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Attraction response of adult *Rhipicephalus appendiculatus* and *Rhipicephalus pulchellus* (Acari: Ixodidae) ticks to extracts from *Calpurnia aurea* (Fabaceae)

P. Nana[a,b], N.K. Maniania[a,*], R.O. Maranga[b], H.L. Kutima[b], H.I. Boga[b], F. Nchu[c], J.N. Eloff[d]

[a] *International Centre of Insect Physiology and Ecology (icipe), PO Box 30772-00100 GPO, Nairobi, Kenya*
[b] *Jomo Kenyatta University of Agriculture and Technology, PO Box 62000-00200, City Square, Nairobi, Kenya*
[c] *Percy Fitz Patrick Institute of African Ornithology, University of Cape Town, Private Bag X03, Rondebosch 7701, Cape Town, South Africa*
[d] *Phytomedicine Programme, Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort 0110, South Africa*

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ABSTRACT

Experiments were carried out to investigate the response of two tick species *Rhipicephalus pulchellus* Gerstaker, 1873 and *Rhipicephalus appendiculatus* Neumann, 1901 to three different extracts (acetone, aqueous and oil) of the dried leaves of *Calpurnia aurea* (Aiton) Benth in both an inverted glass tube and a dual choice T-olfactometer. The oil extract at 50 and 100 mg/ml attracted 46.7% and 65.9% of *R. appendiculatus*, respectively, in the inverted glass tube assay, which was comparable to 47.8% of the attraction-aggregation-attachment pheromone (AAAP) used as positive control. At a dose of 100 mg/ml the oil extract attracted 52.4% of *R. pulchellus* in the T-olfactometer bioassay. The relative attraction of both tick species to plant extract was also tested in semi-field plot experiments using a trap baited with different concentrations of emulsifiable extract of *C. aurea*. A dose of 100 mg/ml attracted 52.2% of *R. pulchellus* and 44.4% of *R. appendiculatus* from a distance of 1 m while 14.4% of *R. pulchellus* and 12.2% of *R. appendiculatus* were attracted from 5 m distance at the same dose. Addition of CO_2 to the plant extract-baited-trap at the dose of 100 mg/ml increased the range of attraction of adult *R. pulchellus* (44.4% from 5 m distance) and up to 33.3% of adult *R. appendiculatus* tick from a distance of 4 m. The results of this study suggest that extracts from *C. aurea* can potentially be used as baits in a trap for the control of ticks in the field.

1. Introduction

Rhipicephalus appendiculatus Neumann, 1901 and *Rhipicephalus pulchellus* Gerstäker, 1873 (Acari: Ixodidae) are blood sucking arthropods endemic to tropical and subtropical regions. They feed on a variety of hosts including cattle, sheep and goat, and transmit theileriosis caused by the protozoan *Theileria parva* Theiler, 1911 (Billiouw et al., 2002). The disease is fatal in cattle and has been reported in 11 countries in eastern, central and southern Africa (Norval et al., 1992). Estimated direct economic loss associated with theileriosis is enormous with 1.1 million cattle deaths due to the disease annually (Mukhebi and Perry, 1992).

Existing methods of tick control rely heavily on chemical acaricides and repellents. Although they have been effective in suppressing tick populations and incidences of tick-borne diseases, their main disadvantages have

* Corresponding author. Tel.: +254 20 8632000; fax: +254 20 8632001/2.
E-mail address: nmaniania@icipe.org (N.K. Maniania).

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been the high costs relative to the value of cattle and cattle products, and the development of tick resistance to various ranges of acaricides (Martins et al., 1995; George, 2000). This has prompted a search for alternative methods of tick control that can be used alone or in combination with other tick control methods in an integrated tick management strategy (Jongejan, 1998). These include the use of predators (Samish and Alekseev, 2001), entomopathogenic nematodes (Reis-Menini et al., 2008), and entomopathogenic fungi (Kaaya and Hassan, 2000; Maranga et al., 2006; Maniania et al., 2007).

The potential of pheromone-mediated tactics for the control of ticks was demonstrated by Norval et al. (1989a) who used a crude extract of *Amblyomma hebraeum* Koch, 1844 male-produced attraction-aggregation-attachment pheromone (AAAP). A delivery method for infection of ticks in vegetation with entomopathogenic fungi was tested by Maranga et al. (2006) and Nchu et al. (2009), and it is a device that can be used to deliver pheromone and carbon dioxide. The use of attraction and aggregation pheromone was recently used by Nchu et al. (2010) to enhance auto-dissemination of entomopathogenic fungus *Metarhizium anisopliae* (Metsch.) Sorok. (Ascomycota: Hypocreales) for the control *Amblyomma variegatum* Fabriscius in the field.

