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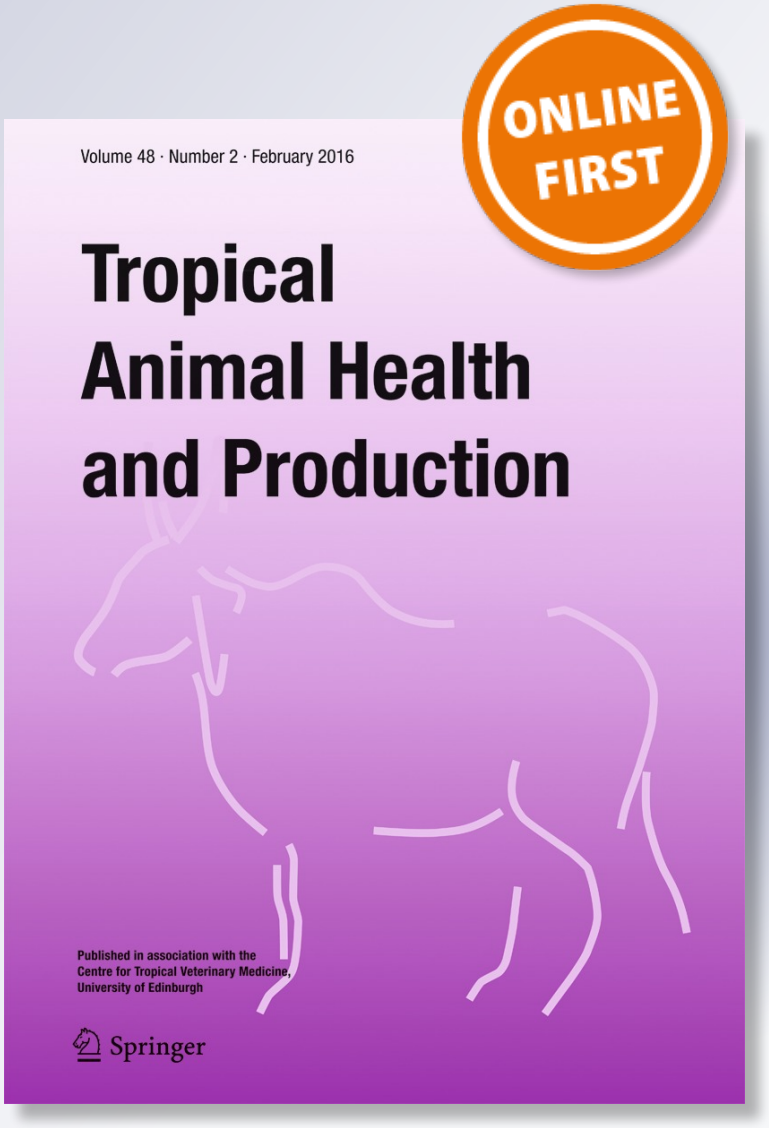

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REGULAR ARTICLES

In vitro acaricidal activity of *Bobgunnia madagascariensis* Desv. against *Amblyomma variegatum* (Fabricius) (Acari: Ixodidae)

Jackson Muyobela[1,2] · **Philip Obed Yobe Nkunika**[2] · **Enala Tembo Mwase**[3]

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Abstract The objective of the study was to determine the acaricidal properties of *Bobgunnia madagascariensis* (Desv.) J.H. Kirkbr. and Wiersema (Leguminosae) against adult *Amblyomma variegatum* (Fabricius) ticks, using *Tephrosia vogelii* Hook.f. (Leguminosae) as a positive control. Plant extracts of both were prepared using methanol, acetone and chloroform as extraction solvents. Methanol leaf extracts of *T. vogelii* (0.014 g) and methanol fruit extracts of *B. madagascariensis* (0.0062 g) gave the highest mean extraction weights among the plant parts and solvents used. In free contact bioassays, only methanol extracts of the bark and leaf material of *T. vogelii* and methanol fruit extracts of *B. madagascariensis* produced 100 % mortality of *A. variegatum* ticks in 24 h. The acaricidal activity of methanol leaf extracts of *T. vogelii* persisted for up to 8 days while that of fruit extracts of *B. madagascariensis* persisted for only 6 days. In topical application bioassays, the toxicity of *T. vogelii* and *B. madagascariensis* extracts was found to be significantly different at 95 % confidence level, with *B. madagascariensis* extracts (LD_{50} 0.030 *w/v*) being more toxic than *T. vogelii* extracts (LD_{50} 0.555 *w/v*). This study has shown that plant extracts of *B. madagascariensis* and *T. vogelii* extracts have significant in vitro acaricidal activity against *A. variegatum* ticks and can thus be considered as alternatives for tick control. Further research is however required on persistence, safety and the required application rates.

