
	P-value (treatment and control)	*	*	*	*	*	*	*	*
	$EC_{50(\%)}$	–	–	–	1.699	4.146	4.747	4.768	8.305

*Significant difference, $P < 0.05$; **no significant difference, $P > 0.05$; – not determined.

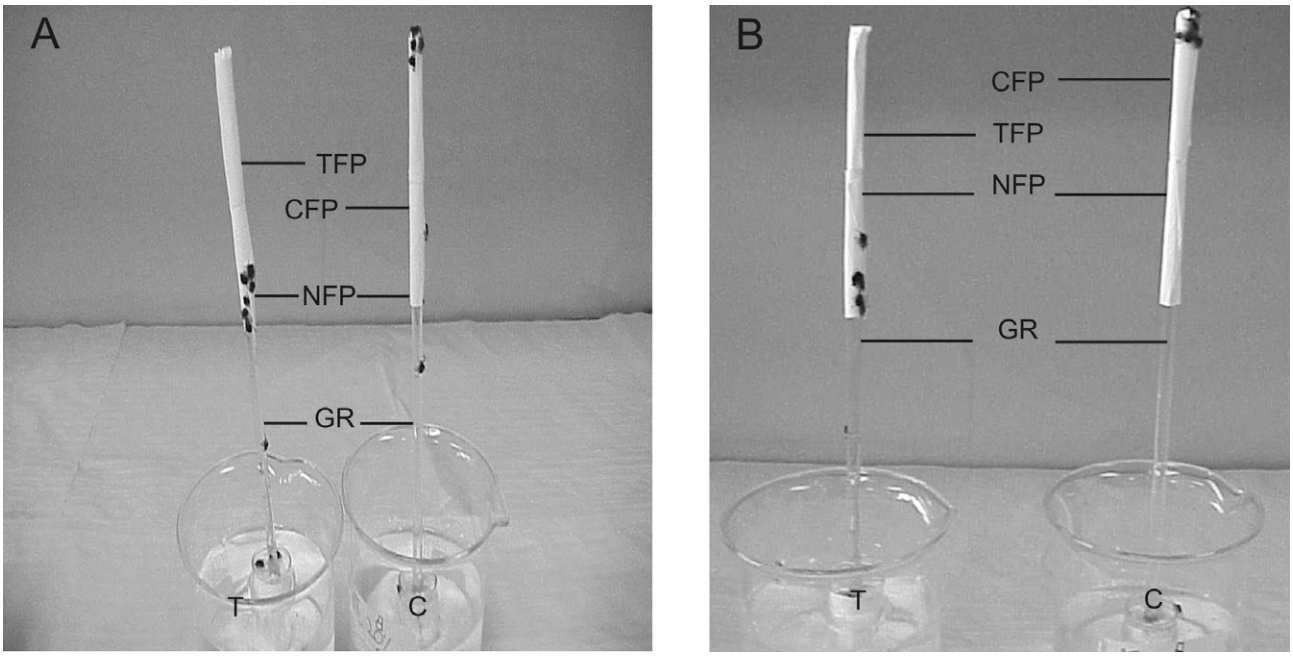

Fig. 2: **Typical example showing questing position of ticks on treatment and control glass rods.** (A) **DEET and** (B) **essential oil of *Lavendula angustifolia.*** TFP, treatment filter paper; CFP, control filter paper; NFP, neutral filter paper; GR, glass rod; T: treatment; C, control.

indicated in our introduction, the recent study by Jaenson *et al.*[8] showed that the essential oil of *L. angustifolia* diluted to 30 % in 1,2-Propanediol had 100 % repellencies against the nymphs of *Ixodes ricinus.* Our results support these findings and further indicate that the repellent effects of the essential oil of *L. angustifolia* are not only specific to *I. ricinus* but are also effective against other tick species such as *H. m. rufipes.* Tick repellence in our study was demonstrated for each of 5 %, 10 % and 20 % concentrations established by diluting the essential oil of *L. angustifolia* in dichloromethane. The repellency appears to be dose-dependent since high repellence persisted for longer periods in higher concentrations than lower once.

Also, data obtained from this study show that the percentage repellency recorded for *L. angustifolia* compared favourably with those obtained for DEET. Previously, Lwande *et al.*[13] also showed that the essential oil of *Gynandropsis gynadra* compared favourably with DEET in repelling *Rhipicephalus appendiculatus* ticks. These findings clearly suggest that some plant species may contain anti-tick agents that may be equally effective against ticks as some of the commercially traded synthetic acaricides. When properly identified and characterised, such plant-based anti-tick agents may enjoy widespread use since it is generally believed that they pose less of a threat to the environment[3,19] than their synthetic counterparts. Furthermore, the use of plant-based products, particularly those from indigenous plants, may save countries the high costs of importing synthetic acaricides[10].

However, in spite of the promising results obtained in this study and elsewhere[8] regarding the anti-tick properties of *L. angustifolia,* more studies are necessary in order to further clarify the anti-tick properties of this plant. Furthermore, toxicity tests on animals should be conducted to determine the side-effects of the extracts of this plant species.

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