Chromolaena odorata (L.) R.M. King & H. Rob. Leaf Extract as Potential Control Agent for *Rhipicephalus microplus* Canestrini, 1888

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ABSTRACT

The huge economic losses due to tick infestation in livestock and the problems associated with the use of synthetic acaricides require cheaper and safer interventions for tick control. This study investigated the potential use of dichloromethane crude extract of *Chromolaena odorata* leaves in the control of *Rhipicephalus microplus* using glass plate repellency bioassay and topical application procedures. The percentage tick repellency at 3.125 mg/ml and higher concentrations of the extract ranged from 33 to 80. The extract exhibited lethal effects on *R. microplus* with LC_{50} of 22.60 mg/ml. The mean percentage tick mortality was significantly higher compared with that of the control at 6.25 mg/ml ($p = 0.044$), 12.5 mg/ml ($p = 0.017$), 25 mg/ml ($p = 0.006$) and 50 mg/ml ($p < 0.0001$) of the extract. Tick mortalities for all concentrations of the extract were significantly higher compared with that of Amiraz 20, a synthetic acaricide commonly used in livestock production. The extract demonstrates potent acaricidal properties and may serve as potential source for developing acaricidal compounds.

Keywords: *Chromolaena odorata*, Leaf Extract, acaricidal, *Rhipicephalus microplus*

Introduction

Tick infestation is a major problem in the livestock industry as it accounts for huge economic losses due to illhealth and death of livestock. The cattle tick, *Rhipicephalus microplus* Canestrini, 1888 (Murrel and Barker, 2003) considered as the most important tick parasite of livestock globally, transmits the protozoan parasites *Babesia bigemina* and *Babesia bovis* (which cause babesiosis) and the bacterium, *Anaplasma marginale* (which causes anaplasmosis) (Costa *et al.*, 2013). Its infestation in cattle and the yield losses thereof have been estimated to be approximately 3.24 billion dollars annually in Brazil (Grisi *et al.*, 2014). This highly reproductive tick (Madder *et al.*, 2011) has been associated with numerous unsuccessful control strategies, extensive and inappropriate use of acaricides with the attendant problems of development of resistant strains (Chen *et al.*, 2007; Nolan, 1990; Reck *et al.*, 2014) and the deposit of harmful residues in milk, meat and in the environment (Balbus *et al.*, 2013; Nonga *et al.*, 2011). At the same time, the use of synthetic accaricides is associated with high cost and low effectiveness.

In recent years, there has been increasing attention on the use of cheaper and more eco-friendly approaches in tick control. The use of ethnobotanical plant extracts and essential oils have been promoted in this regard. Based on a review by Banumathi *et al.* (2017) more than 60 species of plants have been explored against *Rhipicephalus microplus* worldwide with generally high degree of success.

Chromolaena odorata (L.) R.M. King and H. Robinson (Asteraceae), an exotic and invasive plant (Bani, 2002; Vaisakh and Pandey, 2012) originating from the Americas (McFadyen, 1988), has wide distribution in the tropical and subtropical regions of the world including West Africa (Braimah and Timbilla, 2002). *C. odorata* has been reported to have anti-inflammatory, anti-pyretic (Taiwo *et al*., 2000; Vaisakh and Pandey, 2012), analgesic (Vaisakh and Pandey, 2012), and antimicrobial (Vaisakh and Pandey, 2012; Vital and Rivera, 2009) properties, among other relevant medicinal properties. These properties formed the basis for investigating the potential use of *C. odorata* in the control of *Rhipicephalus microplus*.

Materials and methods

Collection and preparation of plant material

Clean and fresh *Chromolaena odorata* leaves were collected and authenticated at the Department of Plant and Environmental Biology (DPEB), University of Ghana. A voucher specimen (voucher number: UGISA-2018-002) was deposited in the DPEB Herbarium.

The leaves were air-dried at room temperature, pulverized using an electric blender, kept in zip lock bags and stored at -20°C. The powdered leaves were weighed in portions of 50 g using an electric balance (Mettler Toledo XS 104, Switzerland).

Preparation of extract and ticks used

Extraction was done sequentially as described by Jeyaseelan *et al.* (2012) using petroleum ether, dichloromethane and ethyl acetate, each of high analytical grade, obtained from Daejung (South Korea). In each case, the extraction was done three times and the filtrates combined and dried at 40°C in an oven. The percentage yield of the extract was calculated using the formula mass of extract obtained / mass of dry C. ordorata, 50 g and stored at -20°C for further test.