Keywords Plant extracts · Free contact bioassay · Topical application bioassay · Tick control · Mortality

Introduction

Amblyomma variegatum (Fabricious, 1794), commonly known as the tropical bont tick, is the commonest and most widely distributed livestock tick in Africa (Walker et al. 2007). It is a three-host tick which transmits the causative agent of ehrlichiosis or heart water to cattle, sheep and goats (Esemu et al. 2013) and is associated with dermatophilosis (Walker, et al. 2007). Indigenous breeds of cattle have generally developed enzootic stability to heart water and are relatively resistant to dermatophilosis. Therefore, the economic impact of this tick is largely due to the actual feeding habit and the serious wounds it causes (Stachurski and Lancelot 2006).

Several studies have demonstrated that there is a positive correlation between calf mortality and teat damage caused by *A. variegatum* (Stachurski 2000 and Mashebe et al. 2014). Formation of scar tissue on teats as a result of feeding by adult *A. variegatum* ticks can result in complete destruction of one or more teats, which leads to a significant reduction in suckling efficiency of calves (Walker et al. 2007). As a result, utilization of milk from dams is inefficient which consequently lowers growth rates and significantly increases calf mortality (Uilenberg 1992). Other significant economic damage accredited to this tick

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is reduction in live weight gain. Pegram and Oosterwijk (1990) estimated a reduction of 48 to 63 g for each engorging female *A. variegatum* tick in central Zambia. It has also been shown that for every ten engorging females of this tick, there is a decrease in live weight gain of 20 kg over a period of 3 months (Pegram et al. 2000). Therefore, *A. variegatum* significantly affects the production and productivity of cattle, especially among resource poor smallholder cattle farmers, hence the need to control this tick.

Currently, the use of synthetic chemical acaricides applied in dips and strays remains the cornerstone of tick control in much of the developing world (George et al. 2004 and Abbas et al. 2014). However, the majority of resource poor smallholder cattle farmers consider the application of chemical acaricides to cattle to be too expensive (Madzimure et al. 2013), which frequently leads to the misuse of products. Inadequate spray volume application, excessive between-treatment intervals and replacement of acaricides with agricultural pesticides are some of the most common examples of acaricide misuse (Bianchi et al. 2003 and Muyobela et al. 2015). Other detrimental effects of the use of chemical acaricides include environmental pollution (Magano et al. 2008), demise of non-target organisms (Habeeb 2010) and resistance to development (Abbas et al. 2014). Consequently, there is a considerable amount of research currently underway to find alternative sustainable and cost-effective methods to control cattle ticks (Opiro et al. 2010).

One alternative to chemical tick control is the use of botanical pesticides (Babar et al. 2012 and Zaman et al. 2012). The opportunity to use local, readily available acaricidal plants would be desirable among resource poor smallholder farmers, as they provide a cheaper and safer alternative to synthetic chemical acaricides (Madzimure et al. 2013). As such, several plants have been demonstrated to have significant acaricidal properties (Habeeb 2010; Opiro et al. 2010; Madzimure et al. 2011, 2013). It appears that the acaricidal properties of *Bobgunnia madagascariensis* (Desv.) J.H. Kirkbr. and Wiersema (Leguminosae) have not been investigated, hence the need for this study.

