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Tropical Animal Health and Production

ISSN 0049-4747

Trop Anim Health Prod DOI 10.1007/s11250-013-0367-6 Tropical
Animal Health
and Production

Published in association with the
Centre for Tropical Veterinary Medicine.
University of Edinburgh

Springer



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Efficacy of *Strychnos spinosa* (Lam.) and *Solanum incanum* L. aqueous fruit extracts against cattle ticks

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Accepted: 28 January 2013

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Abstract The efficacy of *Solanum incanum* and *Strychnos spinosa* aqueous fruit extracts was evaluated against cattle ticks in on-station experiments and laboratory tick bioassays. In the on-station experiment using cattle, fruit extracts were applied at three concentrations 5, 10, and 20 % (*w/v*) and compared with a commercial acaricide, Tickbuster® (amitraz) spray (positive control) and no treatment (negative control). The treatments were applied at weekly intervals for 6 weeks as surface sprays on 32 Mashona cattle in a completely randomized design experiment. Ticks on individual cattle were identified, counted,

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Published online: 28 February 2013

and recorded daily. Peripheral blood samples were collected for parasite screening. In the laboratory, tick bioassays were conducted at four concentrations, 5, 10, 20, and 40 % (w/v) fruit extracts compared to Tickbuster® (amitraz) spray (positive control) and distilled water (negative control). The extracts were incubated with Rhipicephalus (Boophilus) decoloratus tick larvae and mortalities for each treatment level recorded after 24 and 48 h. The 5 % Solanum incanum treatment had higher efficacy ratio (P<0.05) than the other fruit extract concentrations of the same plant species. Efficacy ratio was higher (P<0.05) in the 5 % S. spinosa-treated cattle than in the untreated control but lower (P<0.05) than that for the amitraz treatment. The bioassays indicated that there was a high efficacy ratio for the lowest fruit extract concentrations when ticks were exposed to acaricidal treatments for 48 h compared to 24 h. Overall, the results indicate that Solanum incanum and Strychnos spinosa individually have some acaricidal effect.

Keywords *Strychnos spinosa* · *Solanum incanum* · Tick control · Aqueous fruit extracts · Pesticidal plants · Acaricidal efficacy

Introduction

Most animals kept by resource-poor farmers in developing countries are affected by endemic pathogens (Wanzala et al. 2005). In Zimbabwe, Hargreaves et al. (2004) reported that 36 % of animals in smallholder farming areas of Zimbabwe are sold due to illness. Although ticks and tick-borne diseases are a major challenge for many farmers in developing countries, most of the farmers are not economically endowed to tackle the problem. The majority of these smallholder farmers are located in areas where they cannot easily access modern veterinary information and services. In addition, they often cannot afford the conventional acaricides because of high

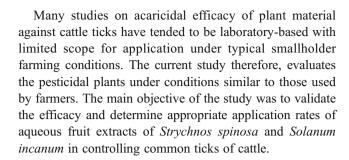


prices (Mwale et al. 2005). Consequently, the opportunity to use local, readily available acaricidal plants to control ticks would be desirable. Some studies have validated the efficacy of ethno-veterinary medicine in smallholder farming areas and reported that it could be cheaper and easier to use than conventional acaricides (Mwale et al. 2005; Wanzala et al. 2005; Madzimure et al. 2011).

In Zambia, smallholder farmers were reported to use Tephrosia vogelii (Hook. f.) (Leguminosae) aqueous leaf extracts as a livestock tick spray (Kaposhi 1992). Topical application of plant-derived infusions, ointments, and dust from tobacco, Nicotiana tabacum L. (Solanaceae) on animals has been reported to drive off some common ectoparasites like tsetse flies, ticks, lice, mange mites, and other arthropods (Wanzala et al. 2005). A survey carried out in Zimbabwe established that smallholder farmers in Centenary (Upper Muzarabani) and Nyanga districts mainly used Strychnos spinosa (Lam.) (Loganiaceae), Lippia javanica (Burm. f.) Spreng. (Verbenaceae), and Solanum incanum L. (Solanaceae) to control cattle ticks (Stevenson et al. 2010). L. javanica aqueous extracts were reported to effectively control ticks on cattle (Madzimure et al. 2011). Farmers should be encouraged to use acaricidal plants that are common to reduce harvesting pressure on scarce plants and minimise disturbance of ecosystems (Wanzala et al. 2005).

