International Scholars Journals

International Journal of Microbiology Research and Reviews ISSN 2329-9800 Vol. 9 (6), pp. 001-005, June, 2020. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Repellent properties of *Nicotiana tabacum* and *Eucalyptus globoidea* against adults of *Hyalomma marginatum rufipes*

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Accepted 07 May, 2020

Scientific evaluation of plants for anti-tick properties is a necessary step towards the development of tick control methods that exclude or involve less of synthetic chemicals. In this study, we evaluated the repellent properties of *Nicotiana tabacum* and *Eucalyptus globoidea* against adults of *Hyalomma marganitum rufipes* using a suitable repellency bioassay. Dichloromethane extract of *E. globoidea* had more repellent potency against adults of *H. m. rufipes* compared to ethyle-acetate extract of *N. tabacum* in all three concentrations (20, 30 and 40% w/v) used. The anti-tick repellent strength of dichloromethane extract of *E. globoidea* compared favourably with that of the commercial arthropod repellent, N, N-diethyltoluamide (DEET) and was dose dependent.

Key words: *Nicotiana tabacum, Eucalyptus globoidea*, *Hyalomma marginatum rufipes*, repellency.

INTRODUCTION

The use of plants or plant-based products for the control of arthropod ectoparasites on livestock is widespread among small scale livestock keepers in Africa (Lwande et al., 1999; Kaaya, 2003; Matlebyane et al., 2010). This practice is typically community-based and as a result, the plant species used for such purposes may vary from one community to another. Furthermore, knowledge on such practices is orally transferred from one generation to another and often lacks scientific validation. A number of studies have so far been conducted to validate the use of plants for tick control. For example, most recently, Zorloni et al. (2010) demonstrated that extracts of *Calpurnia aurea* leaves used by the Borana people of northern Kenya and southern Ethopia to treat louse infestations in humans and calves also have anti-tick properties. Similarly, Magano et al. (2008) and Thembo et al. (2010) demonstrated that *Senna italia* subsp. *arachoides*, used by the Batswana people of southern Africa to improve the health of the livestock, has anti-tick properties. Such studies form a necessary precursor that may lead towards the development of community based tick control programmes. Furthermore, scientific identification of plants with anti-arthropod properties is necessary to avoid indiscriminate harvesting of plants that might not be having anti-arthropod properties.

Although a number of plants have been tested for anti-arthropod properties, more plants that are used in different communities in Africa still remain untested. In this study, the repellency of the traditionally used plant products of tobacco (*Nicotiana tabacum*) and *E. globoidea* which are respectively known in Zulu as *Umdlonti* and *Indlulamithi* was evaluated against adults of *H. m. rufipes*.

H. m. rufipes (the coarse bont-legged tick), is widely distributed in South Africa (Horak and Macivor, 1987) and is of medical and veterinary importance. In addition to causing serious wounds on its hosts through its long mouthparts, this tick also transmits Crimean-Congo Haemorrhagic Fever (CCHF) virus to man (Swanepoel et

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Table 1. Plants and the solvents that extracted well.

Scientific name	Local name	Plant part (g)	Organic solvent (Polarity) (ml)
Nicotiana tabacum	Umdlonti (Tobacco)	Leaves (40)	Ethylacetate (mid polar) (100)
Eucalyptus globoidea	Indlulamithi	Leaves (40)	Dichloromethane (non polar)(100)