A number of plants extracts have been reported to attract ticks of the genera *Rhipicephalus* and these could be used as substitutes for AAAP in the pheromone baited device. These include *Acalypha fruticosa* Forssk (Euphorbiaceae), *Ipomoea spathulata* Hallier (Convolvulaceae), *Solanum incanum* Linnaeus (Solanaceae) (Hassan et al., 1994) and *Calpurnia aurea* Benth (Zorloni et al., 2010). The latter is also used for tick control in western Ethiopia (Regassa, 2000). The present study was motivated by the high level of attraction extracts of *C. aurea* leaves collected in Ethiopia for ticks and the toxicity of the extracts to ticks (Zorloni et al., 2010). Generally, leaves are available throughout the year and grow under a variety of climatic conditions. It could therefore be an important resource for rural communities.

We decided to investigate the attraction of *R. appendiculatus* and *R. pulchellus* adults to different extracts of the leaves of *C. aurea* with the prospects of using them in a baited-trap that is coupled with entomopathogenic fungi (Maranga et al., 2006; Nchu et al., 2009, 2010).

2. Materials and methods

2.1. Plant material

Calpurnia aurea leaves were collected in September 2007 in the Lowveld National Botanical Garden in Nelspruit, South Africa. Identification was performed at the Botanical Garden Herbarium, Pretoria, South Africa, where a voucher specimen was deposited under the number 3206. Leaves were dried in the shade and ground to a fine powder with a McSalib mill (Eloff, 1999). The powder was stored in a closed glass container in the dark.

2.2. Preparation of extracts

Aqueous extract was prepared by maceration of leaf powder (100 g) in 100 ml distilled water maintained at room temperature (23–26 °C) for 24 h. For oil extract, leaf powder (100 g) was macerated in 100 ml of corn oil (Elianto®) all maintained in a warm water bath at 40 °C for 2 h. The mixture was later filtered and different concentrations; 12.5, 25, 50 and 100 mg/ml were prepared by serial dilution in distilled water. Acetone extract was prepared by maceration leaf powder (100 g) in acetone (1:2, w/v) for 6–8 h at room temperature. The extract was evaporated to dryness in vacuo and stored at 4 °C. The dried extract (1 g) was re-suspended in 10 ml of a mixture of acetone, Tween 80, distilled water in a ratio of 1:2:7 and the above concentrations prepared by double dilution. An emulsifiable formulation of the plant extract was used for the field experiment. The dry powder (100 g) of *C. aurea* was macerated in 500 ml of corn oil (Elianto®, BIDCO Oil Refineries Ltd., Nairobi, Kenya) and 100 ml distilled water for 6 h and then placed in a water bath at 40 °C for 2 h. The mixture was later filtered and the different concentrations (12.5, 25, 50 and 100 mg/ml) were obtained by serial dilution with vehicle.

2.3. Pheromone

Attraction-aggregation-attachment-pheromone (AAAP) was included in the inverted glass and T-tube olfactometer bioassays as a positive control. The pheromone was prepared by mixing 0.2 mg of ortho-nitrophenol, 0.1 mg of methyl salicylate and 0.8 mg of nonanoic acid. Tests were carried out using a concentration of 0.02 mg/ml which significantly attracts *A. variegatum* (Nchu et al., 2009). The synthetic compounds used to prepare AAAP were obtained from Sigma–Aldrich Chemie GmbH, Steinheim, Germany.

2.4. Ticks

Two- to three-month-old unfed *R. appendiculatus* and *R. pulchellus* ticks were obtained from the Rearing and Quarantine Unit of the International Centre of Insect Physiology (*icipe*). They were kept in glass vials covered with cotton wool and placed in aluminium tins and maintained at 75% relative humidity and 26 ± 2 °C temperature.

2.5. Bioassays

2.5.1. Inverted glass tube bioassay

The inverted glass tube is a modification of bioassay previously described by Lwande et al. (1999) to evaluate tick repellency. Two test tubes (12 cm long and 1.5 cm diameter) were mounted (with the open end at the bottom), on a wooden stem and fixed on 8 cm × 8 cm platform made of polystyrene. The platform was placed inside a plastic lunch box (20 cm × 10 cm × 8 cm) surrounded by water to prevent ticks from escaping. Approximately 1.5-cm space was left between the mouth of the test tube and the platform. The two test tubes were placed 6-cm apart. The plant extract (0.5 ml) was applied to a piece of cotton wool using

a micropipette. Equal quantity of the solvent used to make the extract was also applied on the other test tube and served as control. AAAP (0.02 mg/ml) was also added as a positive control. Ticks were introduced in a group of 10 (5 males and 5 females) on the platform between the test tubes. Based on their climbing behaviour, ticks that did not climb after 30 min were considered as "no choice" and excluded from the analysis. The number of ticks that climbed the test tubes containing the extract and the controls were counted. After each experiment, the tubes were washed with running tap water and soap, and subsequently rinsed with 70% ethanol and dried. Treatment and control tubes were alternated after every assay by alternating control and test cotton wool. Treatments were randomized and the experiment was replicated 10 times with ticks used only once in each replicate. The experiment was conducted in the laboratory at 23–26 °C and 70 ± 5% RH.

