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Full Length Research Paper

Tick-repellent properties of four plant species against *Rhipicephalus appendiculatus* Neumann (Acarina: Ixodidae) tick species

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The objective of the present study was to investigate the repellence effects of extracts of four plant species on *Rhipicephalus appendiculatus* (Neumann) larvae. The plants were Cissus *adenocucaulis* F*, Cassia didymobotrya Fresen., Kigelia africana(*Lam.) Benth. and *Euphorbia hirta* L. The effects were evaluated by the fingertip repellence bioassay using extracts obtained using three organic solvents of different polarities: methanol, dichloromethane and hexane. The study demonstrated that all extracts evaluated showed a repellence effect that ranged from 43-88%. For all four plant species, the use of different extraction solvents did not significantly vary repellence effect (P>0.05). C. didymobotrya and K. africana showed the best repellence percentages. These indicate the strong potential of these plants for tick control in an integrated tick management system for livestock owned by resource-poor farmers in northern Uganda.

Keywords: ethnobotany, *Cassia didymobotrya, Euphorbia hirta, Kigelia africana*, *Symphostema adedacule*, *Rhipicephalus appendiculatus*

INTRODUCTION

The use of non-insecticide based approaches to the control of insect and other arthropod pests has received special attention with the aim of promoting development of integrated control programs. The non-chemical options include the application of measures that modify habitats, biological control, and plants that have insecticidal properties (Kaaya, 2002). It is now accepted that traditional practices and knowledge on use of natural substances to kill or repel crop pests and parasitic arthropods on livestock have merit, and are used by different societies throughout the world. Furthermore, it is now recognised that this kind of complementary medical approach to the management of livestock health is necessary to boost livestock production at community level (Toyang *et al.,* 1995).

A wide range of plants have always been used for repelling ticks and other biting insects, using varying techniques such as burning plant material for its smoke to repel mosquitoes (Sharma *et al*., 1993; Ansari & Razdan, 1996; Seyoum *et al.,* 2002a). *Ocimum* spp. (Labiatae) have been used traditionally and effectively against mosquitoes (Gbolade *et al.* 2000; de Paula *et al.* 2003; Waka *et al*., 2004), black flies (Aisen *et al., 2004*) and ticks (Mwangi *et al*., 1995a). *Gynandropsis gynandra* (L.) Briq.), a shrubby plant, abundant in Eastern Africa, exhibits repellent and acaricidal properties to larvae, nymphs, and adult *R. appendiculatus* and *A. variegatum* (Malonza *et al*, 1992). Various authors have reported the use of tick-repellent grasses as a possible means of tick

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control (Beesley, 1982), and can be effectively adopted in an integrated control strategy (Kaaya, 2002).

In an earlier study, it was established that extracts of *Cassia didymobotrya* Fresen*, Euphorbia hirta* L., *Kigelia africana (*Lam.) Benth and *Cissus adenocucaulis* F. were toxic to adults of *R. appendiculatus* (Opiro *et al*., unpublished report). These four species were selected for investigation on the basis of a survey conducted among livestock keepers in two districts of Northern Uganda, which identified them as the most promising for controlling tick loads on cattle. This study set out to establish whether extracts of these plant species also exhibited repellent properties.

MATERIALS AND METHODS

The selected plants were collected from their natural habitat, put in a polythene bag and quickly transported to the laboratory to avoid metabolic transformations. The plants were then dried under shade in the laboratory. The dried plants were then ground to powder using a kitchen blender. The powder (100g) was soaked for 3 days in three solvents (500ml each) of increasing polarities: hexane, dichloromethane and methanol. Each solution was subsequently filtered through Whatmann filter papers, and then the filtrate was extracted in an extraction apparatus. Recovered extracts were placed in pre-cleaned, sterilized and oven-dried sample bottles and the top covered with aluminium foil and allowed to dry at 0℃ to remove any toxic solvents remaining in infinitesimal quantities. The dry extract obtained was stored at 4°C in tightly stoppered bottles.

Stock solutions of each extract were prepared by dissolving 0.5gm of the powder extract in a few drops of Dimethyl sulfoxide (DMSO) and then topped up with saline to make solutions of 0.25mg/ml of each extract. The stock solutions were then used for the repellence bioassays.

Tick test materials were obtained from a laboratory colony reared initially from field collected ticks maintained at the ICIPE laboratory in Nairobi. The laboratory-reared colony (larvae) was kept at 27-28℃ and 85-95% relative humidity without illumination and used in the bioassays.

Repellence effects of the extracts were evaluated with a fingertip bioassay similar to those described by Schreck *et al*. (1995), Pretorius *et al*. (2003) and Carroll *et al*. (2005). The fingertip bioassay therefore relies on this host-seeking behavior and the tendency of the tick to climb and hang on a potential host. The tick would not move away or drop to the ground unless some deterrent in term of odour or contact chemical drives it away.