There is paucity of information on the efficacy, persistence, and required rate of application of acaricidal plants (Mathias 2004), particularly when sub-optimal dosages are likely to result in tick resistance (d'Hotman and Hatendi 1998). Many resource-poor farmers in Africa may go for months without tick control for their animals owing to poor acaricide distribution and inability of governments to sustain the free service which existed for decades, thereby increasing the chances of tick-borne disease outbreaks. Tick-borne diseases such as babesiosis, theileriosis, ehrlichiosis, and anaplasmosis can result in high mortality if not detected and treated early. For example, East Coast fever leads to an estimated mortality of about 10 % or 1.1 million cattle in Africa (Norval and Lightfoot 1982; Olwoch et al. 2008).

In many cases, the use of indigenous plants for pest control by resource-poor farmers is cost-effective, easy, and environmentally friendly (Belmain and Stevenson 2001) mainly because the plants are locally available and farmers are already using them. However, their use needs to be optimized since different chemotypes of some pesticidal plant species occur which can lead to highly variable efficacy (Stevenson et al. 2012; Belmain et al. 2012) while safe handling should be promoted widely since there is not always adequate information about the safety of these plants when used for dipping cattle. Nyahangare et al. (2012) reported that some pesticidal plants show acute mammalian toxicity to animals but these were at very high dosages so typical use by farmers is unlikely to expose them to toxic levels.



Materials and methods

Two experiments were carried out to determine acaricidal efficacy of *Strychnos spinosa* and *Solanum incanum*. The first validated the efficacy of the plants against ticks on cattle as claimed by smallholder farmers while the second evaluated the efficacy of the plants on tick larvae in laboratory bioassays.

Experiment 1 Efficacy of *Solanum incanum* and *Strychnos spinosa* in controlling ticks on cattle

Study site

The study site for the field experiment was Henderson Research Station (HRS) as described previously in Madzimure et al. (2011).

Preparations of plant extracts

Strychnos spinosa and Solanum incanum were hand-harvested from the wild at HRS farm. The plant species were positively identified at the National Hebarium and Botanic Garden, Harare, Zimbabwe. The inner soft pulp and seeds of the unripe fruits of Strychnos spinosa were extracted by breaking the hard outer shell of the fruit, crushed at speed 1 for 60 s in a 3-Speed Ultra Power Electric blender (KitchenAid® Appliances, Michigan, USA) to form a viscous homogenous fluid. The fluid was weighed and diluted with tap water at room temperature for 48 h to come up with concentrations of 5, 10, and 20 % (w/v). The extracts were then filtered through a muslin cloth. The Solanum incanum mixture was prepared following the same procedure but using ripe fruits. The rationale for using ripe or unripe fruits and the subsequent preparation method were based on farmer practice according to surveys conducted in Zimbabwe (Stevenson et al. 2010).

Experimental design and procedure

Prior to the commencement of the study, the strategic policy for tick management at HRS was plunge dipping once every week during high rainfall months (December to April) and



once monthly for the rest of the year. The conventional dip used is amitraz. In the current study, animals were not dipped for a month before the experiment in order to allow natural tick infestation to develop. The experiment was conducted during November and December for 6 weeks. Daily rainfall was recorded by a meteorological station at HRS.

Thirty-two Mashona cows from HRS were used in the experiment. Each animal was an experimental unit in a completely randomized design. There were eight treatments and controls, each replicated four times. The treatments consisted of the three application levels; 5, 10, and 20 % (w/v) for each of the two plants which covered the broad range of concentrations reportedly used by farmers; no treatment (negative control); and a positive control of Tickbuster® spray (12.5 % EC amitraz-based compound manufactured by Zimphos Private Ltd, Harare, Zimbabwe), applied at the prescribed (label) dilution rate of 0.2 % v/v. Animals in different treatment groups were kept in separate paddocks which had been managed the same way prior to the study and grazed on pastures naturally infested with ticks.

Full body counts of the different types of ticks on each animal were done prior to spraying the animals with the treatments. A 15-l knapsack sprayer was used to uniformly apply 5 l of water (negative control) or the aqueous suspension of the treatment all over the skin of each animal. The order of spraying the treatment groups to avoid the rub-on effect was as follows: negative control, 5, 10, and 20 % concentrations and lastly the positive control. During spraying, each animal was held in the neck region using a cattle crush. The animals were sprayed weekly for 6 weeks from November to mid-December. Ticks were counted daily inbetween spraying.