2.5.2. Dual choice T-tube olfactometer assay

This assay was adopted from previous work described by Nchu et al. (2009). Briefly, two cubicle glass arms (1 cm^3 × 10 cm length each) and a stem (1 cm^3 × 5 cm length) were connected tightly using hard glue. The extreme end of the arms and stem was connected to a cubicle glass chamber (3 cm^3). The chamber of the stem served as the release point. Air entered each arm of the olfactometer from the respective odour source chamber at a flow rate of 5 ml/s. The plant extract (0.5 ml) and the AAAP (0.5 ml) were applied to 2-cm^2 filter paper using a micropipette. In the negative control treatments, only solvents were applied to the filter paper. One tick was placed in the release chamber at a time and allowed a minimum of 1 s and a maximum of 5 min to make a choice in the assay chamber. Any tick that failed to respond was removed after the maximum allowed period. Twenty ticks (10 males and 10 females) were assayed for each dose and were used only once. The olfactometer was rinsed with 70% ethanol and dried at 40 °C for 10 min after each bioassay. The treatment and control chambers were switched between assays to avoid positional bias. All the assays were conducted in the laboratory at 23–26 °C and 70 ± 5% RH.

2.6. Attraction of ticks to plant extract-baited-trap in semi-field plot experiments with and without CO_2

This experiment was carried out at *icipe*'s Headquarters, Duduville, Nairobi, Kenya, during the month of February 2009 using the protocol described by Nchu et al. (2009). The grass within each plot was cut to a height of about 5 cm. The plot was marked at l m interval using wooden pegs from the centre up to 5 m. The extract was placed in a bare 10-cm diameter circle, prepared at the centre of the plot. A 2-cm^2 rubber sponge impregnated with 1 ml of each dose of emulsifiable formulation of the plant extract was fixed on the top of each of the four wooden pegs per trap. The direction of wind was monitored using a thread attached to a wooden stick (l m) placed outside the circular plot in a position where the wind directed the thread roughly towards the centre of the plot. For the experiment with CO_2, plastic beakers with tops opened containing approximately 70 g of solid CO_2 were placed in the centre of the trap to serve as CO_2 source. Baited-traps with and without CO_2 were placed upwind on the chosen sites to allow ticks to move upwind. Ticks were released at 1, 2, 3, 4 and 5 m downwind from the odour source at an angle of 90° midway between the test and control traps. Ten ticks (five males and five females) were used only once for each distance. Ticks were given a distinct colour spot by painting them with an artist's paint (Rowney Georgian oil colour, London Graphic Centre, London, UK) applied topically and ventrally depending on their distance from the trap. The experiment was replicated three times and was carried out in the morning between 0700 h and 1100 h. The temperature above ground during the experimental period ranged between 25 and 29 °C (under the sun) and the relative humidity between 50% and 70% was recorded using a digital Thermo/Hygrometer (SATO Model PC-5000 TRH-II).

2.7. Statistical analysis

For each test, the total number of ticks responding to plant extract was pooled across replicates. The attractant index was determined using the following formula: (number of ticks in test − number of ticks in control)/(number of ticks in control + number of ticks in test) × 100. The percentage of attraction was analysed using Chi Square Goodness of fit test (G-test) (Sokal and Rohlf, 1981). Since there were no significant differences in response between male and female ticks, data for the two sexes were pooled together. For semi-plot field experiments, the total numbers of ticks responding to plant extract was pooled across replicates and then analysed using ANOVA. Mean separation was carried out using Student–Newman–Keuls test. Ticks that did not respond (no response group) were excluded from the analysis.

3. Results

3.1. Relative attraction of *R. appendiculatus* and *R. pulchellus* in inverted glass tube assay

In the inverted glass tube assay, both sexes of *R. appendiculatus* were attracted to different formulations of *C. aurea* extracts and the AAAP used as a positive control (Table 1). The highest relative attraction values were obtained with the oil extract at a concentrations of 50 mg/ml (χ^2 = 19.6; df = 1; P = 0.001) and 100 mg/ml (χ^2 = 40.8; df = 1; P = 0.05), aqueous extract at a concentration of 25 mg/ml (χ^2 = 8.9; df = 1; P = 0.01), acetone extract at the concentrations of 25 mg/ml (χ^2 = 5.09; df = 1; P = 0.05) and 50 mg/ml (χ^2 = 5.14; df = 1; P = 0.001), and AAAP (χ^2 = 6.40; df = 1; P = 0.05). With the oil extract there was a good (R^2 = 0.85) and an excellent (R^2 = 0.998) dose related correlation between the concentration and the behaviour of *R. appendiculatus* and *R. pulchellus*, respectively. These excellent correlations give confidence in the reproducibility of the methods at least with certain extracts. *R. appendiculatus* were repelled at lower concentrations, but there was no evidence of repellency against *R. pulchellus* with any of the extracts evaluated. In the case of aqueous extract there was a variable dose related reaction. It appeared to be an optimal concentration for