B. madagascariensis, commonly known as the snake bean plant, is widely distributed in Africa (Adedote et al. 2011). It is a wild leguminous tree that is wide spread throughout the Miombo woodland areas of Zambia. This plant has been shown to have significant molluscicidal activity on snail hosts of schistosomiasis (Borel and Hostettmann 1987), antifeedant and contact toxicity against the storage pest *Tribolium casteneum* (Herbst, 1797) (Adeyemi and Amupitan 2010) and larvicidal activity against the mosquito *Culex quinquefasciatus* Say (Adedote et al. 2011). Therefore, the objective of this study was to determine the acaricidal properties of *B. madagascariensis* against adult *A. variegatum* ticks, in an attempt to contribute to the scientific knowledge of plants with acaricidal properties.

Materials and methods

The plant material

B. madagascariensis occurs as small trees or shrubs in tropical and southern Africa. It is about 12-m tall, and its outer bark is dark grey and rough with long narrow cracks. It occurs often on sandy or clay-loam soils in valleys and flood plains in altitudes ranging from 150 to 1750 m. *Tephrosia vogelii* Hook.f. (Leguminosae) was used as a positive control in this study. This was because its acaricidal properties have been established by several researchers (Kaposhi 1992 and Matovu and Olila 2007) and have been found to be comparable to Triatix dip (amitraz) (Gadzirayi et al. 2009). *T. vogelii* is found in widely varying habitats, including savannah-like vegetation, grasslands, forest margins, shrub lands and fallow fields. It occurs in climates with annual rainfall of 850–2650 mm, mean temperature 15.5–26.2 °C and up to an altitude of 2100 m.

Collection of plant material and pre-extraction procedure

Plant samples of *T. vogelii* and *B. madagascriensis* were collected by chopping off 30 cm branch ends with fruits from Longwe agricultural camp, located in Isoka District Zambia, in March 2012. This camp was chosen due to the availability of sufficient plant material and easy access to laboratory facilities. Both plant materials were brought to Isoka District Regional Veterinary laboratory for testing. Materials collected were bark, leaves and fruits. The plant material was then chopped into small pieces and was air dried at room temperature in a shade as described by Matovu and Olila (2007).

Preparation of crude plant extracts

The dried plants of each plant part were separately crushed into finer particles. The particles were then weighed into 5, 10 and 15 g portions which were placed in separate beakers. For each weight category of plant material, three different extracts were prepared after addition of 50 ml of each of the following solvents, methanol, chloroform, and acetone, to give crude mixtures. These mixtures were then allowed to stand overnight prior to filtration. Ten (10) ml of the supernatant from each beaker was filtered out using Whatman no.1 filter paper into separate vials of known weights. For each solvent, a control was prepared by adding the same amount of solvent in each control vial. Solvents in both treatments and controls were then allowed to evaporate completely overnight in a fume chamber. After evaporation of the solvents, thin layers of residues were obtained in treatment vials. The weight of these residues was then determined by subtracting the weight of

empty vials with vials with residues. For replications were prepared for each weight category of crude plant mixture. Plant extracts were prepared at room temperature (22 °C) and at atmospheric pressure.

Collection of ticks

Adult ticks of the species *A. variegatum* were collected from Kantenshya agricultural camp from later March 2012 to late April 2012. This was done in a crush pen mainly from mid and rear sections of cattle from a selected kraal. Ticks collected included adult *A. variegatum* male and female ticks encountered. All adult male ticks encountered were collected but only females that had not significantly fed (6 to 7 mm in size) were collected. Collected ticks were placed in perforated plastic containers with freshly cut grass for providing moisture. Species identification and sex differentiation of ticks were done using the pictorial guide provided by Walker et al. (2007). Engorged females were not collected for this study as their larger size as compared to males produces data that does not follow the probit model when males are included in the analysis (Robertson et al. 2007). To solve this problem, other researchers tend to use larvae of uniform known age to evaluate response of ticks to toxicants. However, for plant-based acaricides to be useful, they must show significant acaricidal activity against adult ticks, as this stage is harder to kill and is also the initiator of tick control in resource poor smallholder cattle farming systems. Tick collection was done at every stage of the bioassays as required.