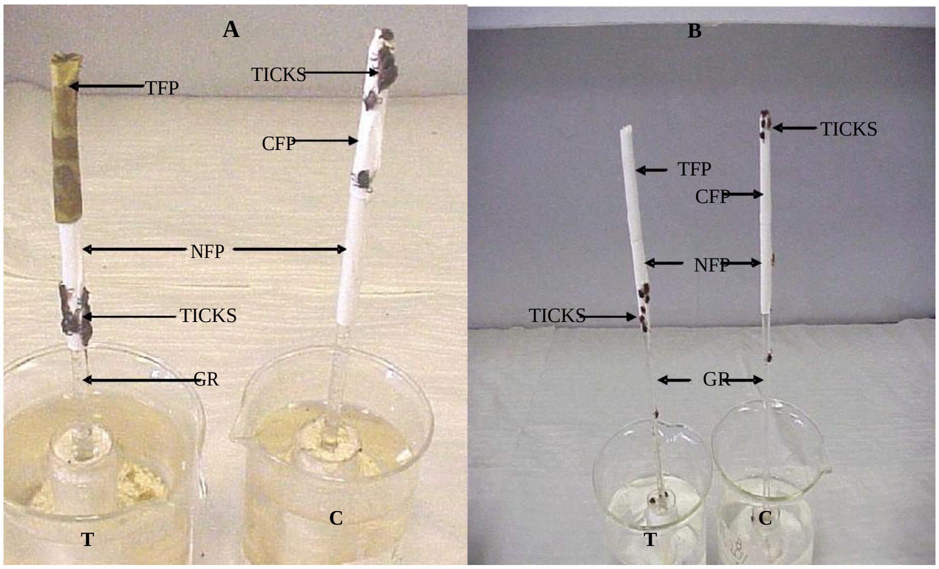

Figure 1: The location of adults*H. m. rufipes*(ticks on treatmentfilter paper, control filter paper, neutralterfi paper and glass **Figure 1.** The location of adults *H. m. rufipes* (ticks on treatment filter paper, control filter paper,
rod) whentesting the repellentefficacy of*Eucalyptusgloboidea*(A) and DEET (B)TFP:treatment. filter paper, CFP: control
neutralfilter paper,filter NFP:paperneutralandfilterglasspaper,GR:rod) glasswhenrod,testingT:treatmenttheandrepellentC:control.efficacy of *Eucalyptus globoidea* (A)
and DEET (B). TFP: treatment filter paper, CFP: control filter paper, NFP: neutral filter paper,
GR: glass rod, T: treatment and C: control.

al., 1983).

MATERIALS AND METHODS

Ticks

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

Selection and preparation of plant extracts

The plant species used in this study, *N. tabacum* and *E. globoidea* were selected based on the information obtained from literature and also personal interviews conducted with traditional practitioners in Utrecht and Pretoria, South Africa.

For each of the three plant species, preliminary extraction was done using methanol, ethyl-acetate and dichloromethane as solvents. The mixture was left for 24 h, following which the extract was filtrated using Whatman No. 1 filter paper. However, only the extracts which extracted well, namely, ethylacetate extract of *N. tabacum* and the dichloromethane extract of *E. globoidea,* were adopted for this study (Table 1). Prior to extraction, each of the plant material was dried and subsequently pounded into powder using ion pestle and mortar for 20 min. The extracts were reconstituted into 20, 30 and 40% w/v concentrations each of which was tested for tick repellency.

Climbing repellency bioassay apparatus

The climbing repellency bioassay and the experimental procedure used in this study are detailed in Mkolo and Magano (2007).

RESULTS

The results obtained in this study are summarized in Tables 2 to 4 and Figure 1. Figure 1A shows a typical observation made for plant extracts that had anti-tick repellent properties. In general, ticks avoided questing on the filter papers treated with repellent plant extracts, thus settling lower on the neutral filter paper. This is unlike the ticks that quested at the top of the control glass rods. Similar, observations were made for DEET (Figure 1B) a commercialized arthropod repellent.

Nicotiana tabacum

Ethyl-acetate extracts of *N. tabacum* at 20 and 30% v/w showed mixed effects of significant and non-significant repellent in the first 40 min (Table 2). However, at 40%

Table 2. Mean percentage repellency of the different concentrations of the ethyl-acetate extract of *N. tabacum* against adults of *H. m. Rufipes.*

Conc. (w/v%)		Time					
		10 min	20 min	30 min	40 min	50 min	60 min
20	Percentage repellency (PR)	88.5	68.96	47.1	52.8	44.9	30.95
	Range of PR (Lowest – Highest)	50 –100	33.3 – 87.5	0 – 85.7	12.5 –100	0 – 87.5	10-67
	P-value	*	*	**	*	**	**
30	Percentage repellency (PR)	72.7	50	53	59.3	53.8	64.7
	Range of PR (Lowest – Highest)	40– 100	25 –100	40 –83.3	40-80	37.5 –80	56- 75
	P-value	*	**	**	*	*	*
40	Percentage repellency (PR)	95.7	92.6	75	58.3	50.9	
	Range of PR (Lowest – Highest)	66.7–100	66.7 –100	40 –100	33.3–100	20-70	0–60
	P-value	*	*	*	*	**	**
	EC_{50} (%)	12.675	16.934	24.023	17.915	29.511	-

*Significant difference, P<0.05; ** No significant difference, P>0.05; - not determined.

v/w the extract showed significant ($p < 0.05$) repellent effects for the first 40 min. Percentage repellency declined with an increase with time up to the 60th min (Table 2). Except for the EC_{50} at the 40th min, this parameter generally increased with an increase in time up to the 50th min. EC_{50} could not be calculated for the 60th min probably due to the low percentage repellencies (that is, 30.95 for 20% v/w and 33.3 for 40% v/w) recorded at this interval (Table 2).

Eucalyptus globoidea

Except for the non-significant repellent effect recorded at the 60th min of the 20% v/w, DCM extract of *E. globoidea*, significant repellent effects against adults of *H. m. rufipes* were recorded in all the tests up to the 120th min (Table 3). The EC_{50} was the lowest (10.50 v/w) at the 30th min and the highest (22.13 v/w) at the 60th min. Except for the records at the 90th and 120th min, the EC_{50} generally increased with increasing time (Table 3).

