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Exploring the Acaricidal Activity of *Cissus quadrangularis* against cattle ticks

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Abstract

Ticks pose a significant challenge in cattle production, leading to accelerated disease transmission and substantial economic losses. Chemical acaricides have limitations, including environmental pollution, inaffordability, and inavailability. Therefore, natural remedies are preferred by most rural farmers in developing countries like Zimbabwe. Cissus quadrangularis (C.quadrangularis) is one of the plants used traditionally for medicinal and ethnoveterinary purposes. The study aimed to evaluate the acaricidal activity of C. quadrangularis extract against cattle ticks. Randomised controlled trials were conducted on forty cattle infested with ticks. The experimental groups (n = 10) were treated with 1%, 3% and 5% C. quadrangularis extract, and the control group (n = 10) were treated with a placebo. The assessment was carried out over a 4–8-week baseline, whereby the extract or placebo was sprayed twice a week for 4 weeks and then weekly for the additional 4 *weeks. Tick counts were performed at baseline (Week 0), 4 weeks, and 8 weeks. Compared to the placebo group, the experimental groups showed a reduction in tick counts at 4 weeks and 8 weeks, respectively. Bioassays (petri dish, tick climbing repellent and fingertip) were also done to evaluate the repellent effects of C.quadrangularis. The assays demonstrated acaricidal activity of C.quadrangularis against cattle ticks, offering a promising natural remedy for tick control that can be considered as an alternative to synthetic acaricides, and for inclusion in the Integrated Pest Management (IPM) methods. Further studies are needed to determine and validate the bioactive compounds in C. quadrangularis that possess acaricidal activity.*

Key words: acaricide; chemical acaricides; natural remedy; placebo; bioactive compounds

Introduction

Income generation and food security are the primary goals of farming in the developing Sub-Saharan countries. Farmers in semi-arid regions, where there is little rainfall, place more emphasis on animal production than crop production. Matabeleland South province (Zimbabwe) is in farming region five, where cattle ranching is the common activity. The peasant farmers in this region, for increased income, means of survival and food security, rear cattle and goats, mostly among other livestock (Chitura et al., 2018; Kunaka et al., 2023). Both smallholder farmers and commercial farmers invest in cattle for an income source, meat and milk production, socio-economic status, as well as for barter trade (Chakale et al., 2022; Tshabalala et al., 2015).

Cattle farming is affected by disease outbreaks, especially tick-borne infections such as heartwater, Theileriosis and Boutonneuse fever (Anjarwalla et al., 2016; Chitura et al., 2018; Mkwanazi et al., 2022). Ticks are persistent pests that not only cause failure in animal production but also transmit diseases, both from animal to animal and from animal to humans. Of all the ectoparasites, ticks are the most significant arthropod disease vectors (Wanzala, 2017), causing severe economic losses through increased livestock morbidity and mortality rates. Examples of tick-borne diseases or tick-associated diseases include bovine dermatophilosis, rickettsioses, lyme, hemorrhagic fever and tick-borne encephalitis (Mugambi et al., 2012; Ndhlovu & Masika, 2013; Wanzala, 2017).

In Zimbabwe, the Department of Veterinary Services (DVS) is making tireless efforts to control pests that affect animal production, especially cattle. The journey of tick control by the DVS started long ago and is still going on. Initially, cattle ticks were controlled by dipping (Hlatshwayo & Mbati, 2005), the use of vaccines, biological pest control (use of entomopathogenic fungi), as well as synthetic chemical control methods (Ndhlovu & Masika, 2013; Mawela et al., 2019), where the animals are being sprayed on the affected parts. All these efforts of tick control have shortcomings, such as inaccessibility to poor farmers due to their cost (Chitura et al., 2018; Marume et al.,2017 Beltrão Molento et al., 2020), poor distribution network (in rural areas these pesticides are rarely sold), inappropriate application may lead to pest resistance and chemical residues in meat or milk that can go up in the food chain (biomagnification) causing death and diseases to untargeted pests including humans (Anjarwalla et al., 2016; Kumar & Nattuthurai, 2012; Wellington et al., 2017). Studies also indicated that overuse or continuous exposure to synthetic pesticides causes human health risks such as cancer, defects in the immune system and the development of metabolic diseases such as diabetes, infertility, as well as disruption of the endocrine system (Anjarwalla et al., 2016). These drawbacks are instigating researchers to find safer, efficient, affordable and accessible pesticides that improve cattle production in both rural and commercial farming.

