Effect of *Chromolaena odorata* (L.) R.M. King & H. Rob. Leaf Extract on Oviposition in *Rhipicephalus microplus* Canestrini, 1888

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Abstract

Rhipicephalus microplus infestation in livestock is associated with animal health and economic losses. There are also environmental safety concerns regarding *Rhipicephalus microplus* control using synthethic acaricides, calling for affordable and safer interventions for their control. This study assessed the effect of dichloromethane extract of *Chromolaena odorata* leaf on oviposition in *Rhipicephalus microplus*. Using topical application procedures, *Chromolaena odorata* leaf extract treatment of *Rhipicephalus microplus* was observed to significantly affect the number of eggs laid, $H(5) = 36.25$, $p < 0.001$. The significant differences in eggs laid were observed between the control group and: 3.125 mg/ml ($p = 0.002$), 12.5 mg/ml ($p = 0.001$), 25 mg/ml ($p < 0.001$), 50 mg/ml ($p < 0.001$) treated groups of tick, but not control and 6.25 mg/ml ($p = 0.077$) treated groups of tick. A dichloromethane extract of *Chromolaena odorata* leaf demonstrates its potential use for effective control of *Rhipicephalus microplus* and may be considered for development of acaricidal compounds.

Keywords: *Chromolaena odorata*, Extract, *Rhipicephalus microplus*, Oviposition

Introduction

The cattle tick, *Rhipicephalus microplus* Canestrini, 1888 (Murrel and Barker, 2003), the most economically important ectoparasite of cattle worldwide (Klafke et al., 2019), is an arthropod of the family Ixodidae. It serves as a vector for the protozoan parasites: *Babesia bigemina* and *Babesia bovis* as well as the bacterium, *Anaplasma marginale* (Guglielmone, 1995; Costa et al., 2013). *B. bigemina* and *B. bovis* are the most prevalent *Babesia* species that infect cattle worldwide (Ganzinelli et al., 2020) and the main cause of bovine babesiosis, a tick-borne hemoprotozoan disease characterized by significant morbidity and mortality worldwide (Ganzinelli et al., 2018; Silva et al., 2018). *A. marginale* causes bovine anaplasmosis, resulting in severe anemia, reduction in weight gain, milk production (Garcia et al., 2022) and mortality in cattle herds (Aubry and Geale, 2011; Machado et al., 2015).

R. microplus is regarded as the most important tick parasite of livestock globally on account of morbidity and mortality in livestock as well as economic losses associated with their infestation and control. These economic losses in the livestock industry are due primarily to increased mortality, reduced milk production, and poor feed conversion (Dantas-Torres and Otranto, 2016; Dantas-Torres et al., 2016). Globally, annual losses caused by *R. microplus* infestation, the associated diseases and its control have been calculated at USD $13.9–18.7 billion (Betancourt, 2017) with potential annual losses estimated to be approximately USD $3.24 billion in Brazil (Grisi et al., 2014). Moreover, *R. microplus* infestation in livestock and their blood feeding damage hides, reducing their quality for the leather industry (Minjauw and McLeod, 2003;

Mondal et al., 2013).

The extensive and inappropriate use of costly synthetic acaricides to control *R. microplus* has resulted in the development of resistant strains (Nolan, 1990; Chen et al., 2007; Reck et al., 2014) and low effectiveness. There is also risk of harmful chemical residues depositing in milk, meat and in the environment (Nonga et al., 2011; Balbus et al., 2013), calling for affordable, safer, and more eco-friendly approaches in *R. microplus* control.