Bioassay procedures

Plant crude extracts were then tested for their anti-tick properties against adult *A. variegatum* using the free contact and topical application method. These bioassays have been successfully used by other researchers (Magano et al. 2008) in screening plants for anti-tick properties. All bioassays were conducted at 25 ± 1 °C and a relative humidity of 60 %.

Free contact

Ten adult ticks (five males and five females) were introduced into each vial of the treatment and control groups. Vials were then closed with a mesh and rubber bands to prevent ticks from crawling out. Tick mortality was then recorded every after 24 h for 8 days. In order to assess the stability of the potent extracts (i.e. the extract that kills all ticks in 24 h), dead ticks were replaced by live ones. Extracts for topical application procedure were selected after 24 h.

Topical application

Only extracts that exhibited mortality in 24 h were used in this bioassay. Preparation was done as in the free contact assay. However, for each treatment, 10 ml of each solution was filtered out and the solvent allowed to evaporate completely. The residues obtained were weighed after which olive oil (Magano et al. 2008) was added in order to obtain three different concentrations (Table 1). Each mixture was stirred gently for 3 min and left to stand overnight to ensure homogeneity. Live ticks were divided into treatment and controls, each with ten ticks. For each treatment group, 10 μl of the crude plant extract was topically applied onto the dorsal surface of the idiosoma using a micropipette. A similar procedure was carried out for control ticks using oil only (control). Four replications were done for each concentration. Tick mortality was recorded after 24 h.

Statistical analysis

Mortality in bioassays was calculated using the formula $Mortality\ (\%) = \left(\frac{Dead\ Tick\ Count}{Total\ tick\ Count}\right) \times 100$ for each replicate. In free contact bioassay, mean mortality for control and

Table 1 Test concentrations of *B. madagascariensis* methanol fruit extracts and *T. vogelii* methanol leaf extracts for topical application bioassay

Species	Plant part	Crude extract weight category (g)	Weight of extract obtained (g)	Volume of olive oil added (ml)	Concentration of residues obtained (*w*/*v*)
B. madagascariensis	Fruit	5	0.035	2.5	0.014
		10	0.0651	2.1	0.031
		15	0.0845	1.3	0.065
T. vogelii	Leaf	5	0.6845	2.5	0.275
		10	1.348	2.1	0.642
		15	2.015	1.3	1.550

Four replications

Table 2 Mean yield (g) of extracts for each extraction solvent per gram of plant material

Species	Weight (g) of extract of		
	Bark	Leaves	Fruit
T. vogelii			
Methanol	0.010 ± 0.05	0.014 ± 0.07	0.03 ± 0.02
Chloroform	0.016 ± 0.008	0.024 ± 0.012	0.0026 ± 0.0013
Acetone	0.014 ± 0.007	0.016 ± 0.008	0.001 ± 0.0005
B. madagascariensis			
Methanol	0.0013 ± 0.0006	0.0023 ± 0.0015	0.0062 ± 0.0025
Chloroform	0.0013 ± 0.0006	0.0017 ± 0.0006	0.0037 ± 0.0015
Acetone	0.00093 ± 0.00012	0.00097 ± 0.00006	0.0013 ± 0.0006

Mean weights shown with their 95 % standard deviation

Four replications

treatment levels was pooled using the formula $PMM\ (\%) = \frac{Mortality\ i + Mortality\ ii \ldots + Mortality\ n}{N}$ where *i*, *ii*,…. *n* denote the 1st, 2nd and the nth sample data, respectively, and *N* stands for the total sample size.