Plant pesticides have been used since time immemorial; our ancestors have been the custodians of this knowledge. Use of plants as efficient acaricides, pesticides or insecticides was developed through long periods of observations as well as trial and error methods (Chakale et al.,2022; Chitura et al., 2018), where different plants were tried against various ailments (Magwede et al., 2014). This intuitive knowledge was passed from generation to generation through word of mouth, observation or apprenticeship (Chitura et al.,2018; Magwede et al., 2014). Without documentation, the knowledge of ethnomedicine can be lost. Furthermore, knowledge on medicinal plants is limited to specific ethnic groups (Chitura et al., 2018); therefore, it is the mandate of this study to document how people in Matabeleland are benefiting from plant acaricides so that other ethnic groups with access to the same plant(s) can benefit.

Plant-based acaricides have been shown to have very good properties over chemical acaricides. For example, studies done by (Adenubi et al., 2018; Anjarwalla et al., 2016; Mkwanazi et al., 2022; Onyango et al., 2018; Quadros et al., 2020; Saravanan, 2022; and Wellington et al., 2017) revealed that they are affordable, easily accessible, biodegradable, eco-friendly, unharmful, and no residues are left in the animal or its product (meat/milk), hence no withdrawal period is required when using plant–based acaricides. Additionally, plants such as *Lippia javanica, Aloe vera, C. quadrangularis, Allium sativum* and *Capsicum annuum* are commonly used traditionally as acaricides or pesticides for both animals and plants, yet their documentation is limited.

C.quadrangularis is an indigenous perennial succulent plant belonging to the Vitaceae family. In the study area, it is commonly known as Veldt grape, *Murunjurunju* or *Intelezi*. All parts of the plant are believed to be medicinal (Hamid & Patil, 2023; Marume et al., 2017; Moto et al., 2018; Onyango et al., 2018), but most acaricidal studies were done on its leaves or stem (Ndlovu & Masika, 2013; Nyahangare, 2015; Wellington et al., 2017). Besides various pharmacological effects identified on *C. quadrangularis*, it is well known for bone, fracture or wound healing in animals (Barbosa et al., 2023; Brahmkshatriya et al., 2015; Camil and Lokesh , 2020; Marume et al., 2017; Samaranayake et al., 2015; Zahan et al., 2022) and for treating diarrhoea in cattle (Barbosa et al., 2023; Bhuvaneswari et al., 2024). Ethnobotanical studies done in Southern Africa, including Zimbabwe, identified C. quadrangularis as a common plant used for tick infections and tick-borne diseases (Magwede et al., 2014; Ndlovu & Masika, 2013; Phaahla et al., 2025), but limited literature is available on its scientific evaluation and validation. This study aimed to evaluate the efficacy of *C. quadrangularis* extract in controlling cattle ticks. Ethnoveterinary practices practised in the study area, especially on tick management, are not documented yet; hence, the thrust of this study. The present study, therefore, was conducted as the first attempt to record and explore the efficacy of C. quadrangularis in managing tick infestations.

Methods and Materials

Plant collection and identification: The stem of *C. quadrangularis* was collected in Gwanda district. The sample was assigned a voucher specimen number, deposited, and positively identified at the National Herbarium and Botanical Garden of Zimbabwe.

Plant preparation: the *C. quadrangularis* stem was then collected, washed to remove dust and any other form of dirt, and chopped into small pieces for easy drying under the shade. The dried parts were then ground in a motor and pestle into fine powder. During trials, fresh stems were crushed and soaked in water for two hours and sprayed or applied to the affected area.

Plant extraction and preparation: Traditional method (infusion) of extraction was used, whereby the plant powder was soaked in water overnight (to allow the active ingredients to dissolve and concentrate) in a closed plastic container. Extracts of different concentrations (1%, 3% and 5%) were prepared. The concentrations were obtained by adding 10g,30g and 50g of plant powder per one litre of water, respectively.

Tick identification: The tick type was identified by the local veterinary officers as *Amblyomma hebraeum,* commonly known as the Bont tick (Fig. 1).

Fig. 1: Example of the type of ticks found infesting cattle in the study area

Ethical Approval: In order to perform animal experiments, ethical approval (MRCZ/A/2986) was obtained from the Medical Research Council of Zimbabwe (MRCZ); hence, the experiment followed the standard protocols approved by the MRCZ. Volunteers for the fingertip assay signed informed consent forms before participation. The researchers explained the risks associated with the study. The participants also confirmed that they have no allergies or skin problems associated with being in contact with *C. quadrangularis* powder, sap or jelly.