DEET

The tick repellent potency of DEET at 5% v/v lasted for one hour. However, at higher concentrations, that is at 10 and 20% v/v it lasted at least for two hours (Table 4).

DISCUSSION

This study describes the repellency of the crude extracts of *N. tabacum*, and *E. globoidea* against adults of *H. m. rufipes* ticks. Between these two plant species, it is the dichloromethane extract of *E. globoidea* that showed stronger tick repellence in all three concentrations (20, 30 and 40% w/v), a result comparable to that of DEET, the commercial arthropod repellent. The effectiveness of the extract was dose dependent as evidenced by an increase in percentage repellency as the concentration increased from 20 to 40% w/v. The strong repellency properties observed with *E. globoidea* in this study appear to be shared with other members of the genus *Eucalyptus*. Most recently, Sengottayan (2007) showed that the leaf extract of *E. tereticornis* have strong insecticidal properties against immature and mature stages of *Anopheles stephensi*.

Although tobacco (*N. tabacum*) is traditionally

Table 3. Mean percentage repellency of the different concentrations of the dichloromethane extract of *E. globoidea* against adults of *H. m. Rufipes.*

Conc. (w/v%)		Time							
		10 min	**20 min**	**30 min**	**40 min**	**50 min**	**60 min**	**90 min**	**120 min**
20	Percentage repellency (PR)	86.8	72.2	76.1	61	55	40	64.5	59.6
	Range of PR (Lowest – Highest)	80-100	40-100	30-100	20-100	44.4 –100	0–80	33.3- 100	22.2-100
	P-value	*	*	*	*	*	**	*	*
30	Percentage repellency (PR)	86	93.1	91.1	91.8	89.8	79.0	78.7	63.3
	Range of PR (Lowest – Highest)	42.8- 100	80- 100	66.7- 100	80- 100	66.7- 100	60- 100	70- 80	50- 100
	P-value	*	*	*	*	*	*	*	*
40	Percentage repellency (PR)	94.3	93	100	97.9	91.8			
	Range of PR (Lowest – Highest)	83.3- 100	71.4- 100	100-100	77.8- 100	90- 100	40-100	80- 100	55.6-100
	P-value	*	*	*	*	*	*	*	*
	EC_{50} (%)	10.504	11.842	13.582	18.029	18.487	22.130	19.110	

*Significant difference, P<0.05; ** No significant difference, P>0.05.

Table 4. Mean percentage repellency of DEET against adults of *H. m. Rufipes.*

Conc. (w/v%)		Time							
		10 min	**20 min**	**30 min**	**40 min**	**50 min**	**60 min**	**90 min**	**120 min**
5	Percentage repellency (PR)	100	97.4	82.5	85.4	63.6	58.7	48.9	31.3
	Range of PR (Lowest – Highest)	100-100	87.5-100	71.4-90	66.7-90	50–75	50–75	10-80	12.5-70
	P-value	*	*	*	*	*	*	**	**
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- 100	100- 100	66.7- 100	66.7- 100	60- 100	66.7- 80	50- 100
	P-value	*	*	*	*	*	*	*	*
20	Percentage repellency (PR)	100	100	100	100	97.2			
	Range of PR (Lowest – Highest)	100- 100	100- 100	100- 100	100- 100	87.5- 100	77.8-100	66.7- 100	40-90
	P-value	*	*	*	*	*	*	*	*
	EC_{50} (%)	-	-	-	1.699	5.045	4.747	4.768	8.305

*Significant difference, P<0.05; ** No significant difference, P>0.05; - not determined.

known as a natural arthropocide (Jacobson and Crosby, 1971), in the present study ethyl-acetate extracts thereof showed a significant degree of tick repellence for the first 40 min only at the 40% w/v concentration. Lower concentrations appeared to have been less effective, presenting a mixture of both significant and non-significant results in the first 40 min of the test. Compared to the dichloromethane extract of *E. globoidea*, the strength of ethyl-acetate extract of *N. tabacum* to repel adults *H. m. rufipes* was lesser. However, the potency of tobacco against ticks may be accentuated when it is mixed with other substances. For example, in Kenya, dried tobacco leaves are mixed with a mineral called "Magadi soda" to form Kupetaba, a commercialised anti-tick product (Dipeolu and Ndungu, 1991).

In summary, the results of this study, further strengthen the widely held view that plant products can be used as an alternative to synthetic repellents or along with other synthetic repellents under the integrated management system. However, the shortfall in the progress made so far is that most studies are based on *in vitro* laboratory models. Future studies must therefore focus on field trials to validate laboratory derived evidence.

ACKNOWLEDGEMENTS

Our appreciation and thanks go to the traditional practitioners who generously shared their knowledge with us. We also thank the University of Limpopo (Medunsa campus) for providing space and the National Research Foundation for funding the research.

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