ORIGINAL ARTICLE

In vitro acaricidal activity of traditionally used medicinal plant against southern cattle tick *Rhipicephalus (Boophilus) microplus*

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Abstract

Background: *Rhipicephalus microplus* (*Boophilus*) is an economy damaging cattle pest, and conventional acaricides face issues like resistance and environmental toxicity. Sustainable alternatives are urgently needed. **Objectives:** The aim of the study is to determine the efficacy of crude *Cassia auriculata* flower extract in increasing the mortality and inhibiting the development of the tick at multiple life stages. **Materials and Methods:** The acaricidal potential of *C. auriculata* flower extract is evaluated against the southern cattle tick, *R. microplus*, by testing four concentrations (25, 50, 75, and 100 µg/µL) across all major tick life stages. Crude flower extract of *C. auriculata* is prepared and applied at concentrations of 25, 50, 75, and 100 µg/µL to eggs, larvae, nymphs, and adult ticks. Mortality and developmental inhibition were measured at each stage. **Results:** A clear dose-dependent response is observed. The lowest dose (25 µg/µL) caused only modest mortality, while 50 and 75 µg/µL resulted in significantly greater tick mortality. The highest concentration (100 µg/µL) produced nearly total mortality in all life stages, including eggs, larvae, nymphs, and adults. **Conclusion:** *C. auriculata* flower extract demonstrates substantial acaricidal efficacy, especially at 100 µg/µL, against all life stages of *R. microplus*. The findings support its potential in integrated pest management as an herbal, eco-friendly, and effective alternative to synthetic acaricides.

Key words: Cassia auriculata, nymph, pest management, Rhipicephalus (Boophilus) microplus, tick

INTRODUCTION

Cattle ticks *Rhipicephalus* (*Boophilus*) *microplus* are a destructive blood-sucking ectoparasite mainly infects the wild animals and causing a serious threat to the health and reproduction of cattle and leads huge economic losses. These small blood-sucking ectoparasites belong to the Ixodidae family, commonly present in tropical and subtropical regions.[1] The warm and humid climate in tropical and subtropical regions provides an ideal environment for tick proliferation, hence, they more prevalent than other regions. Cattle ticks significantly create a serious health-threating disease in cattle and lead health and productivity through direct blood-feeding and by transmitting various comorbidities.[2] The life cycle of the cattle is more complex consisting of four stages egg, larva, nymph, and adult. All the stages of ticks typically infest the cattle via skin irritation, and all the stages are effectively affect the cattle's skin, where they feed on blood for sustenance. The infestation of cattle ticks in livestock leads a great economic loss within the agriculture sector. The severe cattle tick infestation significantly reduces the milk production, cause weight loss, and diminishes the reproductive capacity of cattle.[3] In addition, cattle ticks also act as vector for wide range of pathogens, including protozoa and bacteria, which leads other complications such as babesiosis and anaplasmosis. These diseases not only affect the health, but it also create a severe economic thread like additional cost for treatment and management.[4] Hence, a comprehensive approach is required to control the cattle tick infestation. Currently, both chemical and non-chemical

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methods are practiced. In chemical methods, synthetically derived acaricides are designed to kill or repel ticks. In general, the application of acaricides is the primary care application to control the ticks.[5] However, resistance to acaricides is a growing concern, necessitating rotation of different chemical classes. The integration of various control methods, including biological control (using predators and parasites of ticks), environmental management (reducing tick habitats), and the genetic control (breeding tick-resistant cattle), in combination with chemical control, is essential for sustainable tick management. However, the overuse or misuse of acaricides leads to the development of resistance in tick populations, rendering them less effective over time.[6,7]