All the animals were observed daily for any signs of tickborne or other diseases. Peripheral blood smears were prepared from each animal on a weekly basis, stained with Giemsa, and screened for parasites. The parasites were identified according to the characters described by Levine (1985). Blood for smears was collected from the ear vein. The blood films were prepared according to the method of Kelly (1979) and as used in related studies (Madzimure et al. 2011). They were then examined under a light microscope at a magnification of $\times 1,000$ for the presence of hemoparasites.

Experiment 2 Laboratory efficacy of *Solanum incanum* and *Strychnos spinosa* on *Rhipicephalus* (*Boophilus*) *decoloratus* larvae

Study site

The experiment was carried out at the University of Zimbabwe, Faculty of Veterinary Science Entomology laboratory.

Collection of ticks and preparation of tick larvae

Fully engorged female *R.* (*Boophilus*) *decoloratus* ticks were collected from a cattle herd in a village on the border of the Great Limpopo Transfrontier Park in South East Zimbabwe. There is minimal tick control and no acaricide resistance history in their area. Fully engorged female ticks were transported to the Central Veterinary Laboratory (Department of Veterinary Services in the Ministry of Agriculture, Mechanisation and Irrigation Development, Zimbabwe), in aerated containers with moistened paper. On arrival, the ticks were washed in distilled water to remove eggs laid during transportation and those ticks that had started laying eggs were not used. Up to five clean ticks were placed in a plastic rearing tube, closed firmly with a ventilated stopper and placed in an incubator at 27 to 28 °C and 85 to 95 % relative humidity (RH). All eggs laid were collected within 7 days from commencement of incubation.

Experimental design and procedure

An adaptation of the Soberanes technique (Sobaranes-Céspedes et al. 2002 as described by Miller et al. 2007) was used for the tick bioassays. Each plant (Solanum incanum and Strychnos spinosa) was tested at four concentrations, 5, 10, 20, and 40 % (w/v) and all treatments (including the synthetic acaricide) replicated six times in a completely randomised design. A 10-ml aliquot of each plant extract at each concentration and amitraz (12.5 % EC) were placed in a glass Petri dish (10 cm diameter) containing a 9-cm diameter Whatman no. 1 filter paper cut into two halves. After soaking in the extracts, the filter paper was removed from the Petri dish and approximately 20 tick larvae (14-21 days old) were placed on the wet filter paper. The paper was folded and closed on the sides with steel paper clips to prevent tick larvae escaping taking care to avoid crushing the larvae. Tickbuster® spray (amitraz) was diluted to 0.2 % using tap water and used as a positive control. Distilled water was used as a negative control. The packets containing the larvae were then incubated at 27 to 28 °C and 90±5 % RH. Mortality assessments were conducted at 24 and 48 h. The positive control treatment was done last and placed separately in the incubator to avoid cross-contamination.

Statistical analysis

For experiment 1, the daily tick counts were used to calculate acaricide efficacy ratio per animal using a formula adapted from O'Neill (2006):

acaricide efficacy =
$$1 - \left(\frac{\text{treatment tick count}}{\text{untreated control tick count}}\right)$$

The acaricide efficacy ratios, expressed as a percentage, were subjected to arcsine square root transformations to



normalize the data and then analyzed by the repeated measures analysis of variance (F test) procedure of (SAS 2006). The following model was used:

$$Y_{ijk} = \mu + T_i + W_j + T_i \times W_j + e_{ijk}$$

Where:

 Y_{ijk} The response variable (arcsine-square root transformed tick count efficacy ratio)

M The overall population mean

 T_i The fixed effect of the *i*th treatment (*i*=treatment

1,...8)

 W_j The effect of time post-weekly treatment application (j=week 1,...6)

 $T_i \times W_j$ The interaction of time post-weekly treatment

application and treatment

 e_{iik} The residual error effect

The bioassay data was similarly analyzed as for experiment 1 data based on the following model:

$$A_{ijk} = \mu + T_i + H_j + T_i \times H_j + e_{ijk}$$

Where:

 A_{ijk} The response variable (arcsine-square root transformed tick count efficacy ratio)

 μ The overall mean

 T_i The effect of *i*th treatment (*i*=treatment 1,...9)

 H_j The effect of *j*th time post treatment application

(j=24 h, 48 h)

 $T_i \times H_j$ The effect of interaction of treatment and time

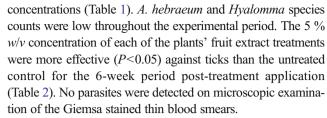
post-treatment application

 e_{ijk} The residual error

For comparison of least square mean efficacy ratios, the PDIFF statistic within the repeated measures ANOVA procedure of SAS (2006) was used. The arcsine-square root transformed tick count efficacy ratios were back-transformed for reporting purposes.