Chromolaena odorata (L.) R.M. King and H. Robinson (Asteraceae) is an exotic and invasive plant (Bani, 2002; Vaisakh and Pandey, 2012) common in the tropical and subtropical regions of the world including West Africa (Braimah and Timbilla, 2002). Its crude extract is reported to have several medicinal properties including wound healing (Raina et al., 2008), antimicrobial (Vaisakh and Pandey, 2012; Vital and Rivera, 2009), antioxidant (Umukoro and Ashorobi, 2006), nematicidal (Thoden et al., 2007) properties as well as anti-malarial activity (Pisutthanan et al., 2005). A previous study, by Aboagye et al. (2022), involving petroleum ether, dichloromethane and ethyl acetate extracts of *Chromolaena odorata* leaves revealed that dichloromethane crude extract of *C. odorata* leaves has repellent and acaricidal effect on *R. microplus*. This finding highlighted the importance of investigating the effect of that extract on the number of eggs laid by *R. microplus*. As a follow up to that study, the effect of dichloromethane crude extract of *C. odorata* leaves on oviposition in engorged *R. microplus* was assessed in this study.

Materials and methods

Preparation of extract and ticks used

Fresh leaves of *C. odorata* were collected from the University of Ghana Botanical Gardens (*5.65115, -0.193*) and air-dried for about two weeks. About 1 kg of the dried leaves were pulverized and extracted *via* maceration with dichloromethane intermittently for 72 hours. At 24-hour intervals, the extract was filtered off and fresh solvent was added to continue the extraction. All the filtrates were combined and concentrated under vacuo on a G3 Heidolph rotary evaporator to give a crude extract of mass 40.5g.

Five different concentrations (3.125, 6.25, 12.5, 25, 50) mg/ml of the crude extract of *C. odorata* leaf were prepared in a two-fold serial dilution of the working concentration using 70% ethanol, precision balance (Mettler Toledo, USA) for weighing and a sonicator (Ultrasonic Washer ASONE, China).

Engorged *Rhipicephalus microplus* used for this study were obtained from cattle ranch in the Ashaiman Municipal District (*5°42'N, 0°02'W*) of southern Ghana. A total of 135 engorged ticks handpicked from cattle were transported in plastic-vented containers to the laboratory for the oviposition study. They were identified based on morphological characteristics described by Walker et al. (2003).

For each of the five concentrations and the control, 20 engorged *R. microplus* with 10 in each transparent glass Petri dish (N=120) were used. Engorged ticks in each group were topically applied with a unique 1 ml concentration of the extract onto their dorsal surfaces. However, topical application (Zorloni et al., 2010) of the control ticks were done using 1 ml 70% ethanol. Following topical application, Petri dishes of similar sizes were used to cover them such that the set-up was aerated whilst preventing the ticks from escaping. The set-up was maintained at average laboratory conditions of 28.6^{o}C and 76% relative humidity, and observed daily for oviposition for two weeks. The total number of eggs laid per tick were counted using dissecting microscope (Cole-Parmer, USA).

Data analysis

The Shapiro Wilk Test in Statistical Package for Social Sciences (SPSS v20) was used to assess normality of the data. The analysis showed a non-normal distribution of the data with outliers in two of the treated groups. The Levenes's Test of equality of error variances showed significant p-value ($p = 0.001$), violating the assumption of homogeneity of variance for Analysis of Variance (ANOVA) Test. Therefore, Kruskal-Wallis test was performed to assess variability of the mean ranks of eggs laid by the groups of *R. microplus*. A post-hoc test was performed to identify the groups of treated ticks accounting for the observed difference in mean ranks of eggs laid.

Results

A Kruskal-Wallis test showed that *C. odorata* leaf extract treatment of *R. microplus* significantly affects the number of eggs laid, $H(5) = 36.25$, $p < 0.001$. The mean and mean rank of eggs laid showed that the control group of ticks laid more eggs than those treated with different concentrations of the extract (Table 1). A Kruskal-Wallis post-hoc tests revealed that the significant differences in eggs laid were observed between the control group (CG) and 3.125mg/ml ($p = 0.002$), CG and 12.5 mg/ml ($p = 0.001$), CG and 25 mg/ml ($p < 0.001$), CG and 50mg/ml ($p < 0.001$) treated groups of tick, but not CG and 6.25 mg/ml ($p = 0.077$) treated group of ticks.