In case of non-chemical approaches, like pasture management strategies is the primary practice to control cattle ticks, rotational grazing and removal of tick habitats are commonly practiced non-chemical methods. In addition, the introduction of biological agents to suppresses the activity of predatory mites or nematodes.[8] The care to control and manage, and mitigate the impact of cattle ticks is currently used practice driven by the recognition of significant economic and public health implications. Recently, several unique technologies have been used, and also development of vaccines against tick-borne diseases, remains crucial in cattle tick management and pervasive ectoparasites.[9] Futhermore, education and awareness campaigns to livestock producers play a key role in promoting the tick management and reducing the prevalence of tick infestations. By implementing sustainable and effective control measures, the agricultural industry minimize the impact of cattle ticks and ensure the health, safety, and productivity of cattle worldwide.[10] Among the various controlling approaches, the use of natural products, mainly acaricides from the plant-based extract as effective strategies for the control of ticks has been focus of research in several countries, due to their eco-friendly nature. Hence, plant-based extracts may use as effective alternatives to many conventional chemical acaricides. Most of the medicinally value plants contain several secondary metabolites with acaricides properties that are responsible for repel or kill ticks. Among these plants, neem (*Azadirachta indica*), eucalyptus (*Eucalyptus* spp.), citronella (*Cymbopogon* spp.), and garlic (*Allium sativum*) are commonly studied in the pest management due to their potential anti-repellent properties.[11,12] The application of plant extracts in the preventing measures involves incorporating them into number of other practices including development of botanical sprays or dips for direct exposure to cattle hides, and also incorporating the plant products into feed supplements to enhance the protecting system against ticks.[13] Plantation of tick-repellent plants in the habitats also helps to provide the natural barrier against tick infestations. *Cassia auriculata* is one of the most used traditional medicinal plant native to India and Sri Lanka, well known perennial shrub called as Tanner's cassia or Avaram senna, reported to their significant importance in the medicine and cultural practices.[14] The bright yellow color flower and slender leaves gives the beauty value to the landscape and offer a myriad of health benefits. In traditional Ayurvedic and Siddha medicine, all the parts of the plants were utilized to treat various ailments ranging from diabetes to skin disorders.[15] The tea prepared from leaves of *C. auriculata* used to treat diuretic and laxative properties and aiding in digestion and detoxification. In addition, its flowers are used in many cosmetic preparations for their skin rejuvenating qualities.[16] Among the various applications of *C. auriculata* shows a remarkable effect against parasites, including ticks and mites. The plant has various secondary metabolites like flavonoids, saponins and tannins, which are responsible their insecticidal properties. Based on the above literature, the present study was designed to evaluate the acaricidal activity of the flower extract of *C. auriculata* against the southern cattle tick *R.* (*Boophilus*) *microplus*.[17]

MATERIALS AND METHODS

Collection and preparation of *C. auriculata* flower extract

The fresh flowers of *C. auriculata* were purchased from the local market, Al Qassim region, Saudi Arabia. The purchased flowers were gently washed with normal tap water and then rinsed with deionized water to remove the impurities and dust, which is followed by shade drying. The dried flowers were crushed into a fine powder and kept in an airtight container. Preparation of flower extract was followed by the method of previous research with slight modification. Initially, 10 g of *C. auriculata* flower powder was added into a cleaned conical flask with 100 mL of double-distilled water and heated at 70°C in a magnetic stirrer. After cooling, the extract is filtered using Whatman No. 1 filter paper and stored in −4°C for further use.[18,19]

Collection of ticks

Engorged adult male and female ticks were collected from Saudi Arabia. Approximately three hundred adults and nymphs were collected by hand-picking method. A few female adults were cleaned in distilled water and allowed to dry on paper. The dried ticks were weighted and adhered with double-sided sticky tape to the lid of a plastic petri dish. This is followed by acclimatization of ticks at 27–28°C and 80–90% relative humidity. The eggs were collected after 14 days and stored in tubes with a cotton cover for moisture and air exchange. The freshly laid eggs were used for further studies. In addition, after 2 weeks of incubation, the egg hatch into the larvae, which is also used for the acaricidal activity.[20-22]