Probit analysis was used to analyse the topical bioassay results with the aid of Polo Plus Probit and Logit Analysis 2.0 software (Le Ora Software 2002). This analysis included probit transformations of percent mortality and natural logarithm transformations of dose. Assessment of goodness of fit was done using the chi-square goodness of fit test and the heterogeneity factor (chi-square divided by the degrees of freedom) of each bioassay. When the heterogeneity factor was greater than 1.0, the data was assumed not to follow the probit model used in the analysis. The likelihood ratio test was used to test whether the regression lines obtained were equal or parallel at 95 % confidence level. Lethal dose ratios at LD_{50} and LD_{90} were used to compare the toxicity of each plant extract against adult *A. variegatum* ticks. Toxicity was considered to be significantly different when the number 1 was not contained in the 95 % confidence interval of the lethal dose ratio (Robertson et al. 2007).

Results

Extraction yields

The mean weights of the plant extracts of *T. vogelii* and *B. madagascariensis* extracted with methanol, acetone and chloroform are shown in Table 2. The highest mean weight of extracts for *T. vogelii* was obtained from leaf material using methanol as an extraction solvent. With regard to *B. madagascariensis*, highest mean weight of extracts was obtained from fruit material using methanol as an extraction solvent.

Free contact bioassays

Only methanol extracts of bark and leaf material of *T. vogelii* and methanol fruit extracts of *B. madagascariensis* obtained from 15 g/50 ml crude mixture produced 100 % mortality of ticks in 24 h (Table 3). Residues obtained from other solvents showed no anti-tick activity. Methanol leaf extracts of *T. vogelii* and methanol fruit extracts of *B. madagascariensis* were selected to be used in topical assays to compare the acaricidal properties of the two plants. As shown in Table 3, the acaricidal activity of methanol leaf extracts of *T. vogelii* persisted for up to 8 days while that of fruit extracts of *B. madagascariensis* persisted for only 6 days.

Topical application bioassays

The chi-square test of goodness of fit for both *T. vogelii* and *B. madagascariensis* gave heterogeneity values of 0.142 and 0.007, respectively. These values were less than the number 1 which indicated that the dose mortality data obtained from both plant extracts fitted the assumptions of the probit model used to analyse the data. The hypothesis of equality of the two regression lines was rejected ($P < 0.05$) while that of parallelism was accepted ($P > 0.05$). This indicated that the regression lines obtained from *T. vogelii* and *B. madagascariensis* extracts were not equal, but were parallel at 95 % confidence level; the

Table 3 Percent mortality of *A. variegatum* ticks caused by crude methanol extracts in contact bioassay. Mortality was taken every 24 h and free ticks introduced. The longer the extracts remained active, the more stable the extracts were

Plant crude mixture	24 h		48 h		72 h		96 h		120 h		144 h		168 h		192 h	
	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T
T. vogelii (leaf)	0	100	0	100	0	100	0	100	0	90	0	80	0	80	0	50
B. madagscariensis (fruit)	0	100	0	90	0	70	0	50	0	20	0	10	0	0	0	0

Four replications

C control, *T* treatment

Table 4 Bioassay results for methanol crude plant extracts of *B. madagascariensis* and *T. vogelii* tested for anti-tick properties against adult *A. variegatum* ticks

Plant extract	*n*	Slope (S.E.)	X^2 (d.f)	H.F.	LD_{50} (95 % CL)	LDR_{50} (95 % CI)
B. madagascariensis	160	3.83 (±0.57)	3.34 (10)	0.33*	0.030 (0.026–0.036)	0.055 (0.042–0.071)
T. vogelii	160	3.01 (±0.49)	5.04 (10)	0.50*	0.555 (0.448–0.682)	

Lethal dose estimates are presented as *w/v* of active ingredient

Four replications

n number of ticks, *S.E.* standard error, *d.f.* degrees of freedom, *H.F.* heterogeneity factor, *95 % CL* 95 % confidence limit, *LDR* lethal dose ratio relative to *B. madagascariensis*, *95 % CI* 95 % confidence interval

*The data followed the probit model ($P < 0.05$)

slopes were not significantly different ($P > 0.05$). The LD_{50} estimates of *B. madagascariensis* and *T. vogelii* methanol are shown in Table 4. Since the 95 % confidence interval of LD_{50} dose ratio did not include one, the relative toxicity of the two plant extracts was significantly different, with *B. madagascariensis* extracts being more toxic than *T. vogelii*. The regression lines of the two plant extracts are shown in Fig. 1.