Field Experimental design: A purposive sampling technique was conducted to select forty cattle infested with ticks. Randomised controlled trials were conducted where a random number generator was used to assign cattle into groups, namely control and experimental groups. The experimental group (n = 10) was treated with 1%, 3% and 5% Cissus quadrangularis extract, respectively, and the control group (n = 10) was treated with a placebo (distilled water). To differentiate the groups of cattle, the cattle were tagged as ACS1 – ACS10 (experimental group 1%), BCS1 – BCS10 (experimental group 3%), CCS1 – CCS10 (experimental group 5%) and P1 – P10 (control group). The tags' meanings were only known to the chief researchers. The assessment was carried out over a 4 - 8-week baseline, whereby the extract or placebo was sprayed twice a week for 4 weeks and then weekly for the additional 4 weeks. Tick counts were recorded at baseline before the experiment (week 0), 4 weeks, and weekly for the following 5 - 8 weeks. Tick counts were done on the most infested parts, such as the perineal region of the cattle. Research assistants were double-blinded as they counted ticks. They were neither told which group was control or experimental nor the meaning of the tags. They were randomly assigned to a group on every count; no one was meant to be in a specific group. Three people were counting each cattle, and the average number was adopted.

Data analysis: Using SPSS software version 23, tick counts at week 0, 4 and 8 were expressed as standard error of means. Tick counts were recorded before and after spraying of *C. quadrangularis* concentration, under the following: (i) live ticks attaching on cattle (before spraying), then after spraying (ii) dropped live ticks, (iii) dead ticks and (iv) remaining ticks (attaching on the cattle). Comparative analysis of the 4 groups was done using Two-way ANOVA. Paired Samples T-test and one-sample T-test were done to assess the efficiency of the *C. quadrangularis* extract in controlling cattle ticks.

In vitro studies: Bioassays to evaluate the repellent effects of *C. quadrangularis* solution were done following the modified methods recorded by Adenubi et al. (2018). Only the petri dish, tick climbing repellency and fingertip bioassays were done. Before the assays, the ticks were acclimatised for 15 minutes (Luseba et al., 2016). ***Petri dish bioassay***: The filter paper was divided into two. The *C. quadrangularis* solution was uniformly applied on the other half, and a placebo was also uniformly applied on the other half. The filter paper was placed in a petri dish, and the ticks were released at the centre so that they could move around the filter paper. At intervals (5,10,15,20,25,30 mins), tick counts were recorded. After 30 minutes, the percentage repellency was calculated. The assay was done in triplicate.

Tick climbing repellent bioassay: Two sticks (rods) were taken as experimental (treated with *C. quadrangularis* solution) and control (placebo), treated with distilled water. The rods were then put in a glass tube with a filter paper attached at the base of each rod. To maintain the number of ticks that had already climbed up the rods, the glass tube was then plugged with a wet cotton cloth. The repellent effect was proven by the number of ticks that failed to climb up the treated rods versus those that managed to climb up the untreated rod at five-minute intervals for thirty minutes. The assay was repeated 3 times to get the average number of ticks repelled.

Fingertip bioassay: Treated and untreated bands were attached (untreated band below the treated band) on the fingertips of three volunteers. The finger tips were then placed vertically, with the untreated part immersed in a tick-infested area. Ticks that managed to climb up to the treated band were considered not repelled, but those that fell off were considered repelled. The number of ticks from both bands was counted at five-minute intervals for 30minutes. The repellency effect was then calculated.

Data analysis: The mean and standard deviation of each assay were calculated using SPSS software version 23. For bioassays, percentage tick repellency was calculated using the formula:

$$\text{Percentage tick repellency} = \left(\frac{[C-T]}{[C]}\right) X\ 100$$

Where: *C is the number of ticks in the control/untreated part*

T is the number of ticks in the experimental/treated part

Average percentage tick repellency for each bioassay was calculated using the following formula:

$$\text{Average tick repellency percentage} = \frac{\sum Pi}{Ni} \times 100$$

Where: $\sum Pi$ is the total number of percentage repellency for intervals

Ni is the number of intervals recorded during the experiment

Results

The results indicated that *C. quadrangularis* is very effective in tick control after three trials using different concentrations (1%, 3% and 5%). The study results indicated that more than half of the ticks that were attaching initially dropped at week 4 in all experimental groups, then dismally at week 8, especially in the 5% concentration group. (Table 1). This simply means the *C. quadrangularis* extract enables the ticks to detach from the host, thereby proving the efficacy of *C.quadrangularis* in controlling cattle ticks.

