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A comparative assessment of the acaricidal activity of *Tephrosia vogelii* on *Rhipicephalus appendiculatus* and *Amblyomma variegatum* in Makoni district, Manicaland Province, Zimbabwe

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Abstract
This study sought to assess the effectiveness of using *Tephrosia vogelii* leaf extract as an acaricide on two tick species *Rhipicephalus appendiculatus* and *Amblyomma variegatum* which are the most prevalent tick species affecting cattle in Makoni District of Zimbabwe. The number of ticks of each of two species, *Rhipicephalus appendiculatus* and *Amblyomma variegatum* were initially counted on each of the twelve cattle before spraying with alcohol and water-based leaf extracts. The number of ticks remaining on each animal after spraying were counted for five days and percentage mortality determined. There was a significant difference in mortality for *R. appendiculatus* and *A. variegatum* (p<0,05) in both alcohol-based leaf extracts and water-based extracts. The study showed that there was higher mortality on *A. variegatum* than *R. appendiculatus* for both extracts this study has shown that plant leaf extracts of *T. vogelii* have significant acaricidal activity against *R. appendiculatus* and *A. variegatum* ticks and can thus be considered as alternatives for tick control.

Keywords: ticks, acaricide, *Tephrosia vogelii, Rhipicephalus appendiculatus, Amblyomma variegatum*

Introduction
One of the major health concerns affecting livestock are ecto-parasites, particularly ticks. Ticks adversely affect animal health and impede development of the livestock industry which forms the major mainstay of the economy in most rural areas. Norval *et al*., (1992) [1] report that, tick-borne diseases (TBDs) can result in severe economic losses due to hide damage, weight loss, milk production reduction, and death of livestock. Heartwater affects ruminants, cattle in particular, and can have high death rate in susceptible animals [2]. Jongejan and Uileuberg (2004) [3] further explain that, indirectly and more importantly, ticks also act as vectors of fatal diseases, for example babesiosis and theileriosis. At least 80% of the world's cattle population are at risk from TBDs [4]. According to Schroeder, (2013) [5], ticks affect cattle directly by causing skin damage, opening up wounds which make the animal susceptible to secondary infection, and cause toxicosis and paralysis, In some instances there is higher reduction of livelihoods, mainly due to TBDs on resource-constrained communal farmers in developing countries of sub-Saharan Africa (SSA), Asia and Latin America [6]. Perry, (2005) [7] identifies, TBDs as one of the most important health and management challenges in Africa ahead of tsetse fly and trypanosomiasis.

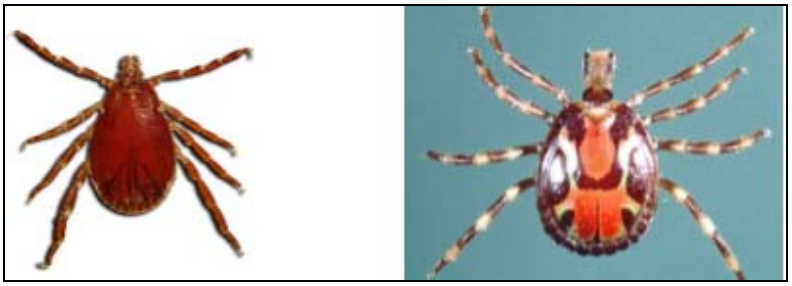
Communal farmers in Zimbabwe regularly dip their cattle using commercial acaricides. The Government of Zimbabwe, through its Department of Veterinary Services is involved in the management and control of ticks where farmers are required to take their cattle to dip tanks once every week in summer and once every two weeks in winter and spring. The dip tanks use synthetic acaricides to kill ticks. Acaricidal compounds that are currently used in tick control for livestock in Zimbabwe are very expensive most farmers are resource-challenged because of economic hardships.

Concerns have been raised on the use of synthetic acaricides in tick control. Some problems like environmental contamination, residues in food and feed, and development of acaricide resistance in ticks have been reported. For example, Lane & Crosskey (1996) [8] has documented tick resistance to chemical acaricides in more than 440 species of insects. There are strains of ticks that have been observed to be resistant to arsenic, a wide range of organophosphates, carbonates, amidines and synthetic pyrethroids [9]. This, therefore, calls for

the need to come up with solutions that are cost effective to the resource-constrained communal farmers.