Discussion

The choice of *Chromolaena odorata* for this study is based on the extensive report of its medicinal properties effective in: wound healing (Raina et al., 2008), the control of microbes (Vital and Windell, 2009), plant-parasitic nematode (Thoden et al., 2007), among others. In particular, Aboagye et al. (2022) demonstrated that, out of petroleum ether, dichloromethane and ethyl acetate extracts of *Chromolaena odorata* leaves, dichloromethane crude extract has repellent and acaricidal effect on *Rhipicephalus microplus*. This informed its use to assess oviposition in *R. microplus*.

R. microplus is a highly reproductive tick (Madder et al., 2011) with each female depositing up to 4500 eggs in the environment (Junquera, 2022) and a mean of 3670 eggs (Davey et al., 2006). In this study, a two-week observation of *R. microplus* treatment using dichloromethane *C. odorata* leaf extract and 70% ethanol (control ticks) revealed significant differences in the number of eggs laid, $H(5) = 36.25$, $p < 0.001$. The control group of engorged *R. microplus* laid more eggs compared with other groups of the tick treated with all concentrations of the extract (Table 1).

R. microplus is a one-host tick whose oviposition takes place in the environment following detachment of the replete female from the host. Blood feeding is key to egg development and oviposition in *R. microplus*. Several studies report strong correlation of engorgement weight of *R. microplus* female (Bennett, 1974; Davey et al., 1980; Davey et al., 2006; Senbill et al., 2018) and other tick species (Iwuala and Okpala, 1977; Drummond et al., 1969a,b) with the number of eggs laid. Ticks used in this study, collected directly from cattle, were still in their blood feeding stage and engorged to different degrees. This may partly explain the generally lower number of eggs produced (Table 1) compared with larger

TABLE 1

Comparison of eggs laid by *R. microplus* two weeks after treatment with dichloromethane *C. odorata* leaf extract

Treatment	Number of ticks, n	Mean eggs laid	SD	Mean rank
Control	20	767.80	313.11	98.45
3.125 mg/ml	20	287.80	201.59	56.62
6.25 mg/ml	20	418.95	315.12	67.72
12.5 mg/ml	20	339.55	398.74	55.12
25 mg/ml	20	180.65	143.39	43.12
50 mg/ml	20	177.50	151.38	41.95

number of eggs reported previously (Davey et al., 2006; Senbill et al., 2018).

The high cost of synthetic acaricides, the problems of resistance in tick species (Raynal et al., 2013; Klafke et al., 2017), environmental and food safety concerns associated with their use (Castro-Janer et al., 2010; Zikankuba et al., 2019) require affordable, safer and readily available natural products such as *C. odorata* extract for tick control. This study demonstrates that dichloromethane crude extract of *C. odorata* leaves reduces egg laying in *R. microplus*. A similar extract is reported to have repellent and acaricidal effect on *R. microplus* (Aboagye et al., 2022). The combined effect of reduction in egg laying and mortality in *R. microplus* following treatment with *C. odorata* extract has the potential of reducing *R. microplus* reproduction, their spread, and infestation in livestock. Therefore, the attendant losses such as high cost of treating tick-related diseases and livestock mortality associated with *R. microplus* infestation in the livestock industry are also expected to reduce. Additionally, there are reports of anti-inflammatory, anti-pyretic (Taiwo et al., 2000; Vaisakh and Pandey, 2012), analgesic (Vaisakh and Pandey, 2012) and antimicrobial (Vital and Rivera, 2009; Vaisakh and Pandey, 2012) properties in *C. odorata*. The use of *C. odorata* extract for tick control may, therefore, reduce tick infestation and associated problems of wound creation during blood feeding, pathogenic infection, and diseases, which undermine livestock production. This intervention may improve body condition of cattle and other affected livestock, and enhance their production and economic gains.

Conclusion

The study demonstrates that dichloromethane leaf extract of *Chromolaena odorata* significantly affect the number of eggs laid by *R. microplus*, H (5) = 36.25, $p < 0.001$. The control group of *R. microplus* laid more eggs compared with all treated groups of the ticks except those treated with 6.25 mg/ml of the extract. The generally lower number of eggs laid compared with those reported in literature may be explained by the use of non replete *R. microplus* females for the study. These findings highlight *C. odorata* leaf as a potential source for development of acaricidal compounds.

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