Table 1
Relative attraction of adult *Rhipicephalus appendiculatus* and *Rhipicephalus pulchellus* (pooled data over replicates) to *Calpurnia aurea* extracts and AAA pheromone in inverted glass bioassays and T-tube olfactometer.

Plant extract (mg/ml)	Inverted glass tube bioassays % Relative attraction[a]		T-tube olfactometer % Relative attraction[a]
	R. appendiculatus	*R. pulchellus*	*R. pulchellus*
Aqueous extract			
12.5	−7.7 a	15.4 a	20.0 a
25	31.8 b	9.1 a	44.4 b
50	−5.9 a	14.3 a	38.5 a
100	16.6 a	13.7 a	22.2 a
Oil extract			
12.5	−16.7 a	2.7 a	13.4 a
25	−15.0 a	8.1 a	31.6 b
50	46.7 b	16.7 a	34.1 b
100	65.9 b	38.1 a	52.4 b
Acetone extract			
12.5	3.0 a	9.1 a	−3.2 a
25	26.8 b	16.2 a	9.5 a
50	19.1 b	4.8 a	0.0 a
100	0 a	12.8 a	8.1 a
AAAP			
0.02 mg/ml	47.8 b	44.2 b	34.9 b

[a] Relative attraction = (number of ticks attracted to test − number of ticks attracted to control)/total × 100; percentages followed by the same letter are not significantly different $P < 0.05$ (Chi-square test).

Table 2
Attraction of adult *Rhipicephalus pulchellus* (pooled data for males and females) to different concentrations of *Calpurnia aurea* in semi-field plot experiments in absence (−) and presence (+) of CO_2.

Distance (m)	Mean percentage ($X \pm$ ESM) tick attraction							
	12.5 mg/ml		25 mg/ml		50 mg/ml		100 mg/ml	
	$-CO_2$	$+CO_2$	$-CO_2$	$+CO_2$	$-CO_2$	$+CO_2$	$-CO_2$	$+CO_2$
1	34.4 ± 2.2cA	34.4 ± 4.0cA	35.5 ± 7.8aA	52.2 ± 6.1bcB	44.4 ± 4.0cA	57.8 ± 2.2bB	52.2 ± 6.2bcA	61.1 ± 6.1bB
2	14.4 ± 4.8bA	32.2 ± 4.0bcA	25.5 ± 4.8aA	40.0 ± 3.8abcA	27.8 ± 6.2bA	47.7 ± 6.1abA	36.7 ± 7.7abA	57.8 ± 4.8bB
3	7.8 ± 2.9abA	22.2 ± 4.0abA	15.5 ± 2.9aAB	34.4 ± 2.9abAB	28.9 ± 5.9bBC	43.3 ± 6.9abBC	34.4 ± 8.0abC	54.4 ± 6.1bC
4	1.1 ± 1.1aA	24.4 ± 2.2abA	5.5 ± 1.1aA	27.8 ± 4.4aA	14.4 ± 2.2aB	42.2 ± 4.0abA	20.0 ± 3.3aBC	47.8 ± 10.5bA
5	0.0 ± 0.0aA	12.2 ± 6.7aA	1.1 ± 1.1aA	24.4 ± 4.0aAB	8.9 ± 1.1aB	37.8 ± 5.6abAB	14.4 ± 2.9aC	44.4 ± 9.7bBC

Means ($X \pm$ ESM) within-column followed by the same lowercase letter and within row bearing the same uppercase letter are not significantly different by Student–Newman–Keul's test ($P = 0.05$).

attraction. Although the oil extract at a concentration of 100 mg/ml gave a similar or higher attraction value as the AAAP ($\chi^2 = 12.19$; df = 1; $P = 0.001$ and $\chi^2 = 6.4$; df = 1; $P = 0.05$, respectively) in Table 1, on the same concentration basis the relative attraction was 3600–5800 times lower than that of AAAP.

3.2. Attraction of *R. pulchellus* in the dual choice T-tube olfactometer

Rhipicephalus appendiculatus ticks did not exhibit any response to the odour source in the dual choice T-tube olfactometer assay and were therefore excluded in this

Table 3
Attraction of adult *Rhipicephalus appendiculatus* (pooled data for males and females) to different concentrations of *Calpurnia aurea* in semi-field plot experiments in absence (−) and presence (+) of CO_2.