Results

For the on-station experiment, ticks that were observed on the cattle in decreasing order of abundance were *Rhipicephalus appendiculatus*, *Rhipicephalus decoloratus*, *Rhipicephalus evertsi evertsi*, *Amblyomma hebraeum*, and *Hyalomma* spp. This was one of the few times that *Amblyomma* spp. was recorded at Henderson Research Station. Time (weeks) postweekly treatment application had an effect (P<0.05) on efficacy ratio. The plant extract treatments had high efficacy ratios in the second and third week of application (Table 1). There was however, no interaction between time (weeks) and treatment. *Solanum incanum* and *Strychnos spinosa* extracts at 5 % w/v were more effective (P<0.05) than the other fruit extract



During the first 2 weeks of the on-station experiment, rainfall was on average less than 4 mm per week. This was followed by a sudden increase in rain during weeks 3 and 4 (15 \pm 0.75 mm), gradually declining to 7.4 \pm 0.37 mm by week 6. From week 4 onwards, it was observed that rain would fall soon after applying the treatments.

In tick bioassays, Strychnos spinosa treatments lacked a clearly defined acaricidal pattern (Table 3). After 48 h of incubation, the 5 % Strychnos spinosa and Solanum incanum treatments had higher efficacy ratios (P<0.05) than the other fruit extract concentrations of the same plant species. Most Solanum incanum treatments had higher acaricidal efficacy ratios (P<0.05) than those of Strychnos spinosa except the 10 % concentration after 24 h of incubation. In general, the acaricidal efficacy ratio of Solanum incanum increased with an increase in incubation period. Solanum incanum fruit extract concentration had no clearly defined acaricidal effect pattern but was generally high (P < 0.05) at lower concentration after 48 h of incubation. The efficacy ratios of Solanum incanum fruit extracts treatments were generally higher (P< 0.05) than those for Strychnos spinosa. There was no difference in efficacy ratios between the positive control and the 5 % Solanum incanum after 24 h of incubation. The positive control had 100 % efficacy ratio even after 24 h of incubation while the negative control had no effect on ticks (Table 3).

Discussion

The fruit extracts of Strychnos spinosa and Solanum incanum were acaricidal on cattle ticks, but they did not show the classical dose dependence that normally occurs with conventional insecticides (Belmain et al. 2001). We had anticipated that the highest acaricidal efficacy ratio would be from the highest plant fruit extract concentration, but the lowest concentration (5 % w/v) for both Strychnos spinosa and Solanum incanum were the most effective. Mordue (Luntz) et al. (1998) reported a similar trend in which anti-feedant azadirachtin plant had the higher efficacy at lower concentration (0.001 mg/l) than higher concentration of 4 mg/l. It could be that the plant chemicals that are biologically active are not very water soluble (Hoet et al. 2004). If the concentration of plant material is very high the polar compounds in the plant materials 'occupy' more of the available 'space' in the water extract to the exclusion of the non-polar compounds (Belmain et al. 2012). There is only so much a specific volume of water can



Table 1 Least square mean efficacy ratios (%) against ticks on cattle for 6 weeks (November–December) after weekly applications of the acaricidal plants' fruit extracts, *Strychnos spinosa* and *Solanum incanum* and control treatments (*n*=4/treatment)

Treatment	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Treated (amitraz) control	86.0ª	98.0 ^a	96.0ª	92.0ª	80.0 ^a	75.0 ^a
Untreated control	0.0^{b}	29.0^{d}	31.0°	2.0^{b}	0.0^{b}	0.0^{b}
5 % Solanum incanum	$4.0^{\rm b}$	45.0 ^{bc}	65.0 ^{ab}	18.0 ^b	0.0^{b}	0.0^{b}
10 % Solanum incanum	0.0^{b}	32.0 ^{bcd}	36.0^{b}	$0.0^{\rm b}$	0.0^{b}	0.0^{b}
20 % Solanum incanum	0.0^{b}	47.0 ^{bc}	58.0 ^{ab}	0.0^{b}	0.0^{b}	0.0^{b}
5 % Strychnos spinosa	0.0^{b}	55.0 ^{bc}	60.0 ^{ab}	24.0^{b}	13.0 ^b	7.0 ^b
10 % Strychnos spinosa	0.0^{b}	27.0 ^{bcd}	38.0 ^b	1.0 ^b	0.0^{b}	0.0^{b}
20 % Strychnos spinosa	0.0^{b}	16.0 ^{cd}	39.0 ^b	0.0^{b}	0.0^{b}	0.0^{b}
±SEM	0.02	0.09	0.10	0.07	0.051	0.03

