RESEARCH ARTICLE

Evaluation of Plant-Based Acaricides for Tick Control in Cattle: A Sustainable Alternative

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Abstract

Tick infestations pose a major challenge, reducing animal health, productivity, and profitability while also transmitting haemoprotozoan and rickettsial diseases. Excessive reliance on chemical acaricides has led to environmental concerns, harm to non-target species, and the emergence of acaricide-resistant ticks, necessitating sustainable alternatives. This study evaluated the acaricidal efficacy of deltamethrin as standard treatment, polyherbal readymade spray, aqueous extracts from *Annona squamosa* (sugar apple), and *Polyalthia longifolia* (Ashoka) as eco-friendly tick control measures. A total of 71 cattle was selected and assessed for tick counts, haematological parameters, and cortisol levels. The study revealed the highest tick reduction with polyherbal readymade spray, followed by the combined and individual plant extracts with significant improvement in haematological parameters in treated groups by Day 28, while untreated cattle showed reverse trend. Similarly, cortisol level, elevated due to infestation, normalized in treated groups but worsened in controls, indicating persistent stress. These findings highlight the potential of plant-based acaricides as effective and sustainable alternatives for tick management in cattle.

Keywords: Cattle, Cortisol levels, Haematological analysis, Plant-based acaricides, Tick control.

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Introduction

Livestock is a crucial pillar of India's agricultural economy, contributing significantly to GDP and rural livelihoods. However, the sector faces substantial economic losses due to ectoparasites, particularly ticks, which negatively impact animal health, productivity, and profitability. Belsare *et al.* (2025) recorded an alarming overall prevalence of 53.30% tick infestation in cattle of Western Maharashtra, notably calves under one year showed even greater susceptibility (70.00%), and among different demographics, animals with poor BCS (76.4%, crossbreds (59.70%), and female cattle (54.60%) experienced higher infestation levels compared to those with good BCS (23.6%), non-descript / native breeds (45.70%) and males (48.80%). Ticks serve as vectors for various haemoprotozoan and rickettsial diseases, exacerbating the burden on livestock farmers. While chemical acaricides have been the primary method of tick control, their extensive use has led to environmental pollution, harm to non-target species, and the emergence of acaricide-resistant tick populations. This necessitates the exploration of sustainable, eco-friendly alternatives for tick management, particularly leveraging India's rich heritage of medicinal plants and ethno-veterinary practices (Kaur *et al*., 2015; Varadharajan and Gnanasekar, 2019; Khillare and Kaushal, 2021).

The plant-based acaricides derived from *Annona squamosa* and *Polyalthia longifolia* present a promising natural alternative for tick control. These plants are known for their bioactive compounds with insecticidal, antimicrobial, and anti-inflammatory properties. *A. squamosa*, commonly known as sugar apple, has demonstrated insecticidal activity, while *P. longifolia*, or Ashoka, possesses significant antimicrobial and insecticidal properties (Katkar *et al*., 2010; Kumar *et al*., 2021). This study aims to evaluate the efficacy of extracts from these plants, both individually and in combination, against tick-infested cattle.

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Materials and Methods

Collection, Identification, and Processing of Plant Materials

The present study focused on the collection, identification, processing, and extraction of *Annona squamosa* and *Polyalthia longifolia* leaves for further experimental use, selecting these plants based on their traditional medicinal significance. Leaves were collected from the Medicinal Plant Unit at the Campus of Veterinary College, Kamdhenu University, Anand, and their identification and authentication were verified by the Department of Genetics and Plant Breeding, B.A. College of Agriculture, Anand Agricultural University, Anand. After collection, the leaves were thoroughly washed with distilled water to remove unwanted particles, shade-dried for about a week to retain phytochemical properties, and ground into fine powder using a mechanical grinder before storage in airtight containers to prevent contamination. For aqueous extraction, 100 g of dried leaf powder from each plant was soaked in 1 litre of distilled water with intermittent shaking every 2 h over 48 h, followed by filtration using muslin cloth. The filtrates were then concentrated in a water bath at 50°C until the solvent completely evaporated, and the resulting dried extracts were stored in airtight glass containers at 4°C to maintain stability and potency for future experimental applications.