Acaricidal activity

The acaricidal activity of crude flower extract of *C. auriculata* refers to its potential to kill *R. (Boophilus) microplus*. Crude flower extract of *C. auriculata* underscores its potential as a natural alternative to synthetic acaricides in pest control applications, with the added benefits of being eco-friendly and potentially safer for humans and the environment.[23]

Egg immersion test

Egg immersion test is done following the methodology of the literature support, with slight modification. Different concentrations (25, 50, 75, 100 µg/mL) of crude flower extract of *C. auriculata* were added into a tick egg placed in tubes. Distilled water is considered as control, and the tubes were incubated for 24 h at 27–28°C with 80–90% relative humidity.[24]

Larval immersion test

Larval immersion test is followed by the methodology of literature support with slight modification. Different concentrations (25, 50, 75, 100 µg/mL) of crude flower extract of *C. auriculata* were prepared, and approximately 50 larvae were added to each tube.[25] Distilled water is considered as the control, and the tubes are closed immediately after the larvae were added and shaken vigorously for a few seconds. Afterward, the tubes were opened, and the larvae were transformed into filter paper placed in a petridish. The petridish was incubated for 24 h at 27–28°C with 80–90% relative humidity.[26]

Nymph immersion test

Nymph immersion test was done following the method of the literature support with slight modification. Engorged nymphs from *R. (Boophilus) microplus* were split into three replicates at four concentrations. The nymphs were submerged in different concentrations (25, 50, 75, 100 µg/mL) of crude flower extract of *C. auriculata*. In control, nymphs were submerged in distilled water. The previously mentioned conditions were used for the nymphs' incubation, and the mortality of the tick was monitored regularly.[27,28]

Adult immersion test (AIT)

The acaricidal activity of crude flower extract of *C. auriculata* was tested against adult engorged females by AIT. Roughly fifty engorged ticks were kept in each petri dish. Ticks are separated into five groups treated against different concentrations of flower extract, and a control. The ticks were individually kept in a petri plate with holes, and they were incubated for 24 h, and maintained the humidity and temperature levels.[29,30] After experimentation, the morphological alterations of dead ticks were observed under a stereo microscope. The percentage of mortality was calculated using the following formula.

Number of dead ticks

Mortality % = Total number of ticks × 100.

Statistical analysis

The obtained data (mean ± standard deviation) were analyzed to test various concentrations of crude flower extract of *C. auriculata* on tick mortality using Microsoft Excel and Tukey's honest significant difference test. $P \leq 0.05$ is considered the significance level to categorize noteworthy variances among the treated and control groups, in which all the comparative graphs of statistical analysis are plotted. A triplicate assay was carried out for each experiment.

RESULTS

The efficacy of crude flower extract of *C. auriculata* against *R.* (*Boophilus*) *microplus* was determined by calculating the percentage of egg, larvae, nymph, and adult mortality. Live ticks treated with various concentrations lay considerably changes ($P < 0.001$) compared to control ticks.

Egg immersion

The microscopic image of *R. (Boophilus) microplus* egg shows the developmental stage in the control and not any changes in those treated with the *C. auriculata* flower extract. In addition, shrinkage of the egg and undeveloping embryo was also observed in the treated group [Figure 1].

Larval immersion

The larvae of *R. (Boophilus) microplus* show 80% mortality in 100 µg/µL concentration. As the concentration of *C. auriculata* flower extract increases, the rate of mortality also increases simultaneously. Meanwhile, the control larvae do not express any physical changes or mortality [Figure 2a].

Nymph immersion test

The toxicity results against *R. (Boophilus) microplus* nymph exposed to *C. auriculata* flower extract at different concentrations (25, 50, 75, and 100 µg/mL) are presented in Figure. Nymph of *R. (Boophilus) microplus* after exposure to the flower extract resulted higher percentage of mortality in the highest concentration (75, 50 µg/µL) compared to the non-treated control group [Figure 2b].