A total number of 235 adult male and female *Rhipicephalus microplus* were handpicked from cattle at the Ashaiman cattle ranch in the Ashaiman Municipal District (*5°42′N, 0°02′W*). The ticks were placed in plastic-vented containers covered with nets and transported to the laboratory for the bioassay. The tick identification was based on morphological characteristics.

Repellency and toxicity assays

The glass plate repellency bioassay (Mkolo *et al*., 2011) was used for this study. Five Whatman number one filter papers were trimmed to fit a 9.2 cm diameter glass plate and divided into two equal halves. The *C. odorata* leaf extracts were redissolved in absolute ethanol and sonicated using a sonicator (Ultrasonic Washer ASONE, China). Varying concentrations (3.25-50.00) mg/ml of the extract were prepared in a two-fold serial dilution of the working concentration using absolute ethanol. Half of each filter paper was applied with 1 ml of the extract with unique concentration. The other half was applied with 1 ml absolute ethanol and served as the control. Treated and control filter papers were allowed to air-dry for 10 minutes. Ten adult ticks (1:1 sex ratio) were placed in the centre of each glass plate with the filter paper and covered with a transparent Petri dish of similar size to prevent the ticks from escaping while at the same time allowing air in the Petri dish. Counts of ticks present on each half of the filter paper were done after 10 minutes. The experiment was repeated thrice, moving the ticks to the center after every 10 minutes and counting ticks in each case.

Toxicity assessment was done by topical application (Zorloni *et al*., 2010) of 1 ml of the extract onto the dorsal surface of ten adult ticks (1:1 sex ratio) per group of two replicates for each concentration. Control ticks were topically applied with 1 ml of absolute ethanol. Using Amiraz 20 formulation information, 1 ml was topically applied to another group of the ticks. The set-up was maintained at 29.9°C and 74% relative humidity, with tick mortality assessed after 24 hours of treatment. Ticks were classified as dead when there was no response to human breath after 30s and no movement following gentle touching.

Data analysis

The average number of ticks repelled or attracted by each concentration was calculated. Repellency index (RI) was calculated using the formula (Nc - Nt/ Nc + Nt)*100 c is subscript to the N % (Pascual-Villalobos and Robledo, 1998; Lwande *et al*., 1999), where Nc and Nt refer to the average number of ticks on the control and treated halves of the filter paper respectively.

Shapiro-Wilk test revealed that the dose-response data were normally distributed. Therefore, Probit analysis (Finney, 1971), in statistical package for social sciences, SPSS (version 16), was done to determine the effective concentration of the extract that killed 50% (LC_{50}) of the ticks after 24 hours of exposure. The mortalities of treated and control groups of ticks were compared using N-1 Chi-squared test in MedCalc Software 15.6.

Results

A sequential extraction of compounds present in *C. odorata* was performed using three different solvents of varying polarities to ensure the availability of a broad range of compounds for testing. While all three solvent extracts were obtained, the dichloromethane fraction showed the most promise and hence served as the focus of the study.

The tick repellency indices calculated for the various concentrations of *C. odorata* dichloromethane leaf extract (CoDLE) were 33% (3.125 mg/ml), 47% (6.25 mg/ml), 47% (12.5 mg/ml), 67% (25 mg/ml) and 80% (50 mg/ml). A good fit of Probit regression line was observed as demonstrated in Table 1. The effective concentration of the extract that killed 50% (LC_{50}) of the ticks after 24 hours of exposure was 22.60 mg/ml (Table 1). Table 2 compares *R. microplus* mortality 24 hours after treatment with the extract and Amiraz 20.

Table 1: Effect of *C. odorata* dichloromethane leaf extract on *R. microplus* after 24 hours of exposure

Tick species	Number of ticks	LC_{50} mg/ml	Slope ± SE	c^2 (df)	p-value
Rhipicephalus microplus	20	22.60	4.95 ± 3.57	2.71 (2)	0.257

c^2: Pearson's Chi-square

LC_{50} mg/ml: Lethal concentration

Table 2: Comparison of *R. microplus* mortality 24 hours after treatment with CoDLE and Amiraz 20