Therapeutic Management

A total of 71 cattle including 8 healthy as well as 63 tick infested cattle divided into seven groups were used to evaluate the acaricidal activity of a polyherbal readymade spray (*Acorus calamus, Allium sativum, Azadirachta indica, Curcuma longa, Ocimum tenuiflorum*, and *Zingiber officinale*) and aqueous plant extracts of *Annona squamosa* and *Polyalthia longifolia* leaves individually and in combination as outlined in Table 1.

Table 1: Different Group of Animals

Groups	Animals	Treatments
Group 1 (Healthy Control)	8	Healthy Animals.
Group 2 (NegativeControl)	8	Tick infested cattle treated with distilled water.
Group 3 (Standard Control)	8	Tick infested cattle treated with deltamethrin @ 2.0 mL/liter aqueous diluted solution for the first day and repeated on Day 16.
Group 4	8	Tick infested cattle treated with 7% aqueous extract of *Annona squamosa* once a day for the first four days and repeated on days 8, 12, 16, 20, 24, and 28.
Group 5	8	Tick infested cattle treated with 7% aqueous extract of *Polyalthia longifolia* once in a day for the first four days and repeated on days 8, 12, 16, 20, 24, and 28.
Group 6	8	Tick infested cattle treated with combination (1:1) of both plant aqueous extract (*Annona squamosa* and *Polyalthia longifolia*) once in a day for the first four days and repeated on days 8, 12, 16, 20, 24, and 28.
Group 7	23	Tick infested cattle treated with polyherbal readymade spray for the first four days and repeated on days 8, 12, 16, 20, 24 and 28.

Haematological Parameters and Cortisol Estimation

Blood samples were collected from tick-infested cattle before treatment (day 0) and post-treatment on days 16 and 28. Six mL of blood was drawn into K_3EDTA vials as well as in clot activator vials to analyze haemoglobin, packed cell volume, erythrocyte and leucocyte counts and, sera obtained from blood collected in clot activator vials, centrifuged at 1650 g for 3 min, were analyzed for cortisol levels by using RIA technique.

Counting of Ticks

Tick burden was estimated using the one-side tick count method, where the total ticks on one side were doubled to extrapolate the full-body count (Miraballes *et al*., 2022). Ticks were counted before treatment (day 0) and post-treatment on days 4, 16, 24 and 28.

Estimation of Efficacy

The efficacy of the treatment groups was calculated by comparing the number of ticks present before and after the treatment (Katuri *et al.,* 2017).

$$\% \text{ Efficacy} = \frac{\text{No. of ticks pre-treatment} - \text{No. of ticks post-treatment}}{\text{No. of ticks pre-treatment}} \times 100$$

Statistical Analyses

The data generated was analyzed to derive means and standard errors of various parameters studied, and statistically analyzed using two way analysis of variance and Duncan's post hoc test at $p < 0.05$ as per Snedecor and Cochran (1994) using IBM sioftware.

Results and Discussion

In-Vivo Evaluation of Acaricidal Activity

The 7% aqueous extracts of *Annona squamosa* and *Polyalthia longifolia* leaves, individually and combined, were evaluated in comparison with deltamethrin and herbal readymade spray for *in vivo* acaricidal efficacy in cattle, following protocols from Parte *et al.* (2014), Ilham *et al.* (2014), and Varadharajan and Gnanasekar (2019). The efficacy of the extracts was assessed by evaluating the reduction in tick counts within the observed area at different treatment intervals. The *in-vivo* acaricidal efficacy was assessed for different groups over Days 4, 16, 24, and 28. Group 2 (Negative control) showed no acaricidal activity throughout the study period (0 ± 0 for all day). Group 3 (Standard control, Deltamethrin) demonstrated the highest efficacy (%) against cattle ticks, starting from