Adult immersion

In the current study, the AIT was done against *R. (Boophilus) microplus* using the flower extract of *C. auriculata*, to evaluate the acaricidal activity. The mortality percentage

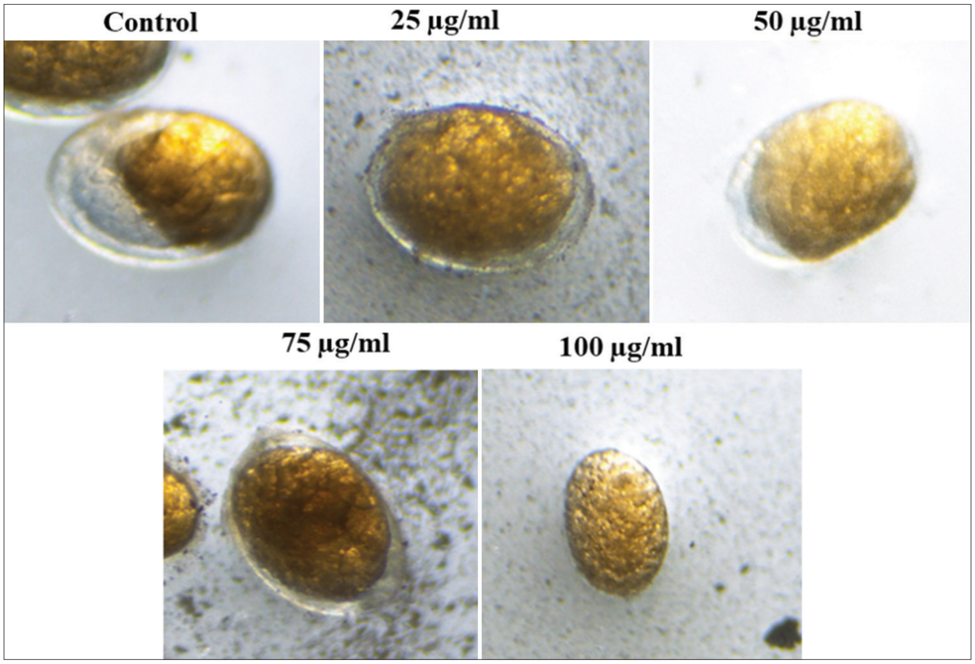

Figure 1: Acaricidal activity of egg of *Rhipicephalus (Boophilus) microplus* in control and treated with different concentrations of *Cassia auriculata* crude flower extract

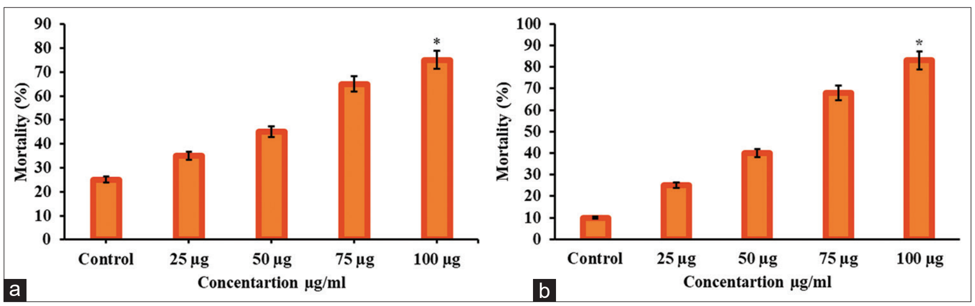

Figure 2: Acaricidal activity of (a) larva and (b) nymph of *Rhipicephalus (Boophilus) microplus* in control and treated with different concentrations of *Cassia auriculata* crude flower extract

was assessed by the death of the tick. Flower extract resulted higher percentage of tick mortality (78%) compared to the non-treated control [Figure 3].