Discussion

The results from plant extraction experiments indicated that the highest mean weight of extracts was obtained from the fruits of *B. madagascariensis* and the leaves of *T. vogelii*, using methanol as an extraction solvent. These extracts exhibited 100 % mortality against adult *A. variegatum* ticks in free contact bioassays, which

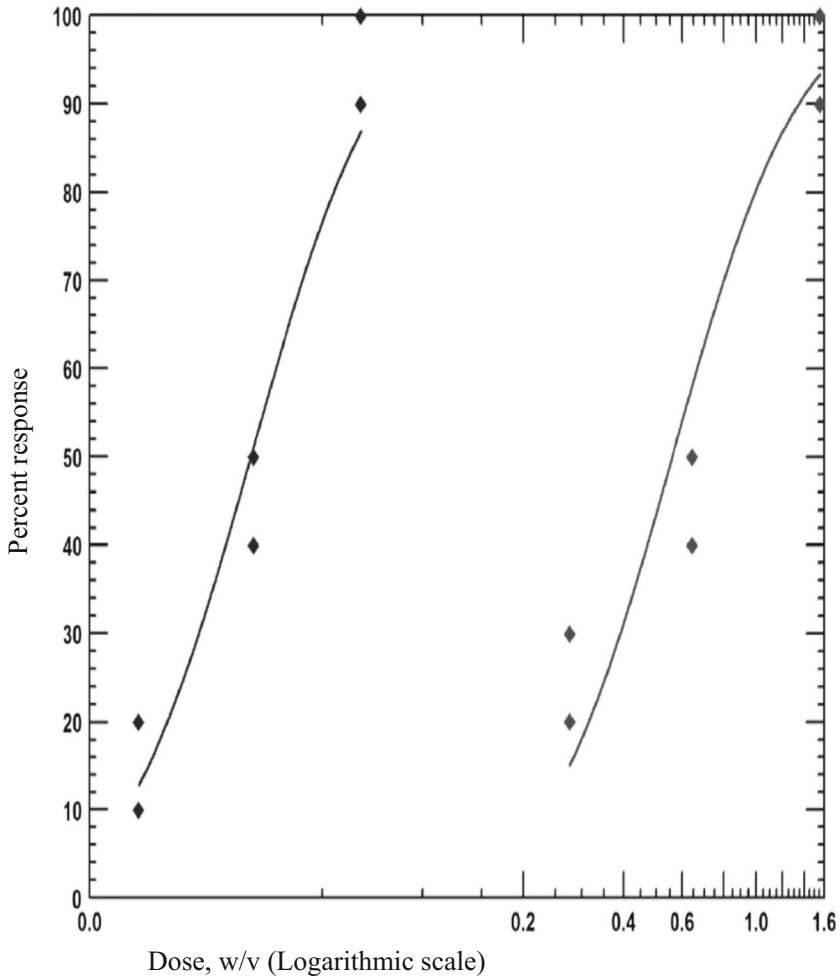

Fig. 1 Topical application assays of *T. vogelii* (*right*) and *B. madagascariensis* (*left*) methanol extracts tested against adult *A. variegatum* ticks. Four replications

indicated that the leaves of *T. vogelii* and the fruits of *B. madagascariensis* accumulate relatively higher active ingredients than the other plant parts tested. These results agree with those reported by Matovu and Olila (2007), who observed high acaricidal activity in methanol leaf extracts of *T. vogelii* against nymph and adult ticks. The results also agree with the observations presented by Stevenson et al. (2010), who outlined the various pesticidal properties of the pods of *B. madagascariensis*. Furthermore, since acaricidal activity was only observed in methanol extracts, the results of the present study support the general notion that the pesticidal active ingredients of plants are more soluble in polar than in non-polar solvents (Mi-Kyeong et al. 2004).