31.59 ± 3.01 on Day 4, increasing to 38.37 ± 3.75 on Day 16, 95.92 ± 2.00 on Day 24, and peaking at 98.22 ± 0.76 on Day 28. Group 4 (extract of *Annona squamosa*) and 5 (extract of *Polyalthia longifolia*) exhibited a gradual increase in efficacy (%) from Day 4 to Day 28. Group 6 (1:1 Combination of *Annona squamosa* and *Polyalthia longifolia* extracts) showed a consistent increase in acaricidal efficacy (%), starting from 17.12 ± 2.23 on Day 4, reaching 33.68 ± 1.84 on Day 16, 63.24 ± 2.51 on Day 24, and 81.24 ± 4.02 on Day 28. Group 7 (polyherbal readymade spray) gradual increase in efficacy (%) from 19.47 ± 1.1 on Day 4 to 96.35 ± 0.75 on Day 28. A significant decrease in average tick counts was recorded in all treatment groups (3, 4, 5, 6 and 7), whereas no significant reduction was observed in the control group (group 2), and no ticks were noted in healthy control group 1. On day 28, group 3 showed the highest reduction in tick counts, followed by groups 7, 6, 4, and 5, with no notable decrease in group 2 (Table 2). Parte *et al.* (2014), and Varadharajan and Gnanasekar (2019) found the combination of *Azadirachta indica*, *Annona squamosa*, and *Polyalthia longifolia* extracts having the highest acaridical efficacy, followed by *A. squamosa*, while *Polyalthia longifolia* was the least effective.

Table 2: *In-vivo* evaluation of per cent acaricidal efficacy (Mean ± SE)

Groups	Post-treatment acaricidal efficacy (%)			
	Day 4	Day 16	Day 24	Day 28
Group 3	31.59 ± 3.01^{Ac}	38.37 ± 3.75^{Aa}	95.92 ± 2.00^{Ba}	98.22 ± 0.76^{Ba}
Group 4	12.42 ± 1.28^{Aa}	32.74 ± 1.67^{Ba}	47.67 ± 2.37^{Cd}	71.91 ± 3.54^{Dc}
Group 5	15.30 ± 1.91^{Aab}	33.61 ± 2.87^{Ba}	54.47 ± 4.25^{Cd}	66.81 ± 2.85^{Dc}
Group 6	17.12 ± 2.23^{Aab}	33.68 ± 1.84^{Ba}	63.24 ± 2.51^{Cc}	81.24 ± 4.02^{Db}
Group 7	19.47 ± 1.1^{Ab}	67.59 ± 2.22^{Bb}	86.01 ± 2.06^{Cb}	96.35 ± 0.75^{Da}

Values within the row with superscripts A, B, C, D and within column with superscripts a, b, c, d differ significantly (p<0.01).

Haematological Alterations

The haematological data indicate significant variations among the experimental groups over time (Table 3). Haemoglobin (Hb), total erythrocytes count (TEC), and packed cell volume (PCV) remained stable in Group 1 (healthy control) across all time points. Group 2 (Negative control) had Hb 6.39 ± 0.34 g/dL, TEC 6.22 ± 0.53 ×10^6/µL, and PCV 19.00 ± 1.21% on Day 0, and it showed a significant decline in Hb, and non-significant change in TEC, and PCV on Days 16 and 28. In contrast, Group 3 (Deltamethrin) showed significant improvement in Hb from 6.69 ± 0.23 to 9.65 ± 0.2 g/dL, TEC from 6.07 ± 0.51 to 9.87 ± 0.29 ×10^6/µL, and PCV from 22.94 ± 0.8 34.70 ± 0.72 % between day 4 and 28 post-treatment. Similarly, Group 4 and 5 (Aqueous plant extracts) also showed significant improvement in these haematological parameters. The most notable recovery was in Group 6 (Combination), where Hb increased from 6.58 ± 0.21 to 11.20 ± 0.32 g/dL, TEC from 5.63 ± 0.32 to 10.74 ± 0.46 ×10^6/µL, and PCV from 18.18 ± 1.23 to 34.43 ± 1.21%. Group 7 (Polyherbal commercial spray) followed a similar trend to that of group 6. Total leucocytes count (TLC, 10^3/µL), however, showed no significant changes across all groups (Table 3). Kumar *et al*. (2010) and Kachhawa *et al*. (2016) observed a decline in haemoglobin, TEC, and PCV in tick-infested animals, while Bhikane *et al*. (2018) reported similar reductions in TEC and PCV but found no significant change in haemoglobin levels. Goswami *et al*. (2024) further confirmed a significant decrease (p<0.05) in haemoglobin, PCV and TEC in tick infested cattle.