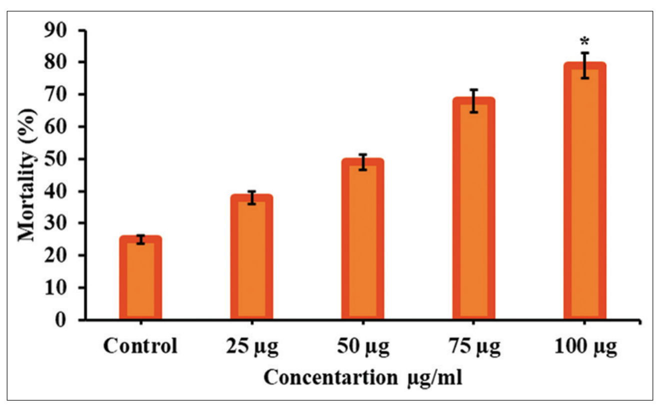

Figure 3: Acaricidal activity of adult *Rhipicephalus (Boophilus) microplus* in control and treated with different concentrations of *Cassia auriculata* crude flower extract

Morphological changes

In the present study, after experimentation, no morphological changes were observed in the control group. However, *R. (Boophilus) microplus* showed morphological alterations after treatment with the crude flower extract of *C. auriculata*. After experimentation, outward shrinkage, minor reduction, and hardness in the dorsal shield (scutum), complete loss of hardness in the dorsal scutum, and complete shrinkage in the whole body of the tick were observed [Figure 4].

DISCUSSION

The southern cattle tick, *R.* (*Boophilus*) *microplus*, is a major ectoparasite affecting livestock worldwide, particularly in tropical and subtropical regions.[31] This tick species is a vector for numerous pathogens, including *Babesia bovis* and *Anaplasma marginale*, causing significant economic losses in cattle industries due to decreased productivity and

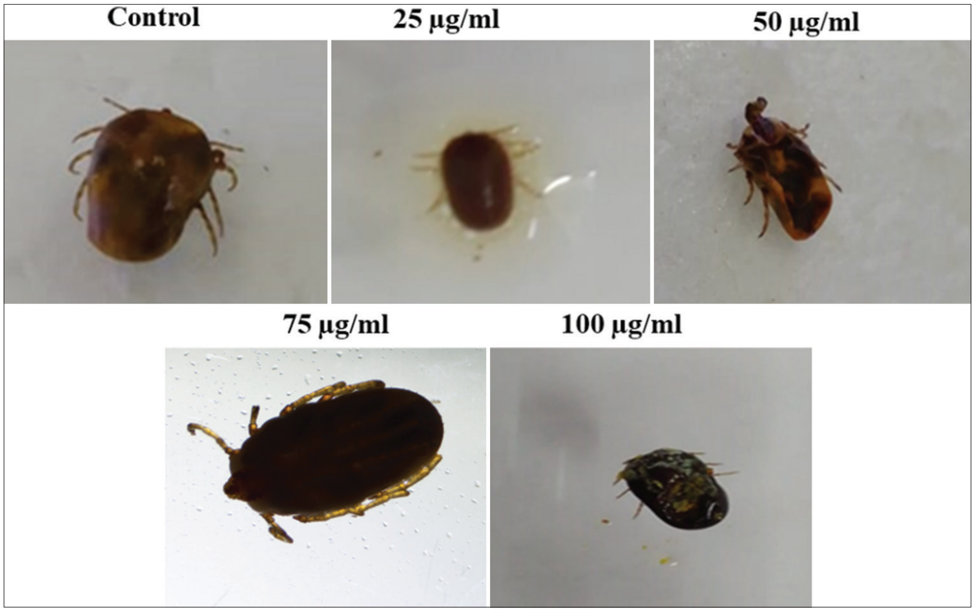

Figure 4: Morphological changes of *Rhipicephalus (Boophilus) microplus* treated with different concentrations of *Cassia auriculata* crude flower extract, and the arrow indicates the reduction of scutum