Distance (m)	Mean percentage ($X \pm$ ESM) tick attraction							
	12.5 mg/ml		25 mg/ml		50 mg/ml		100 mg/ml	
	$-CO_2$	$+CO_2$	$-CO_2$	$+CO_2$	$-CO_2$	$+CO_2$	$-CO_2$	$+CO_2$
1	23.3 ± 3.8bA	27.7 ± 2.9cA	22.2 ± 1.1cA	41.1 ± 4.0bAB	37.8 ± 4.8cB	48.9 ± 6.2aAB	44.4 ± 1.1cB	55.5 ± 7.2cBC
2	7.9 ± 1.1aA	26.6 ± 1.9bcA	13.3 ± 5.1bcA	36.6 ± 3.7abAB	24.4 ± 2.9bAB	51.1 ± 2.9aB	34.4 ± 7.3bcB	47.8 ± 5.9bcB
3	3.3 ± 1.9aA	26.7 ± 7.0abcA	8.9 ± 1.1abA	36.7 ± 5.1abA	18.9 ± 2.2bB	41.1 ± 8.9aA	28.9 ± 2.2bC	45.6 ± 4.8bA
4	2.2 ± 1.1aA	22.2 ± 11.2abcA	4.4 ± 1.1aA	25.6 ± 6.2abA	5.6 ± 2.9aA	26.7 ± 9.6aA	14.4 ± 2.2aB	33.3 ± 3.3abA
5	2.2 ± 2.2aA	11.1 ± 2.9aA	3.3 ± 2.0aA	20.0 ± 6.9aA	7.8 ± 1.1aAB	26.7 ± 6.7aA	12.2 ± 2.2aB	27.8 ± 2.9aA

Means ($X \pm$ ESM) within-column followed by the same lowercase letter and within row bearing the same uppercase letter are not significantly different by Student–Newman–Keul's test ($P < 0.05$).

experiment. With the exception of the acetone extract at the concentration of 12.5 mg/ml which was repulsive, both sexes of *R. pulchellus* were attracted to all the concentrations of the three formulations of *C. aurea* extract and the AAAP (Table 1). The aqueous extract at the concentration of 25 mg/ml ($\chi^2 = 14.22$; df = 1; $P = 0.001$), oil extract at the concentration of 100 mg/ml ($\chi^2 = 23.04$; df = 1; $P = 0.001$) and AAAP at 0.02 mg ($\chi^2 = 47.16$; df = 1; $P = 0.001$) were the most attractive (Table 1). There was again a good dose response between concentration and attraction ($R^2 = 0.88$) with the oil extract.

3.3. Attraction of adult ticks to plant extract-baited-trap in semi-field plots

The response of *R. pulchellus* and *R. appendiculatus* adult ticks to the plant extract varied with the dose and distance of release, in the absence or presence of CO_2. More ticks were attracted from 1 m distance than from 5 m at all the doses tested. For example, at the concentration of 12.5 mg/ml without CO_2, 34.4% of *R. pulchellus* ticks were attracted from 1 m while no single tick was attracted from a distance of 5 m (Table 2). However, at the dose of 12.5 mg/ml without CO_2, 23.3% of *R. appendiculatus* ticks were attracted at 1 m compared to 2.2% from 5 m distance. The dose of 100 mg/ml in the absence of CO_2, attracted 52.2% and 14.4% of *R. pulchellus* ticks at 1 and 5 m, respectively, while 55.5% and 27.8% of *R. appendiculatus* were attracted at 1 and 5 m, respectively (Table 3). A similar trend in tick response was observed when CO_2 was added to the baited-trap, but there was a significant increase in the number of ticks attracted to the trap compared with responses obtained with no CO_2 added. For instance at 100 mg/ml, 1.2 and 3-fold increase in tick attraction was recorded from 1 and 5 m, respectively, with *R. pulchellus* (Table 2). There was a good correlation between the distance from the trap and the attraction of the ticks ($R^2 = 0.92$); similarly there was a good correlation between the dose and relative attraction ($R^2 = 0.96$).

4. Discussion

Both *R. pulchellus* and *R. appendiculatus* adults were attracted to extracts from *C. aurea* in the inverted glass tube bioassay, which is in accordance to their climbing behaviour. In nature, *Rhipicephalus* ticks must climb onto an appropriate object such as grass or weeds where they can encounter a host. However, in the T-tube olfactometer assay, only *R. pulchellus* was attracted to the plant extracts, but not *R. appendiculatus*. There is no clear explanation to this differential behaviour between the two tick species. It was however observed that *R. pulchellus* ticks were more active than *R. appendiculatus*. In the inverted glass tube bioassays, *R. pulchellus* often clumped on top of the stem, suggesting a type of aggregation behaviour. Similar observations were made by Browning (1976) when comparing aggregation behaviour of *R. pulchellus* and *R. appendiculatus* on grass stems in the field in Kenya.