The boundary of the treated area, which encircles the finger along the prominent basal and the middle dorsal creases of the first and second joints, was marked with a fine-tipped pen. By means of a pipette, about 1ml of extract or control was evenly applied completely around the second phalanx of the left forefinger. The solution was allowed to dry for 10 min. A vial containing the test stages of ticks (i.e. larvae) was opened in a smaller petri dish. The treated finger was held horizontally and 10 unfed larvae were transferred singly with forceps to the dorsal surface of the untreated distal segment of the finger between the base of the finger nail and the joint. The finger was then tilted to vertical position with the tip pointing down. The locations of the ticks were recorded at 10 min after the ticks were released on the fingertip. Ticks on the untreated fingertip and those that fell or dropped from the finger 3–4 cm below were considered repelled. Ticks on the treated area and those that crossed it were considered not to have been repelled.

Before each bioassay, the finger was thoroughly washed with soap and rinsed with water. . Furthermore, prior to the application of each extract and control, the larvae were screened for tenacity and readiness to climb by placing them on the tip of an untreated finger until they climbed ~0.5cm. Those that climbed were then used in the bioassays because they were confirmed as being in the proper physiological state i.e. showing appetence behaviour (Dautel, 2004). There was a control with water and solvent for each group. The extract and control were randomly tested and each had 3 replicates. The repellence was calculated as a percentage using the formula:

$$repellence = \frac{no\ of\ ticks\ on\ untreated\ part\ of\ fingertip + no\ of\ ticks\ that\ drop\ to\ the\ ground}{total\ number\ of\ ticks\ placed\ onto\ the\ finger} X 100$$

Data obtained in the repellence bioassays were analysed using General Linear Model (GLM) of SAS (Version 9.2; 2002-2008 by SAS Institute Inc., Cary, NC, and USA.SAS). Means were separated by Tukey test at 5% probability level. Prior to analyses, diagnostic check was performed that necessitated the response variable (percent repellence) to be transformed using arcsine transformation [y'= 100*ASIN (SQRT((y+0.5)/100)*22/28] to stabilize the variances.

RESULTS

All the extracts of the different plants showed repellence effects during the bioassays. The controls did not exhibit any significant repellence effects and therefore were dropped from the analyses. There was a significant interaction effect between solvent and plant species (Table 1).

C. didymobotrya under all the solvents had the highest repellence, followed by *K. africana.* However, *E. hirta* and *C. adenocucaulis* caused the lowest repellence*,* (Table 2).

There was a slight variation in the effects of solvents; the use of Methanol exhibited the highest repellence effects, followed by dichloromethane and hexane for plant species *C. didymobotrya* and *K. africana.* These

Table 1: Analysis of variance for (%) repellence of Rhipicephalus appendiculatus under different solvent and plant species

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	2	0.815005	0.407503	0.23	0.7951
Solvent	2	77.183974	38.591987	21.94	<.0001
Plant_sp	3	5222.490695	1740.830232	989.61	<.0001
Solvent*Plant_sp	6	60.197449	10.032908	5.70	0.0011
Error	22	38.700178	1.759099		
R-Square	Coeff Var	Root MSE	Grand Mean _repellence		
0.992832	1.977471	1.326310	67.07104		

Table 2: Average repellence percentages (mean+ SE) of R. appendiculatus larvae (Values obtained after three replicates**)**

Solvent	C. didymobotrya	E. hirta	K. africana	C. adenocucaulis
Hexane	81.32^{a}±0.619	42.16^{b}±+1.061	70.80^{c}±+0.631	47.90^{d}±0.672
Methanol	87.67^{b}±0.720	45.54^{c}±0.788	76.38^{f}±0.357	52.61^{e}±0.676
Dichloromethane	83.80^{a}±0.358	41.64^{b}±0.415	72.29^{c}±0.621	46.45^{d}±1.444

*Values within the same column followed by different letters are statistically different (P<0.05)