Table 1: Comparative analysis of Tick counts at week 0, 4 & 8

Treatment	Weeks					
	Week 0		Week 4		Week 8	
	Dropped	Attached	Dropped	Attached	Dropped	Attached
Placebo	0	14±01	1±0.58	13±0.58	1±0.58	13±0.58
CS 1%	0	14±01	7±01	7±01	8±01	6±01
CS 3%	0	14±01	7±1.15	7±1.15	10±0.58	4±0.58
CS 5%	0	14±01	11±0.58	3±0.58	13±0.58	1±0.58

Comparing with the control group, the study also proved that *C. quadrangularis* is very efficient, since the number of dropping ticks at week 8 is far more than the number recorded in the control group (Table 1 and Fig. 2). This was also recorded on the attached ticks, at week 8 only (1±0.58) One tick was seen attaching on the 5% treatment and 13±0.58 were seen attaching to the placebo (control group). The 5% treatment proved to be the best; therefore, its efficacy was evaluated and shown in Fig. 3, and also the concentration was used in repellent bioassay analysis.

The graphical comparative analysis (Fig. 2) of the three levels of *C.quadrangularis* concentrations of 1%, 3% and 5% shows that the three concentration levels exhibit different mean numbers of ticks dropped. At week 4, the placebo group had the least number of ticks dropped, approximately 10%, while the CS1% and CS3% concentrations had an equal number of ticks dropped at approximately 50%, yet the CS5% concentration resulted in the highest number of ticks dropped at approximately 80%.