The role of plants in integrated tick control has been the focus of attention in the last two decades (Kaaya, 2000; Wanzala et al., 2005; Abduz Zahir et al., 2009; Zorloni et al., 2010). Some plants have strong acaricidal and/or repellent properties (Kaaya et al., 1995; Mwangi et al., 1995; Zorloni et al., 2010), while others elicit attraction in some ticks (Hassan et al., 1994; Zorloni et al., 2010). Plants produce several compounds including secondary metabolites in variable concentrations in different plant parts, the primary role of which is chemical defence to arthropods (Chadwick and Marsh, 1990). Because ticks do not attack plants, the effect of plant extracts on ticks does not have an ecological basis. Since these metabolites vary in polarity, their solubilities in different solvents will also vary, hence our use of different solvents (distilled water, acetone and oil) to extract these components for screening in the assays. Water was used to evaluate the traditional use of the plant, acetone was used because it is the best extractant for antimicrobial compounds in plants (Eloff, 1998), and oil was used because volatile compounds are usually non-polar and would be extracted by the oil.

In the present study, *R. appendiculatus* adults were significantly attracted to aqueous and acetone extracts in glass tube assay, while *R. pulchellus* were weakly attracted. There was however a decrease in attractancy at higher concentrations of the aqueous and acetone extracts. Topical application of acetone and aqueous extracts from *C. aurea* at 10% and 20% concentration resulted in 100% and 50% mortalities of *R. pulchellus* adult ticks (Zorloni et al., 2010). These concentrations correspond to 12.5 and 25 mg/ml used in our study, but interestingly, no mortality was observed in the treated ticks. In topical application, the plant extract permeates through the cuticle and if the plant extracts contain toxic compounds it might lead to the death of the tick. In our case, ticks were tested for their sensory perception/response to attractants in the extracts. The difference in these results could be explained by the dissimilarity in the method of application. There was a very good dose related response between the concentration of the oil extracts and the attractancy with both tick species.

Although no attempts were made in this study to identify the compound(s) responsible for attraction, *C. aurea* contains considerable amounts of phenolic compounds (Adedapo et al., 2008). These phenolic components are believed to play a key role in the attraction behaviour of over 12 species of ixodid ticks (Wood et al., 1975; McDowell and Wallade, 1986; Yoder and Stevens, 2000).

The relative attraction of 52.4% and 65.9%, respectively, of *R. pulchellus* and *R. appendiculatus*, observed at the concentration of 100 mg/ml of oil extract was not significantly different from the one obtained with AAAP at optimal dose of attraction (0.02 mg/ml) reported for *A. variegatum* (Nchu et al., 2009). It might therefore be conceivable to use high concentrations of plant extracts of *C. aurea* as substitutes to AAAP to attract and infect *R. pulchellus* and *R. appendiculatus* adult ticks in the auto-dissemination approach (Nchu et al., 2009, 2010). In the semi-field plot experiments, there was a good dose related attraction of both tick species at all the distances from the odour source. In all cases, the presence of CO_2 significantly increased the number of ticks attracted over the 4-h observation period. Carbon dioxide has been reported to stimulate certain tick species such as *A. variegatum* to move, sometimes

towards the source (Norval et al., 1989b; Maranga et al., 2003).

The extracts of *C. aurea* leaves did not perform as well as AAAP in attracting ticks in the *in vitro* studies at the same dose level. The dose that showed maximum attraction for AAAP was 0.02 mg/ml and the most effective dose with the plant extract was 100 mg/ml. This dose could be further optimized by varying the formulation. In the semi-field plot experiments *C. aurea* suspensions work well and they could be used as a cheap way of controlling ticks in rural settings because the cost of preparing the plant extract is so low. However, there might also be the need to screen for more potent plant extracts. For instance, Hassan et al. (1994) observed natural attraction of ticks to the leaves of *Acalypha fruticosa* Forsk. var. *villosa* Hutch and laboratory bioassays were able to demonstrate the attraction of ticks to the odour from the plant. Ethno-veterinary leads could be valuable in selecting species to be screened. Zorloni (2008) evaluated the activity of 28 plant species used to control ticks on animals in southern Ethiopia and in most cases there were good activities. *Calpurnia aurea* was one of the most interesting species. It is pleasing that similar activities were found in *C. aurea* collected from Ethiopia and South Africa growing under widely different environmental conditions.

Tick control using only acaricides is costly for the resource-poor African farmer. As such, application of fungi in combination with a tick-attractant (pheromone or kairomone) seems to be a promising low-cost alternative that can be used by such farmers to control ticks (Nchu et al., 2009, 2010). A low quantity of inoculum is required and treatments could be applied in spot-sprays instead of total cover.