Treatment	Mean number of tick mortality	Mean percentage tick mortality	**MDct (χ^2 [df]; p-value)	***MDat (χ^2 [df]; p-value)
Control	0.5	5		
*Amiraz 20	0	0	5 (0.49 [1]; 0.485)	
3.125 mg/ml	3.5	35	30 (2.67 [1]; 0.102)	35 (4.03 [1]; 0.045)
6.25 mg/ml	4.5	45	40 (4.05 [1]; 0.044)	45 (5.52 [1]; 0.019)
12.5 mg/ml	5.5	55	50 (5.66 [1]; 0.017)	55 (7.21 [1]; 0.0073
25 mg/ml	6.5	65	60 (7.52 [1]; 0.006)	65 (9.15 [1]; 0.0025
50 mg/ml	10	100	95 (17.19 [1]; <0.0001)	100 (19 [1]; <0.0001

*Formulation: 1 litre of Amiraz 20: 1100 litres of water (i.e. 0.125 ml of Amiraz 20: 137.5 ml distilled water).

**MDct: Mean difference of control vs. treatment

***MDat: Mean difference of Amiraz 20 vs. treatment

Discussion

This study assessed the effect of dichloromethane leaf extract of *Chromolaena odorata* and Amiraz 20, an acaricide on the Ghanaian market on the other hand, on adult cattle tick (*Rhipicephalus microplus*). From the data obtained, repellency increased with increasing concentration of the extract, with the lowest and highest concentrations repelling 30% and 80% of tick population respectively. Similarly, increasing concentration of the extract resulted in increasing mortality of the ticks after 24 hours of exposure (Table 2). However, Amiraz 20 and the control produced comparable effect on the ticks. Within 24 hours of exposure to the ticks, the extract demonstrated high toxicity with 22.60 mg/ml (LC_{50}) killing 50% of the ticks (Table 1). Similar acaricidal activity of essential oil from *C. odorata* leaf against *Rhipicephalus lunulatus* has been observed (Tedonkeng *et al.*, 2004).

Although *C. odorata* is invasive (Bani, 2002; Vaisakh and Pandey, 2012) and allelopathic (Hu and Zhang, 2013), it has important medicinal properties that may serve varied and useful purposes to enhance economic gains. The antimicrobial (Vital and Rivera, 2009; Vaisakh and Pandey, 2012), anthelminthic (Panda *et al.*, 2010) and wound healing (Raina *et al.*, 2008; Ayyanar and Ignacimuthu, 2009; Panda *et al.*, 2010) properties of *C. odorata* (characteristic of ethanolic and methanolic extracts, respectively) highlight the potential practical application of the relevant active compounds. Injuries, microbial and secondary bacterial infections associated with blood-feeding activities of ticks in general undermine the health of livestock. The acaricidal activity observed in this study, among other medicinal properties of *C. odorata* leaf extracts, may improve upon the health and body condition of livestock and enhance economic gains from animal husbandry. Thus, further work to isolate and characterize the active compound(s) would be beneficial for the livestock industry.

Interestingly, all concentrations of the extract were more effective in killing the ticks within 24 hours of exposure compared with Amiraz 20, which was as good as the control group (Table 2). Additionally, significant tick mortalities were observed for all concentrations of the extract except 3.125 mg/ml when compared with the control group. These findings suggest that dichloromethane leaf extract of *C. odorata* is highly toxic to *R. microplus* and may serve as target for developing acaricidal compounds. Amiraz 20 contains 20% (w/v) amitraz, a broad spectrum insecticide and acaricide used in veterinary medicine, agriculture and horticulture throughout the world since 1974 (Gupta, 2007). There are reports of development of amitraz resistance in field populations of *R. (B.) microplus* in several parts of the world (Singh *et al.*, 2015; Rosado-Aguilar *et al.*, 2008; Jonsson and Hope, 2007; Li *et al.*, 2005). These findings call for further investigation into the resistance status of *R. microplus* to Amiraz 20.

Conclusion

The study reveals that the dichloromethane leaf extract of *Chromolaena odorata* has potent acaricidal property with 22.60 mg/ml (LC_{50}), killing 50% of cattle tick population 24 hours after exposure to the extract. All concentrations of the extract except 3.125 mg/ml were more effective in killing the ticks studied in comparison with the control group. Interestingly, Amiraz 20 was comparable to the control group of ticks in respect of their effect on tick mortalities. These findings, while highlighting the potential of the extract for developing effective acaricidal compounds, also call for investigation into the resistance status of the cattle tick to Amiraz 20.

Acknowledgements

The authors are grateful to the Department of Animal Biology and Conservation Science (DABCS) and Department of Chemistry, University of Ghana, for the logistical support. The contributions of Messrs. Peter Ohemeng, Patrick Agoayabu and Abdul-Razak Mohammed are duly acknowledged.

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