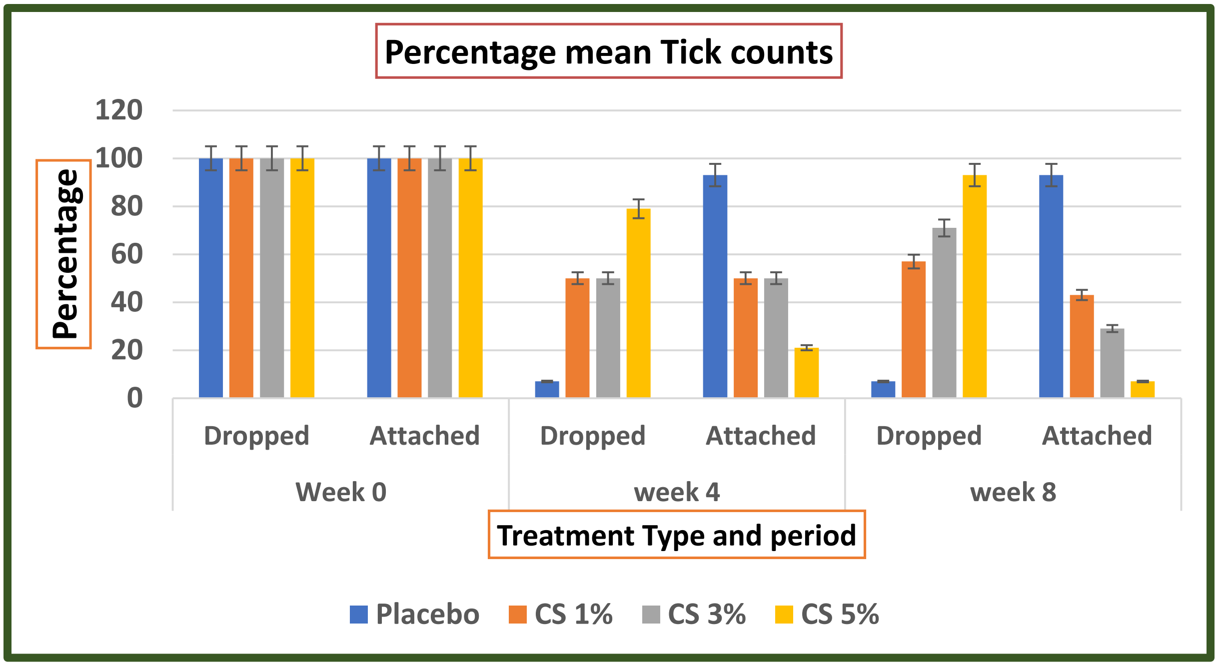

Fig 2: Comparative mean number of ticks by relative status at week 4 and week 8

The number of ticks attached remained high in the placebo group, with approximately 90% ticks still attached at week 4. At week 8, CS1%, CS3%, and CS5% had approximately 50%, 70%, and 90% of the ticks dropped, respectively, while in the placebo group, the same number of ticks had dropped as at week 4, indicating no change in the placebo group. The number of ticks still attached at week 4 indicates a major difference in the effect of these three levels of *C. quadrangularis* concentrations, with CS1% leaving approximately 41% attached, CS3% leaving approximately 30% attached, and CS5% leaving approximately less than 10% of the ticks attached.

Furthermore, the Analysis of Variance (ANOVA) was used to test whether there were differences in the effectiveness of the 3 levels (1%, 3% & 5%) of *C. quadrangularis* concentration in treating ticks on cattle. The null hypothesis assumed equality of the three *C. quadrangularis* concentration levels mean effect, while the study hypothesis assumed that at least two of the three *C. quadrangularis* concentration levels mean effect are not equal. From the ANOVA table, we obtained a high F-value of 3.75, which is greater than the F-Critical value of 3.38. Since our F-statistic value is greater than the F-critical value, 3.75 > 3.38, we reject the null hypothesis and conclude that at least two concentration levels of *C. quadrangularis* have statistically different mean effects on controlling tick infestation on cattle. The P-value of 0.01675 is less than the significance level of 0.05 (P=0.01675 < 0.05), which implies that there are statistically significant differences between the effectiveness of the three concentration levels of *C. quadrangularis* in controlling ticks on cattle. Therefore, we conclude that, at 5% level of significance, the three *C. quadrangularis* concentration levels (1%, 3%, & 5%) have statistically significant differences in their effectiveness in cattle tick control.

Fig. 3 also agrees with Fig. 2 above on the fact that at week 8, there were no ticks (whether dead or live attached to the plant leaf) at 5% concentration treatment. The efficacy of the 5% *quadrangularis* concentration acaricidal solution was also evidenced in bioassay experiments that proved the repellent effect of *C. quadrangularis* (Table 2 and Fig. 4).