The present study has provided evidence of attraction of *R. pulchellus* and *R. appendiculatus* to *C. aurea* leaf extracts, thereby the prospects of using them in combination with entomopathogenic fungi in a trap system for auto-dissemination of fungal conidia in the field. Safety of this plant for the environment makes it an ideal component of integrated pest management systems (Liang et al., 2003). Future research will concentrate on the screening for more potent plant extracts attractive to ticks, and identification and compatibility studies of active components with entomopathogenic fungi.

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References

Abduz Zahir, A., Abdul Rahuman, A., Kamaraj, C., Bagavan, A., Elango, G., Sangaran, A., Senthil Kumar, B., 2009. Laboratory determination of efficacy of indigenous plant extracts for parasites control. Parasitol. Res. 105, 453–461.

Adedapo, A.A., Jimoh, F.O., Koduru, S., Afolayan, J.A., Masika, P., 2008. Antibacterial and antioxidant properties of the methanol extracts of leaves and stems of *Calpurnia aurea*. BMC Complement. Altern. Med. 8 (53), 1–8.

Billiouw, M., Vercruysse, J., Marcotty, T., Speybroeck, N., Chaka, G., Berkvens, D., 2002. *Theileria parva* epidemics: a case study in eastern Zambia. Vet. Parasitol. 107, 51–63.

Browning, T.O., 1976. The aggregation of questing ticks, *Rhipicephalus pulchellus*, on grass stems, with observation on *Rhipicephalus appendiculatus*. Physiol. Entomol. 1, 107–114.

Chadwick, D.J., Marsh, J., 1990. Bioactive compounds from plants. In: Eds Ciba foundation Symposium 154. John Wiley and Sons, Chichester, pp. 2–21.

Eloff, J.N., 1998. Which extractant should be used for the screening and isolation of antimicrobial components from plants? J. Ethnopharmacol. 60, 1–8.

Eloff, J.N., 1999. It is possible to use herbarium specimens to screen for antimicrobial components in some plants. J. Ethnopharmacol. 67, 355–360.

George, J.E., 2000. Present and future technologies for tick control. Ann. N. Y. Acad. Sci. 916, 583–588.

Hassan, S.M., Dipeolu, O.O., Malonza, M.M., 1994. Natural attraction of livestock ticks by the leaves of shrub. Trop. Anim. Prod. 26, 87–91.

Jongejan, F., 1998. Integrated control of ticks and tick-borne diseases (ICTTD). Parasitol. Today 14, 173–176.

Kaaya, G.P., 2000. The potential for antitick plants as components of an integrated tick control strategy. Ann. N. Y. Acad. Sci. 916, 576–582.

Kaaya, G.P., Hassan, S., 2000. Entomogenous fungi as promising biopesticides for tick control. Exp. Appl. Acarol. 24 (12), 913–926.

Kaaya, G.P., Mwangi, E.N., Malonza, M.M., 1995. Acaricidal activity of *Margaritaria discoidea* (Euphorbiaceae) plant extracts against the ticks *Rhipicephalus appendiculatus* and *Amblyomma variegatum* (Ixodidae). Int. J. Acarol. Vol.21, 123–129.

Liang, G.M., Chen, W., Liu, T.X., 2003. Effects of three neem-based insecticides on diamondback moth (Lepidoptera: plutellidae). Crop Protect. 22, 333–340.

Lwande, W., Ndakala, A.J., Hassanali, A., Moreka, L., Nyandat, E., Ndungu, M., Amiani, H., Gitu, P.M., Malonza, M.M., Punyua, D.K., 1999. *Gynandropsis ginandra* essential oil and its constituents as tick (*Rhipicephalus appendiculatus*) repellents. Phytochemistry 50, 401–405.

Maniania, N.K., Nchu, F., Ekesi, S., 2007. Fungal pathogen for biocontrol of ticks. In: Ekesi, S., Maniania, N.K. (Eds.), Use of Entomopathogenic Fungi in Biological Pest Management. Research Signpost, Kerala, pp. 275–294.

Maranga, R.O., Hassanali, A., Kaaya, G.P., Mueke, J.M., 2003. Attraction of *Amblyomma variegatum* (ticks) to the attraction-agregation-attachment-pheromone with or without carbon dioxide. Exp. Appl. Acarol. 29, 121–130.

Maranga, R.O., Hassanali, A., Kaaya, G.P., Mueke, J.M., 2006. Performance of a prototype baited trap in attracting and infecting the tick *Amblyomma variegatum* Acari: Ixodidae) in field experiments. Exp. Appl. Acarol. 38, 211–218.