increased veterinary costs.[4,32] In general practice, to control *R. microplus* involving synthetic acaricides, which have led to preventing the development of resistance, environmental contamination, and concerns over residues in animal products. Hence, there is a great need for exploring the alternative control approaches, including use of medicinal plants extracts and plant-derived compounds. *C. auriculata*, commonly known as Tanner's Cassia or Avaram, is one of the most often used medicinal plants in traditional medicine, it also has promising acaricidal properties. In the traditional medicine system, *C. auriculata* used in various therapeutic properties, including anti-microbial, anti-inflammatory, and anti-diabetic properties.[14,15] The acaricidal effects of *C. auriculata* against cattle tick have been a subject of scientific investigation mainly targeting all stages of the tick, such as egg, larva, nymph, and adult. The bioactive compounds present in *C. auriculata,* such as flavonoids, saponins, and tannins in various parts of the plant, including leaves, flowers, and park. These compounds are well known to disrupt the various pharmacological processes of ticks, finally leads the mortality and interference of reproductive process. For instance, the flavonoids form *C. auriculata* disrupt the enzymatic pathways which is crucial for the tick's survival and reproduction. The results of these experiments demonstrate that its promising acaricidal activity against cattle tick *R. (Boophilus) microplus*. The observed a dose-dependent activity against cattle tick suggests a specific mode of action, which significantly involving interrupting the vital physiological processes for tick survival. The present study demonstrates the crude flower extract of *C. auriculata* exhibited significant acaricidal activity across all the tested concentrations, in the highest concentration 100 µg/mL shows highest mortality than other concentrations. These findings suggest that the plant extract of *C. auriculata* causes increased mortality rates in a dose-dependent manner, and indicating that the higher concentration of the extract was more effective in killing the ticks. At low concentration 25 µg/mL, the extract caused notable mortality but was less effective compared to the higher concentrations. At 50 µg/mL, the concentration shows a significant rise in mortality, particularly targeting the larvae and nymph states, which are more susceptible to acaricidal agents due to their smaller size and thinner cuticle layers. Whereas the 75 µg/mL concentration showed significant improvement in efficiency, with a substantial increase in mortality in all life stages. These findings demonstrate the bioactive compounds rich in higher concentrations in the extract are sufficiently potent to disrupt the physiological processes of the ticks and lead the high mortality rates. A concentration of 100 µg/mL resulted the highest mortality rate compared to others. The adult ticks are typically more resistant to the chemical reagents due to their thick and large size cuticle; however, the plants extract shows significant mortality in all life stages of ticks, which indicating the plant extract comprehensive efficacy.

The mechanism behind the acaricidal activity of *C. auriculata* crude flower extract attributed the presence of various secondary metabolites. These bioactive compounds likely interact with the tick's nervous system, metabolic processes, and cuticle, and finally lead the death. For instance, the flavonoids present in the plant extract possess significant neurotoxic properties, which can interfere with tick's nervous system and cause paralysis and death. The bioactive compounds such as tannins and saponins, in the plant extract, can disrupt the integrity of the tick's cuticle, leading dehydration and mortality. In the early stage of life cycle, the egg stage exhibited the least sensitivity to the extract, which is consistent with the general observation that eggs are often more resistant to acaricidal agents due to their protective chorion. However, even at this stage, higher concentration (75 and 100 µg/ml) significant reduction in hatching rates, which indicating the plant extract effectively penetrated the egg chorion and affect the development of embryo. The significant findings of the present study demonstrated the potential effect of potential of *C. auriculata* crude flower extract against cattle tick. The dose-dependent increase in mortality highlights the importance of optimizing the optimal concentration of extract which effective tick control. This could be particularly advantageous in the development of sustainable tick control strategies and also eco-friendly nature, and significantly reduce the application of synthetic acaricides that often lead to resistance issues and environmental contamination. The previous reports also supported the present findings; the extract from *A. indica* at 80 mg/mL concentration showed the lowest LC_{50} and LC99 values compared to *Ricinus communis* and *Calotropis gigantea* against *R. (Boophilus) microplus*. The study carried out with ethanolic extract of *C. auriculata* significantly decreased egg hatchability, and interpreting the life cycle at an early stage.[33] In addition, larval packet tests experiment indicated that the exposure to *C. auriculata* extracts showed a high mortality rate among the larvae. These findings suggest that the bioactive compounds present in the flower extract may act as potent neurotoxins and significantly disrupt the cuticle integrity of the larvae and leads the death. The flower extract of *C. auriculata* also create a remarkable acaricidal activity against the nymphal and adult stage of *R. microplus.*