There is a keen interest in the development of alternative eco-friendly anti-tick natural products [10]. Amongst the natural products, some plant extracts such as *Jatropha curcas* [11], *Cassia fistula* [12] and *Ricinus communis* [10] have been shown to have significant activity against economically important tick species including those which are acaricide resistant [10]. The fish poison bean, *Tephrosia vogelii*, is a leguminous plant that grows wild in much of sub-Saharan Africa. It has been used by Samburu and Maasai pastoralists in Kenya to rid their livestock of ticks [13]. The efficacy of *T. vogelii* has been demonstrated in Zimbabwe at commercial level on dairy cattle, but has not considered for the local communal breeds of cattle hence the need for this research. The main purpose of this study was to evaluate the effectiveness of *T. vogelii* extracts in the control of ticks on indigenous Mashona breeds of cattle in Zimbabwe's Eastern Highlands district of Makoni.

Fig 1: Photograph of *T. vogelii* plant (photo by Cephas Madoma)



(*Source*: http://www.alanrwalker.com/assets/PDF/tickguide-africa.pdf.)

Fig 2: Photos of (a) *Rhipicephalus appendiculatus* and (b) *Amblyomma variegatum*.

Materials and methods

Study area

The study area was St Benedict's, situated in Makoni District (Fig 3.) of Manicaland province. The area is situated 146km South East of the capital Harare, Zimbabwe at an altitude of 800 to 1219 m above sea level, 18^0 40' S latitude and $32^0$16′ E longitude. St Benedict's is found in agro- ecological zone II. It is characterised by an average annual rainfall between 600mm to 800mm and a mean annual temperature of 27 ^{0}C. Maximum rainfall being experienced between November and March and is sufficient for both crop and livestock production. The land is surrounded by the mountains and uncultivated lowlands and cover with grassland dominated with *Panicum* species shrubs and some leguminous plants. The major agricultural enterprises in the area are crop and animal production.

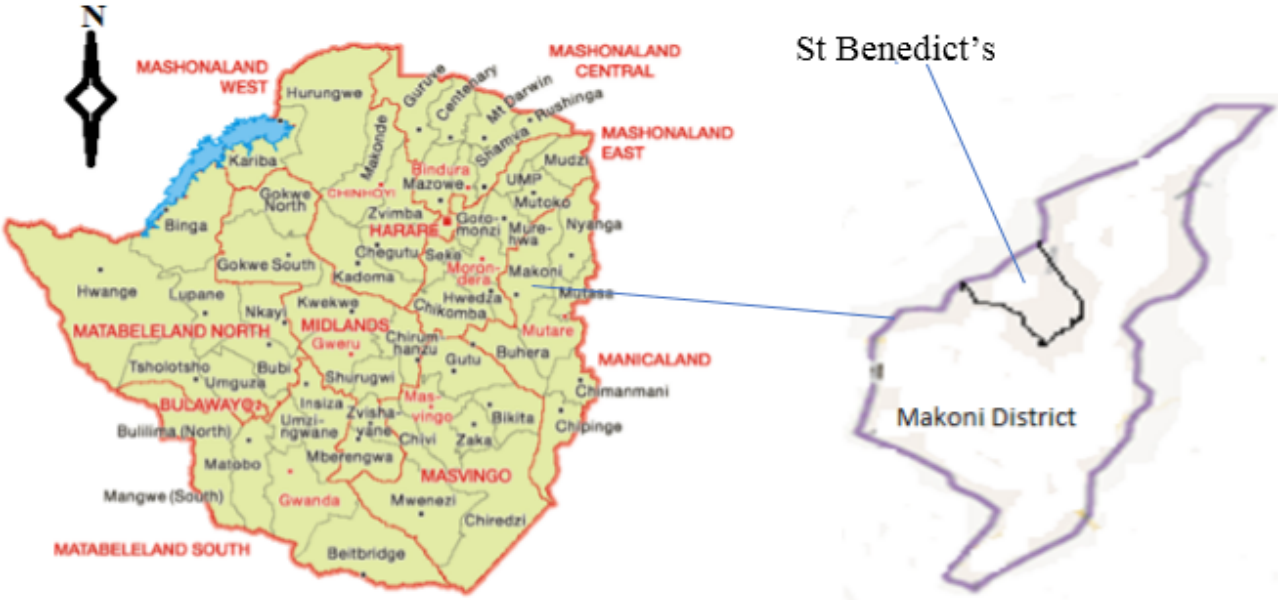


Fig 3. Map showing the study area St Benedicts in Makoni District

Identification of tick species infesting cattle

An initial survey was conducted to establish two dominant tick species infesting cattle in the area. Two tick species were identified in consultation with the Department of Veterinary Services in the District in March 2017. The two tick species were *Rhipicephalus appendiculatus* and *Amblyomma variegatum*.

Determination of sample size

To determine the sample size of ticks to be used during the study, an average tick load of each of the two-tick species on each animal was determined through a physical count of twelve randomly selected cattle to determine the number of ticks on each animal.