Probit analysis of topical application bioassays of methanol fruit extracts of *B. madagascariensis* and methanol leaf extracts of *T. vogelii* on adult *A. variegatum* ticks produced probit regression lines that were not equal but were parallel, as the slopes of the two lines were not significantly different at 95 % confidence level. Since the slope of a probit regression line estimates the change in activity per unit change in dose or concentration (Robertson et al. 2007 and Heong et al. 2010), parallel lines suggested that the relative potency of the two extracts tested was not significantly different (Robertson and Rappaport 1979). Therefore, for every unit change in dose, the unit change in mortality was not significantly different for the two extracts. The estimated LD_{50} of the two plant extracts was found to be significantly different, with the LD_{50} of *T. vogelii* (0.585) being higher than the LD_{50} of *B. madagascariensis* (0.031). This result indicated that the toxicity of *B. madagascariensis* was higher than that of *T. vogelii*.

The chemical compounds conferring acaricidal activity to *A. variegatum* in methanol fruit extracts of *B. madagascariensis* and methanol leaf extracts of *T. vogelii* were not isolated in this study. However, Stevenson et al. (2010) reported the presence of highly glycosylated flavonoids in methanol extracts of the pods of *B. madagascariensis*. These compounds were probably responsible for the observed acaricidal activity of *B. madagascariensis* observed in this study, as the other major chemical constituents of the pods, saponins, have been associated with molluscicidal activity (Borel and Hostettmann 1987). The presence of rotenoids, deguelin, rotenone, sarcolobine, tephrosin and α-toxicarol, in the leaves of *T. vogelii*, has been reported to be responsible for its pest control efficacy (Stevenson et al. 2012). Kalume et al. (2012) reported that the rotenoid content of *T. vogelii* leaves was associated with its acaricidal activity against the cattle tick *Rhipicephalus appendiculatus*. Therefore, these compounds were mostly likely responsible for the observed acaricidal activity of *T. vogelii* leaf extracts.

It has been recently reported that there are two distinct chemo-varieties of *T. vogelii* under cultivation in Africa (Stevenson et al. 2012). Chemotype 1 (C1) contains the rotenoids that confer pest control activity to *T. vogelii*, while chemotype 2 (C2) lacks rotenoids, being characterized by prenylated flavonones, and is therefore unsuited for pest control. Consequently, it is highly likely that if C2 was used in this study, *T. vogelii* would not have exhibited any acaricidal properties. Therefore, the results presented here only apply to C1.

The variability of the pesticidal efficacy of the leaves *T. vogelii* C1 has also been reported. Belmain et al. (2012) attributed this variability to the seasonal variation of rotenoids with respect to the relative abundance of tephrosin, deguelin and rotenone. These researchers recommended that *T. vogelii* C1 leaves should be harvested in January, when deguelin is highest in content, as rotenone production is relatively constant. Tephrosin levels are low in January but it is less bioactive than dequelin and rotenone (Belmain et al. 2012). Since the leaf material for this study was collected in March 2012, the implication is that the material collected in January may likely exhibit a shift in the *T. vogelii* probit regression line to the left, thereby reducing the value of LD_{50} estimated. Any shift to the left of the probit regression line would increase the toxicity of *T. vogelii* extracts, making its toxicity to be more similar to *B. madagascariensis* extracts. However, if leaf material was collected in June when deguelin content is lowest (Belmain et al. 2012), the *T. vogelii* probit regression line may shift to the right, thereby reducing the toxicity of *T. vogelii*. Therefore, LD_{50} of *T. vogelii* leaf extracts may vary depending on the rotenoid concentration (Fang and Casida 1999), which in turn depends on the season.

The practical implications of the results presented in this study are that extracts of the pods of *B. madagascariensis* are effective for tick control and can be used by resource poor smallholder cattle farmers. However, further research is required on persistence, safety and the required application rates when using water as an extraction solvent.

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Compliance with ethical standards

Statement of human and animal rights All applicable international and national guidelines for the care and use of animals were followed.

Conflict of interest The authors declare that they have no conflict of interest.

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