Within a column, means with different superscripts differ (P<0.05) SEM standard error of mean

accommodate in terms of dissolved compounds. Other authors reported an artifact of higher concentrations clogging the sprayer nozzles and, therefore, not dispensing the intended dose (Rovesti and Deseo 2009). The finding that lower concentrations were more effective against ticks may lead to optimal use of the plants by farmers and sustainable harvesting.

The delayed effectiveness of the plant treatments to the second and third weeks of the cattle experiment supports the observed time and treatment interaction effect. This was further supported by the higher mortality in tick bioassays after 48 h of exposure to fruit extract concentrations compared to 24 h. It is hypothesized that the acaricides might have killed or repelled female ticks before they fed on the cattle blood. A female hard tick lays thousands of eggs once in its lifetime. Blood is required to support the development of eggs. Hence less progeny is produced if the female is repelled before it feeds for long on the blood. Absence of engorged ticks on the animals treated with commercial and plant-based acaricides might suggest that the treatments were effective. However, it is not clear why the

Table 2 Least square mean weekly acaricidal efficacy ratios (%) for the 6-week acaricide application period (November–December; n=4)

Treatments	Mean acaricide efficacy ratio		
Treated (amitraz) control	87.7ª		
Untreated control	$0.0^{\rm e}$		
5 % Solanum incanum	22.0^{bc}		
10 % Solanum incanum	11.3 ^{cd}		
20 % Solanum incanum	17.4 ^{bcd}		
5 % Strychnos spinosa	26.4 ^b		
10 % Strychnos spinosa	$11.0^{\rm cd}$		
20 % Strychnos spinosa	9.1 ^d		
±SEM	2.73		

Within a column, means with different superscript letters differ (P<0.05) SEM standard error of the mean

efficacy of all treatments decreased after the third week. The plausible explanation could be the washing away of the acaricides by rainfall during the study period which was light but continuous after the application of the treatments. This also explains why Tickbuster® spray had decreased efficacy after the third week contrary to improved efficacy with consecutive applications reported by Madzimure et al. (2011). The experiment was deliberately conducted during the rainy season to provide extreme conditions for testing the plant material since ticks are most prolific at this time of the year and hence pose a serious threat to livestock health.

The acaricidal effects of Solanum incanum could be due to the presence of toxic glycoalkaloids such as solasonine, alkamines such as nitrosamines and carcinogenic glycosides (Katsvanga et al. 2006; Manase et al. 2012). While glycoalkaloids could be responsible for the activity and are partially water soluble they would easily be outcompeted for solution by sugars and other polar compounds. When there are more sugars that are dissolved, less glycoalkaloid will be dissolved hence the extract becomes less potent. Other compounds isolated from the fruits include the alkaloids solasodine and solamargine, and the steroidal sapogenins diosgenin and yamogenin (Anonymous 2012). Manase et al. (2012) also isolated a new spirostanol saponin, along with four known saponins, dioscin, protodioscin, methyl-protodioscin, and indioside D, and one known steroid glycoalkaloid solamargine from Solanum spp. The current findings agree with in vitro efficacy tests on R. decoloratus, where solamargine resulted in 30 to 100 % mortality (Anonymous 2012). These results are also consistent with the pesticidal properties of Solanum spp. that were confirmed against the vegetable aphid, Brevicoryne brassicae (Katsvanga et al. 2006; Muzemu et al. 2011). It is not known whether Solanum incanum fruit extract act like many acaricidal plants such as the Neem tree (Azadirachta indica) that have been reported to disrupt the mating and oviposition of insects and prevent eclosion of their eggs and moulting of their nymphs



Table 3 Least square mean efficacy ratios (%; ±SEM) against tick larvae after 24 and 48 h treatment with plant fruit aqueous extracts and Tickbuster® (amitraz)