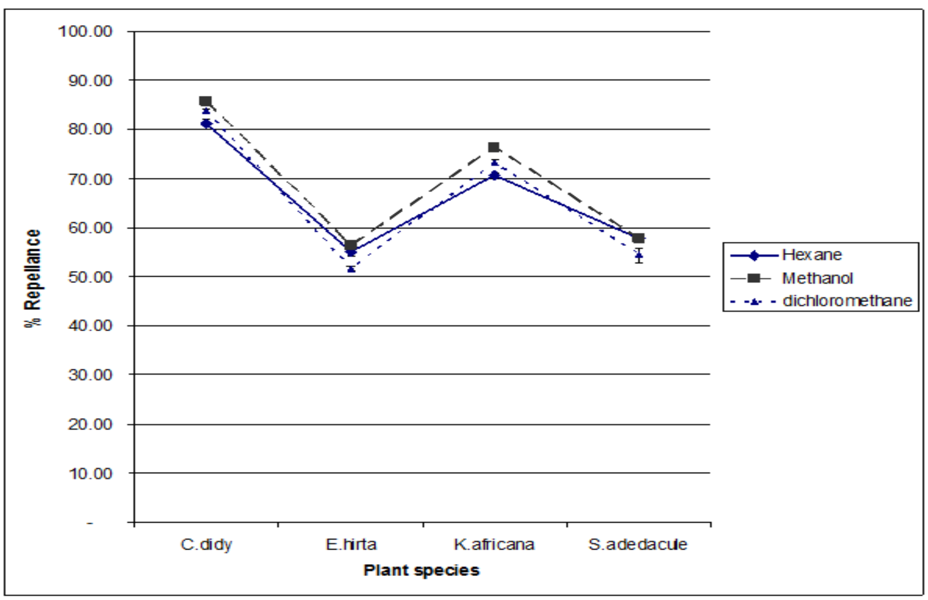

Figure 1: Repellence (%) of plant species extracts to ticks under 3 solvents

trends in solvent reaction were not maintained for plant species *E. hirta* and *C. adenocucaulis,* which could explain the interactions effects between plant species and solvents (Figure 1).

Discussions and Conclusions

The present study has demonstrated that extracts from all four plant species had repellence effects. A repellent – usually a volatile – is a chemical that causes an organism to make orientated movement away from the stimulus source, whereas an attractant – usually a volatile –is a chemical that causes an organism to make orientated movements towards the stimulus source (Jaenson *et al.*, 2005). This study confirms that the plant species contain volatile substances that are responsible for the repellence effect that can be extracted with different organic solvents. Nevertheless the chemical compounds that are involved were not identified. None of these plants had been investigated for tick repellent properties before, but literature shows the potential of families of these species to emit Isoprene and monoterpene. Much as the role for isoprene emission by plants is unknown, most secondary compounds produced by plants serve a protective role. For example, monoterpene production by plants is related in part to repulsion of herbivores (Grispoon *et al.,* 1991). These compounds could have played a part in the tick repellent properties, but studies need to be done first to confirm this hypothesis.

The repellence percentages obtained for *C. didymobotrya* and *K. africana* are quite high and compare well with those for other plants with tick repellence properties such as *Gynandropsis gynandra* (Malonza *et al*., 1992) and Molasses grass, *Melinis minutiflora* (Mwangi *et al*., 1995b). Although the degree of repellence exhibited was subject to the solvent used for extraction, both *C. didymobotrya* and *K. africana* showed consistently strong repellent properties. Like with previous *in vivo* experiments, it was not possible to determine whether ticks detected the repellent by contact or olfactory chemoreception. Thus, research to investigate these sensory channels may be important.

Since this study has demonstrated the potential of these plants to be repellent or lethal to ticks, there is a possibility for incorporating the control of *R. appendiculatus* ticks as part of an integral control in livestock and pasture management. . Farmers can be encouraged to plant the seeds of plants in large numbers around cattle pens and inside grazing fields, especially the communal ones, if follow-up studies establish that the whole plants other than only extracts can also act as repellents to ticks. However, it has several limiting factors that must be taken into consideration; one of them may be the odour and flavour of plant when cattle is grazed; on the other hand it is possible that this strong odours might transfer onto meat and milk although this has to be demonstrated. If its direct use in the field is limited or restricted to strategic handling schemes then it can be difficult to apply in the field. Alternatively, it is possible to use the extract on hoofs and body of the animal to prevent the infestation with larvae, though this potential use faces the challenges of rapid denaturing by the ultraviolet sunrays, a phenomenon that commonly occurs with these compounds (Francisco *et al.,* 2004). There is need to investigate the longevity of repellency, the optimum amount of plant to be used, and methods that will increase the effectiveness of this method in the field.

In addition to the above, phytochemical analysis to determine the active principles of the plants that are responsible for the repellent activities are urgently called for. This would help in identifying the spectrum of activity of the extracts as well as determining their mechanism of action. These studies can explore, among other factors, the isolation and identification of the products, variability due to the plants or the environment, and synergism due to mixtures of compounds in crude extracts. The next approach should therefore concentrate efforts in the promising repellent extracts (*K. africana and C. didymobotrya*) to fractionate and isolate active compounds.

Nonetheless, the potential of using these botanicals in an integrated tick management strategy is viable given the high repellence percentages. Its use can fit into the concept of anti-tick pasture proposed by Dipeolu *et al.,* (1992). These could significantly reduce the cost and environmental effects of using conventional chemical acaricides.

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