Extraction of *T. vogelii* extracts.

Mature leaves were plucked in the first week of February 2017 and then weighed up to 50grams. Plant leaves were first air dried, then re-weighed to give a mass of 50grams and ground to powder. During preparation of extracts, each 50gram of sample the dried plant leaves were soaked in 100ml of cold water and ethanol respectively then left for an hour to allow the active ingredients to be exuded into the solvents. At the end of soaking period, the extracts were filtered using a sieve to remove the large pieces of leaf material and the filtrates were collected.

Application of extracts on the ticks

The acaricidal activity of extracts and fractions from leaves of *Tephrosia vogelii* was carried out on cattle ticks. The leaf extracts were applied direct on affected areas on cattle using two litre spray guns. The number of ticks of each species per animal were counted from day one up to day five.

Data analysis

Data analysis was done using MINITAB software. The comparison of the mortality rates between the tick species was made by means of a t-test for both alcohol and water-based leaf extracts. Analysis of variance (ANOVA) was carried out at 5% level of significance to test for any significant differences among extracts for the two-tick species.

Results

The raw data for the study is summarised in Table 1 and Table 2. The average mortality for *R. appendiculatus* for the water-based extract was 32.16 % and for *A. variegatum* 71.67%, while the average mortality for the alcohol-based extract was 51.67% for *R. appendiculatus* and 91.16% for *A. variegatum* (Table 3).

Table 1: Tick counts of *R. appendiculatus* and *A. variegatum* after spraying cattle with water based *T. vogelii* extract.

Animal number	Counting intervals/days	Tick count for *R. appendiculatus.*	Tick count for *A. variegatum.*
	0	17	13
	1	17	10
1	2	17	8
	3	15	8
	4	14	4
	5	12	3
% mortality		29%	77%
	0	22	16
	1	21	14
2	2	21	14
	3	19	11
	4	18	9
	5	17	6
% mortality		23%	63%
	0	12	13
3	1	10	10
	2	10	8
	3	9	6
	4	9	5
	5	7	2
% mortality		42%	77%
	0	33	21
	1	33	19
4	2	33	15
	3	32	12
	4	31	7
	5	31	5
% mortality		6%	76%
	0	21	27
	1	20	26
5	2	18	22
	3	17	19
	4	16	16
	5	14	14
% mortality		30%	46%
	0	8	11
	1	8	9

6	2	7	6
	3	4	5
	4	4	3
	5	3	1
% mortality		63%	91%

Table 2: Tick counts of *R. appendiculatus* and *A. variegatum* after spraying cattle with alcohol-based *T. vogelii* extract.

Number of animals.	Counting intervals/days	Tick count for *R. appendiculatus.*	Tick count for *A. variegatum.*
	1	15	15
	2	14	11
1	3	12	8
	4	11	4
	5	9	2
% mortality		40%	89%
	0	9	7
	1	8	1
2	2	8	3
	3	6	1
	4	4	1
	5	3	1
% mortality		67%	100%
	0	13	8
	1	11	2
3	2	9	2
	3	9	2
	4	8	0
	5	6	0
% mortality		54%	75%
	0	9	6
	1	9	5
4	2	9	2
	3	7	1
	4	5	0
	5	4	0
% mortality		56%	100%
	0	16	9
	1	15	5
5	2	14	4
	3	14	2
	4	12	1
	5	9	0
% mortality		43%	100%
	0	14	6
	1	12	6
6	2	10	3
	3	9	2
	4	7	1
	5	7	1
% mortality		50%	83%

Table 3: Average mortality rates for water and alcohol-based extracts for *R. appendiculatus* and *A. variegatum.*

Tick Species	Average Mortality (%)		
	Water Based Extract	Alcohol Based Extract	Overall Mortality
R. appendiculatus	32.16	51.67	41.95
A. variegatum	71.67	91.16	81.35

Results of the two-sample t-test showed that there was a significant difference at 5% level of significance between the mortality for *R. appendiculatus* and *A. variegatum* ($P<0.05$). Generally, *T. vogelii* extracts were more effective against *A. variegatum* than *R. appendiculatus*. This is evidenced by the high average mortality of 81.35% in *A. variegatum* as compared to 41.95% in *R. appendiculatus* (Table 3).

Further comparisons within each species for the two extracts (treatment) were carried out to check for significant differences at 5% level of significance. Significant differences among the extracts for *R. appendiculatus* and *A. variegatum* were observed as shown in Table 4 and Table 5 respectively. Generally, the alcohol-based extract is more effective on both *R. appendiculatus* and *A. variegatum*.