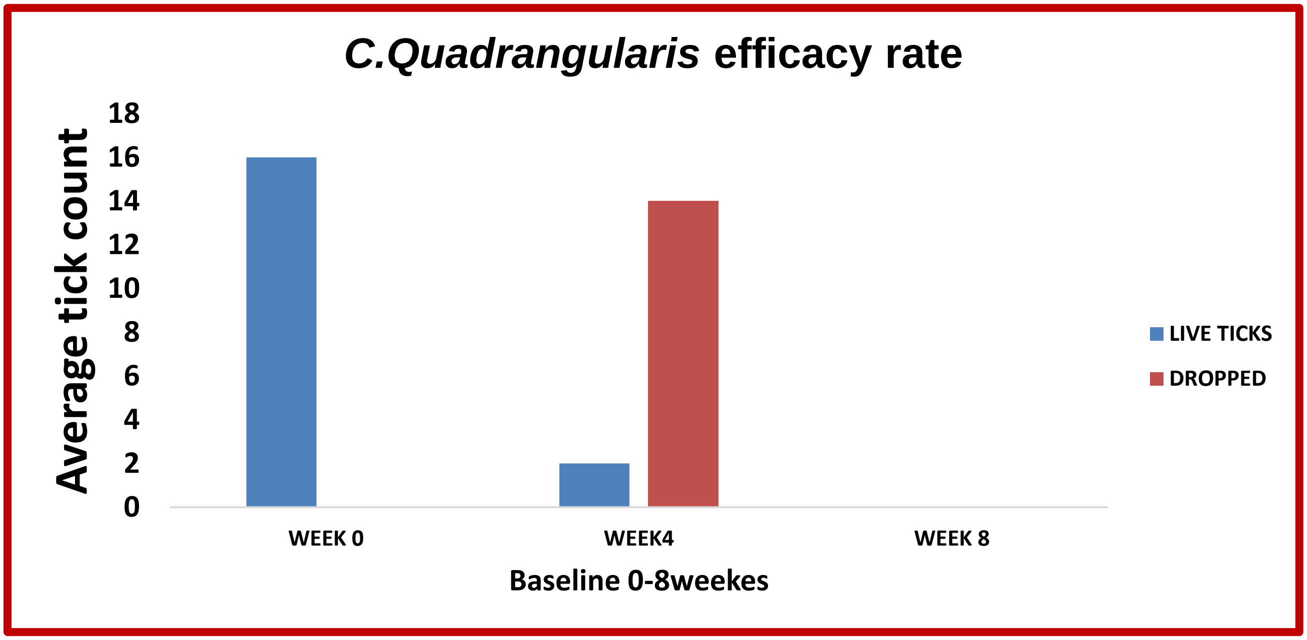

Fig. 3: Overall efficacy rate of *C. quadrangularis* acaricide

The ticks were repelled from the treated solution rather than the untreated/placebo, as shown in Table 2:

Table 2: Percentage Repellent effect of 5% *C. quadrangularis* acaricide

	Total Ticks	climbed	Repelled
Petri dish	100	7±0.58	93±0.58
Tick Climbing	100	3±0.58	97±0.58
Finger Tip	100	7±0.58	93±0.58

The results on bioassays indicate that the average repelled ticks per assay is over 90% (petri dish -93%, tick climbing 97% and fingertip 93%). On the same note, the percentage tick repellency (Fig. 4) was 89% for the Petri dish and Finger Tip assays, and 100% for the Tick climbing repellency assay. These results give enough evidence on the effectiveness of *C. quadrangularis* acaricidal activity since the percentage repellent effect is almost < 89%.

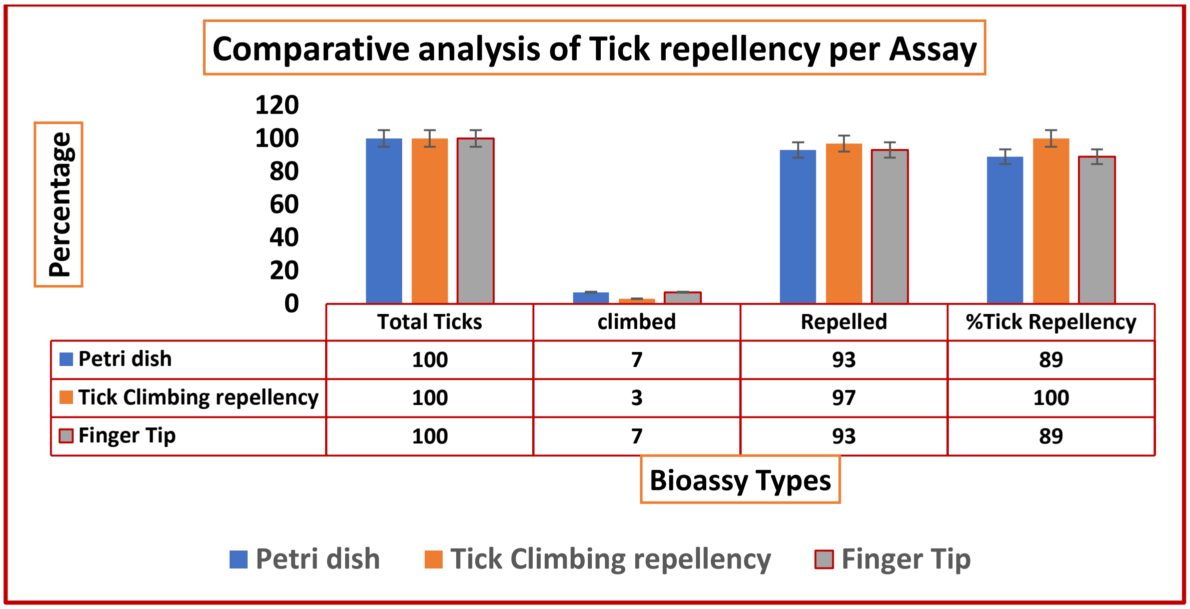

Fig. 4: Comparative analysis of Tick repellency per Assay

Discussion

Use of trials in order to come up with valid results on the use of *C. quadrangularis* was done to validate the efficacy of the plant and to support the results of some studies, such as Magwede et al. (2018), where *C.quadrangularis* was the most mentioned plant for controlling ticks. Also, Fouche et al. (2017) tested thirteen plants, and the study proved that *C. quadrangularis* (1%) was the most effective acaricidal plant among the tested and was less toxic towards both the targeted and other organisms.