Martins, J.R., Corrêa, B.L., Ceresér, V.H., et al., 1995. A Situation Report on Resistance to Acaricides by the Cattle Tick Boophilus microplus in the State of Rio Grande do Sul Southern Brazil. In: Rodriguez, C.S., Fragoso, S.H. (Eds.), Resistencia y Control en Garrapatas y Moscas de Importancia Veterinaria III. Seminario Internacional de Parasitologia Animal, Acapulco, Mexico, pp. 1–8.

McDowell, P.G., Wallade, S.M., 1986. 2,6-Dichlorophenol in the tick *Rhipicephalus appendiculatus* Neumann: a reappraisal. J. Chem. Ecol. 12 (1), 1986.

Mukhebi, A.W., Perry, B.D., 1992. Economic implications of the control of east coast fever in eastern central and southern Africa. In: Kategile, J.A., Mubi, S. (Eds.), Proceedings of the Workshop held in Kadoma Hotel, Zimbabwe, July 20–23, 1992. International Livestock Centre for Africa (ILCA), Addis Ababa, Ethiopia, http://www.fao.org/Wairdocs/ILRI/x5485E/x5485e0h.htm.

Mwangi, E.N., Hassanali, A., Essuman, S., Myandat, E., Moreka, L., Kimondo, M., 1995. Repellent and acaricidal properties of *Ocimum suave* against *Rhipicephalus appendiculatus* ticks. Exp. Appl. Acarol. 19 (1), 11–18.

Nchu, F., Maniania, N.K., Toure, A., Hassanali, A., Eloff, J.N., 2009. The use of a semiochemical bait to enhance exposure of *Amblyomma variegatum*

(Acari: Ixodidae) to *Metarhizium anisopliae* (Ascomycota: Hypocreales). Vet. Parasitol. 160 (3–4), 279–284.
Nchu, F., Maniania, N.K., Hassanali, A., Eloff, J.N., 2010. Performance of a *Metarhizium anisopliae*-treated semiochemical-baited trap in reducing *Amblyomma variegatum* populations in the field. Vet. Parasitol. 169 (3–4), 367–372.
Norval, R.A.I., Andrew, H.R., Yunker, C.E., 1989. Pheromone mediation of host selection in bont tick *A. hebraeum* (Koch). Science 243, 364–365.
Norval, R.A.I., Butler, J.F., Yunker, C.E., 1989b. Use of carbon dioxide and natural or synthetic aggregation-attachment pheromone of the bont ticks, *Amblyomma hebraeum*, to attract and trap unfed adults in the field. Exp. Appl. Acarol. 7, 171–180.
Norval, R.A., Perry, B.D., Young, A.S., 1992. The Epidemiology of Theileriosis in Africa. Academic Press, pp. 481.
Regassa, A., 2000. The use of herbal preparations for tick control in western Ethiopia. J. S. Afr. Vet. Assoc. 71, 240–243.
Reis-Menini, C.M., Prata, M.C., Furlong, J., Silva, E.R., 2008. Compatibility between the entomopathogenic nematode *Steinernema glaseri* (Rhabditida: Steinernematidae) and an acaricide in the control of *Rhipicephalus* (*Boophilus*) microplus (Acari: Ixodidae). Parasitol. Res. 103 (6), 1391–1396.
Samish, M., Alekseev, E., 2001. Arthropods as predators of ticks (Ixodoidea). J. Med. Entomol. 38 (1), 1–11.
Sokal, R.R., Rohlf, F.J., 1981. Biometry: The Principles and Practice of Statistics in Biological Research, 2nd ed. Freeman and Company, New York, pp. 859.
Wanzala, W., Zessin, K.H., Kyule, N.M., Baumann, M.P.O., Mathias, E., Hassanali, A., 2005. Ethnoveterinary medicine: a critical review of its evolution, perception, understanding and the way forward. Livest. Res. Rural Dev. 17 (11), 1–31.
Wood, W.F., Leahy, S.M.G., Galun, R., Prestwich, G.D., Meinwald, J., Purnell, R.E., Payne, R.C., 1975. Phenols as pheromones of ixodid ticks: a general phenomenon? J. Chem. Ecol. 1 (4), 501–509.
Yoder, J.A., Stevens, W.B., 2000. Attraction of immature stages of the American dog tick (*Dermacentor variabilis*) to 2,6-dichlorophenol. Exp. Appl. Acarol. 24, 159–164.
Zorloni, A., 2008. Evaluation of plants used for the control of animal ectoparasitoses in southern Ethiopia (Oromiya and Somali regions). M.Sc. Thesis. Phytomedicine Programme, University of Pretoria.
Zorloni, A., Penzhorn, B.L., Eloff, J.N., 2010. Extract of *Calpurnia aurea* leaves from Southern Ethiopia attract and immobilise or killed ticks. Vet. Parasitol. 168 (1–2), 160–164.