Table 4: One-way ANOVA for *R. appendiculatus* mortality versus extracts

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F-value	P-value
Extract	1	1141	1141	4.96	0.05
Error	10	2304	230.4		
Total	11	3445			

Table 5: One-way ANOVA for *A. variegatum* mortality versus extracts

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F-value	P-value
Extract	1	1141	1141	6.52	0.029
Error	10	1750	175		
Total	11	2891			

Discussion

Generally, the results showed that *T. vogelii* is effective as an ethno-veterinary acaricide against both *R. appendiculatus* and *A. variegatum,* with *A. variegatum* being the most susceptible species. These results are similar to those obtained by Kalume *et al*., (2012) [14] who observed a mortality of 95% and 100% using concentrations of 10 and 20 mg/ml of leaves of two varieties of *Tephrosia vogelii* against *R. appendiculatus*. A comparative assessment of the effect of ethanolic extract of *T. vogelii* and synthetic acaricide on *A. variegatum* was also carried out by Dougnon *et al*., (2014) [15] on Borgou cattle in Benin using 50g of leaf powder per 500 ml of ethanol. They reported a high mortality of *A. variegatum.* Gadzirayi *et al*., (2009) [16] also reported similar results using various concentrations ranging from 50 to 100 g of leaves of *T. vogelii.* Therefore, the use of *Tephrosia* leaves' extract as a low cost acaricide in Zimbabwe is very encouraging, considering the high cost of commercially manufactured acaricide and their undesirable environmental effects and unacceptable risks to non-target organisms. Moreover *T. vogelii* as a biopesticide is particularly attractive on the grounds of its low toxicity to mammals and is easily biodegradable [17].

The ethanolic leaf extract of *T. vogelii* exhibited a higher level of mortality on both *R. appendiculatus* and *A. variegatum.* Matovu and Olila, (2007) [18] have indicated that extracts of *Tephrosia vogelii* with chloroform, methanol (alcohol), petroleum ether and water can be effectively used in the field to control all genera of ticks. Uddin and Khanna, (1979) [19] and Dougnon *et al*., (2014) [15] explain that the acaricidal activity is probably due to the presence of the retinoid compounds, rotenone and deguelin contained in *T. vogelii* plant. According to Kalume *et al*., (2012) [14], the contents in the two main rotenoid compounds identified in the plant namely rotenone and deguelin differ markedly as demonstrated by HPLC analysis with the amount of deguelin higher when compared with rotenone. Kalume *et al*., (2012) [14] further explain that the acaricide activity of *T. vogelii* could be due to the high content of deguelin. However, the exact mode of action of deguelin on tick remains poorly understood and further studies on its mode of action need to be investigated. Matovu and Olila, (2007) [18] have also reported also that leaves of *T. vogelii* accumulate relatively high amounts of the active ingredients compared to the roots. This may explain why leaves should be preferred for effective pest control. In addition, there is need to maintain the life of the plant and thus ensure a sustainable harvest. *T.vogelii* will produce good results only if mixed with organic solvents which allow maximum exudation of the rotenone and deguelin. In cold water the active ingredients are almost incompletely dissolved except if the mixture is left standing for some days to exude.

A. variegatum was the most affected species than the *R. appendiculatus* because it is more sensitive to *T. vogelii* than the *R. appendiculatus* and it is located everywhere on cattle while *R. appendiculatus* mostly favours in the ears where the *T. vogelii* leaf extracts were difficult to reach. The anatomy of *R. appendiculatus* was partially insensitive to *T. vogelii* leaf extracts. There are many species of ticks and some of them completely resistant to the leaf extracts for example *R. microplus.* The acaricide resistance in ticks was conferred primarily by two major physiological mechanisms, target site insensitivity and metabolic detoxification [20]. Higher tick mortality may also have been contributed by immaturity of ticks. *A. variegatum* was the most affected species may be because of its soft scutum (dorsal shield).

Conclusion

Based on the results of the study, we can conclude that the use of *T. vogelii* leaf extract as a low-cost acaricide has produced impressive results and therefore its application by communal farmers in Zimbabwe should be encouraged. *T. vogelii* generally is user friendly because it causes no harm to the user and causes no threats to the environment. Moreover *T. vogelii* is a legume whose cultivation will enhance soil fertility. Farmers could use alcohol-based extracts for them to achieve best results.

Further research could also be done to determine the chemical composition of the ethnoveterinary acaricides since this can also be a contributing factor to the effectiveness of the acaricides. These acaricides can be affected by temperatures and others can be quickly removed by rain water.

Only two species of ticks were investigated but there are many types of ticks which need to be investigated also. This study should be carried in other districts or areas of Zimbabwe to establish if the same pattern observed is also emerging.

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