Treatment	Time		
	24 h	48 h	
Distilled water (negative control)	0.0 ^{a1}	0.0 ^{a1}	
Tickbuster (positive control)	100.0 ^{c1}	100.0 ^{c1}	
5 % Strychnos spinosa	1.7 ^{a1}	24.4 ^{b2}	
10 % Strychnos spinosa	5.8 ^{a1}	0.0^{a1}	
20 % Strychnos spinosa	0.0^{a1}	0.0^{a1}	
40 % Strychnos spinosa	2.5 ^{a1}	5.4 ^{a1}	
5 % Solanum incanum	26.7 ^{b1}	100.0^{c2}	
10 % Solanum incanum	0.0^{a1}	71.8 ^{c2}	
20 % Solanum incanum	55.8 ^{b1}	39.9 ^{b1}	
40 % Solanum incanum	45.0 ^{b1}	45.0 ^{c1}	
±SEM	7.39	7.39	

Within a column means with different superscript letters differ (P< 0.05). Within a row, means with different superscript numbers differ (P<0.05)

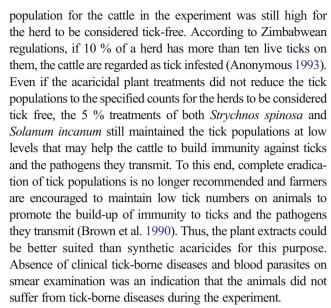
SEM standard error of the mean

(Saxena 1993). There is a need to verify the active ingredients in *Solanum incanum* in order to establish potential mechanisms of action. Knowledge of the chemistry of plants can be used to optimize applications (Stevenson et al. 2009). While the present studies confirm that *Solanum incanum* is effective in controlling tick numbers, further laboratory experiments with isolated compounds are required to determine if the plant extracts can reduce tick feeding, moulting, fecundity, and viability of eggs; and whether the extracts repel or kill the adults.

Tick mortality on cattle exposed to *Strychnos spinosa* could be due to the secoiridoids, kingiside, flavonoids, quinines, and loganin (Hoet, et al. 2004; Nyahangare et al. 2012) which are found in the fruit pulp without the seeds. However, kingiside was tested and found not to be effective against cattle ticks. Previous studies showed that rats fed on *Strychnos spinosa* fruit pulp had signs of strychnine poisoning (Nyahangare et al. 2012) as reported by other authors (Philippe et al. 2004; Makarovsky et al. 2008).

Although cattle that were treated with *Strychnos spinosa* fruit extracts had more ticks when compared to the conventional acaricide, amitraz, it is possible that aqueous extraction, despite being cheap and feasible for resource-poor farmers, was not the best extraction method. Insect toxins in plants are often only partially soluble in water thus extraction by alcohol or the addition of a detergent during extraction may produce a more potent extract than simply using water (Hoet et al. 2004; Belmain et al. 2012). Future studies could explore the use of different types of alcohols normally used as liquor in rural retail outlets.

While different tick species populations were significantly reduced by the plant fruit extract treatments, the total tick



For sustainable and wide-scale use of the pesticidal plants studied, it is paramount to introduce sustainable production, harvesting, and processing methods. There is potential for sustainable production of some pesticidal plants using modern propagation techniques (Sarasan et al. 2011). As also with synthetic pesticides, farmers still need to observe practical safety procedures when handling pesticidal plants.

Conclusions

Solanum incanum and Strychnos spinosa fruit extracts are acaricidal, and the 5 % (w/v) concentrations, have better acaricidal effects on cattle ticks than higher concentrations. Use of the more effective lower concentrations may lead to optimal use of the plants by farmers and sustainable harvesting. The plant fruit extract treatments were not as effective as the synthetic commercial acaricide, amitraz, but can be a viable option in the absence of the acaricide. The tick bioassays showed that there was higher tick mortality when ticks were exposed to acaricidal treatments for 48 than 24 h. Overall, farmers can use these acaricidal plants when they cannot access the commercial acaricides and to provide build-up of tick immunity in cattle.

Acknowledgments The work reported was financed by the European Union, 9th European Development Fund through the Southern African Development Community (SADC) Secretariat's Implementation and Coordination of Agricultural Research and Training (ICART) project and the Africa Caribbean and Pacific Science and Technology program. The contents of this document are the sole responsibility of the authors and can under no circumstances be regarded as reflecting the position of the SADC Secretariat or the European Union. The authors are grateful to Henderson Research Station for supplying the test cattle, animal handling facilities, and technical support. Dr. M. Munyangani of Mazowe Veterinary College provided technical expertise in screening cattle blood samples for parasites. We also thank the Central Veterinary Laboratory staff in the Department of Veterinary



Services, Ministry of Agriculture, Mechanisation and Irrigation Development, Zimbabwe; for collecting and rearing the ticks.

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