In vitro immersion test results also demonstrated the treated group nymphs exhibit reduced motility due to the disruption of hormonal changes, which are crucial for motility. The exposure of adult with plant extract also reported that the impaired reproductive capabilities including reduced oviposition and lower egg viability. These findings suggested that the bioactive compounds present in the flower extract are responsible for the interruption and malfunction in the reproductive organs and alters the hormonal changes. Furthermore, the acaricidal properties of *C. auriculata* are comparable to other chemical acaricides, but the nature of less likely to contribute to resistance development and biodegradable nature, flower extract has added advantages. Hence, the application of plant-based acaricides makes them as potent attractive alternatives for integrated pest management programs. In the present study, acaricidal activity of *C. auriculata* crude flower extract against *R.* (*Boophilus*) *microplus* is well-documented, with supporting evidence of efficient acaricidal activity across various developmental stages of the tick. The bioactive compounds in the flower extract play a crucial role in physiological processes in ticks and leading mortality and reduced reproduction. Due to the disadvantage of chemical and synthetic acaricides, *C. auriculata* represents a promising natural alternative for management of *R. microplus* and contributing to more sustainable and environmentally friendly pest management strategies. Hence, further research is needed to focus the isolation and characterization of potent bioactive compounds from *C. auriculata* which are responsible for their acaricidal activity, and development of optimized extraction methods to enhance the efficacy of *C. auriculata* in flied trials. Additional investigations also need to be know the mechanism of action and development of formulation strategies for practical application in the livestock management. Overall findings of this study demonstrated that the potential acaricidal activity of *C. auriculata* also suggests that a novel source of potent acaricidal compounds for sustainable tick control. Further research will focus on the field evaluation and the formulation of standardized plant extracts to ensure consistency and efficiency in practical applications.

CONCLUSION

The present study carried out to evaluate the acaricidal activity of *C. auriculata* flower extracts against the southern cattle tick *R.* (*Boophilus*) *microplus* with various developmental stages of cattle tick, including egg, larvae, nymph, and adult. The results revealed that the flower extracts exhibit significant efficacy with increasing concentrations, leading to higher mortality rates. The different concentrations from low to high dose (25, 50, 75, and 100 µg/mL) show significant efficacy with increasing concentrations leading to higher mortality rates in dose dose-dependent manner increase acaricidal effects. The highest concentration, 100 ug/mL led to a substantial reduction in hatching rates, suggesting that interference with embryonic development. When the larvae exposed to the same concentration exhibited significantly increased mortality, likely due to the extracts penetration and disruption of their cuticle and internal development. In case if nymphs, the highest exposure group showed the highest mortality, which can be attributed to the impact of extract on their motility and development. Adult exposed to the highest concentration depicted the highest mortality rate, which revealed that the flower extract effectively disrupting the feeding and reproductive capacity of tick and finally leads the death. The overall finding indicates the *C. auriculata* flower extract exhibited high efficiency as an acaricide, particularly at higher concentrations. This suggests its potential.

This suggests its potential effectiveness as a natural and eco-friendly alternative to synthetic acaricides for controlling tick infestations. Further research in the field of isolating the bioactive compounds present in the extract and their specific modes of action could provide in-depth understanding into its acaricidal efficacy and pave the way for developing effective and potent target-specific treatments. In addition, field trials and estimations of any potential non-target effects are crucial to confirm its safety and practical applicability in diverse environmental settings.

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