Additionally, the use of stem in our experiment proved to be more efficient. Although the whole plant (meaning every part) of *C. quadrangularis* is both medicinal and acaricidal (Onyango et al., 2018), we preferred to use the stem for its flesh and succulent nature. Various studies, such as Chenniappan et al. (2020), Luseba et al. (2016), Nyahangare et al. (2019 and Zaki et al. (2020, used the stem in their experiments, and their results proved that the stem of *C. quadrangularis* possesses both pharmacological and acaricidal properties. Ndhlovu and Masika (2017) also used the *C. quadrangularis* stem in their experiment to control cattle ticks and dermatophilosis in Zhombe, and their results proved that *C. quadrangularis* is efficient, environmentally benign and safe for users (Saravanan, 2022), since no itching or skin irritation complaints were received during the study.

In addition, the results indicated that the use of infusion as a preparation method is effective. One of the trials done during this study was to compare the topical application and spraying of the extracts, and it proved that spraying is more effective than topical application. In contrast to Ndhlovu and Masika (2013), where the stem infusion was rubbed on the affected area, the current study proved that spraying is most effective, though the results produced by both experiments were similar. Extraction by infusion has proved to be easy, cheaper (especially in rural areas) and most effective since it allows bioactive compounds to be completely extracted in water (Magwede et al., 2014; Ndhlovu & Masika, 2017).

The results of bioassays proved that *C. quadrangularis* acaricidal solution is eco-friendly and can deter as well as repel pests (Nyahangare et al., 2019). During the observations, no other creatures were observed dead; thus, controlling ticks using *C. quadrangularis* does not kill other beneficial organisms, but only affects the targeted ones. These results are in support of various studies such as Anjarwalla et al. (2016); Taha & Baioumy Ali (2020), Quadros et al. (2020) and Wellington et al. (2017). This observation on the use of *C. quadrangularis* as an acaricide that is cheap, easy to prepare, efficient, safe and environmentally friendly was also reported by (Anjarwalla et al., 2016; Taha & Baioumy Ali, 2020; Ndhlovu & Masika, 2013; Nyahangare et al., 2019; Quadros et al., 2020).

Furthermore, the results also showed that the *C. quadrangularis* remedy does not kill the pests, but has repellent and deterrent activity (Anjarwalla et al., 2016; Quadros et al., 2020; Taha & Baioumy, 2020). It can be argued that the bioassay done for *C. quadrangularis* acaricide was for thirty minutes, but those recorded in Adenubi et al. (2018) were done for sixty minutes. This can be attributed to the fact that for the petri dish bio assay, there were no ticks in the treated side at 15 minutes, and on tick climbing, no tick was recorded at 20 minutes. Then, on a fingertip bioassay, the repellent effect was seen at ten-minute intervals; hence, 30 minutes was necessary. The *C.*

quadrangularis solution proved to be very effective and safe for use within a short period through the bioassays done. The results of bioassays strongly support the results of field trials, as they also show that *C. quadrangularis* is very effective in repelling pests such as ticks (Fouche et al., 2016**;** Ndhlovu & Masika, 2013; Nyahangare et al., 2019).

Although the study proved the efficacy of *C. quadrangularis* in different aqueous concentrations, further studies are recommended, as these are preliminary results limited to one study area and tick type. Organic solvents should be tested on the efficacy of *C. quadrangularis*, as well as its acaricidal effect on other types of cattle and ticks found in different locations.

Conclusions

C.quadrangularis treatment demonstrated significant acaricidal activity against ticks on cattle, offering a promising natural remedy for tick control that can be used as an alternative to chemical acaricides. It is natural, efficient, easily accessible and very easy to prepare, which makes it a better alternative to the chemical acaricides in the market. Also, by exhibiting tick control agents, the *C. quadrangularis* extract solution can be included in the Integrated Pest Management (IPM) method. Based on this, the following recommendations can be made:

- Documentation of natural remedies of acaricides made from other plants should be promoted so that rural farmers have a choice in tick control
- Further studies are needed to determine and validate the bioactive compounds in *C. quadrangularis* that have potential acaricidal activity.
- Use of plant-based acaricides can be incorporated and used as an alternative to chemical acaricides.
- Conservation of indigenous plants should be prioritised to promote both sustainable Agriculture and livelihoods
- In vitro and in vivo studies on the safety and efficacy of plant-based acaricides such as *C. quadrangularis* for the standardisation of these acaricidal agents.

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