Cortisol Levels

Group 1 (Healthy) cattle had a cortisol level of 12.06 ± 0.91 ng/mL. In Group 2 (Negative control), cortisol levels began at 21.65 ± 2.19 ng/mL on Day 0, increased gradually to 27.59 ± 1.1 ng/mL by Day 28. In Group 3 (Deltamethrin), cortisol levels decreased from 21.14 ± 0.72 ng/mL on Day 0 to 16.1 ± 1.32 ng/mL on Day 16, and 11.42 ± 0.77 ng/mL on Day 28. Group 4 and 5 showed a similar reduction from 23.16 ± 0.76 and 20.16 ± 1.93 ng/mL on Day 0 to 10.30 ± 0.43 and 10.23 ± 0.62 ng/mL on Day 28, respectively. Group 6 and 7 also showed a similar drop in cortisol levels. On Days 0 and 16, cortisol levels in all treatment groups (3, 4, 5, 6 and 7) and the control group (Group 2) were significantly higher compared to the healthy group (Group 1). By Day 28, cortisol levels in the treatment groups returned to levels comparable to the healthy group, with no significant differences observed (Table 4). These findings suggest that effective tick control not only reduces parasitic load but also plays a crucial role in mitigating stress in cattle.

Conclusion

The study evaluated the acaricidal efficacy of a polyherbal readymade spray, a 7% aqueous extract of *Annona squamosa*, and a 7% aqueous extract of *Polyalthia longifolia* against standard deltamethrin on tick-infested cattle. Among the treatments, the herbal readymade spray demonstrated the highest tick reduction, at par with deltamethrtin by day 28, followed by the combined and individual extracts.

Haematological parameters (Hb, TEC, and PCV) were significantly lower in tick-infested cattle, but recovered to healthy levels in treated groups by Day 28, while the control group showed further decline. Additionally, cortisol levels were initially elevated in tick-infested cattle, but normalized in treated groups by Day 28, while the control group exhibited a further increase, indicating persistent stress. The effect of the herbal treatments highlights their potential as alternatives to synthetic acaricides for managing ticks in cattle.

Table 3: Haematological parameters estimation in different groups (Mean ± SE)

Parameters	Groups	Day 0	Day 16	Day 28
Haemoglobin (Hb, g/dL)	Group 1	10.67 ± 0.39^{a}		
	Group 2	6.39 ± 0.34^{Bb}	5.06 ± 0.29^{Ac}	5.15 ± 0.34^{Ac}
	Group 3	6.69 ± 0.23^{Ab}	8.13 ± 0.29^{Bb}	9.65 ± 0.2^{Cb}
	Group 4	6.28 ± 0.5^{Abc}	8.66 ± 0.53^{Bb}	10.29 ± 0.35^{Cab}
	Group 5	6.61 ± 0.1^{Ab}	8.55 ± 0.31^{Bb}	9.56 ± 0.23^{Cb}
	Group 6	6.58 ± 0.21^{Ab}	8.93 ± 0.15^{Bb}	11.20 ± 0.32^{Ca}
	Group 7	5.47 ± 0.18^{Ac}	8.59 ± 0.26^{Bb}	9.37 ± 0.26^{Cb}
Total erythrocytes (10^6/µL)	Group 1	10.52 ± 0.35^{a}		
	Group 2	6.22 ± 0.53^{b}	5.92 ± 0.41^{e}	6.51 ± 0.15^{b}
	Group 3	6.07 ± 0.51^{Ab}	7.00 ± 0.2^{Acd}	9.87 ± 0.29^{Ba}
	Group 4	5.26 ± 0.65^{Ab}	7.42 ± 0.58^{Bbc}	10.33 ± 0.68^{Ca}
	Group 5	5.25 ± 0.36^{Ab}	6.42 ± 0.16^{Bde}	7.43 ± 0.18^{Cb}
	Group 6	5.63 ± 0.32^{Ab}	8.23 ± 0.23^{Bb}	10.74 ± 0.46^{Ca}
	Group 7	5.16 ± 0.21^{Ab}	7.25 ± 0.19^{Bcd}	9.69 ± 0.26^{Ca}
Packed cell volume (%)	Group 1	29.34 ± 0.6^{a}		
	Group 2	19.00 ± 1.21^{cd}	20.29 ± 1.3^{d}	15.78 ± 1.2^{c}
	Group 3	22.94 ± 0.8^{Ab}	26.69 ± 0.83^{Bab}	34.70 ± 0.72^{Cb}
	Group 4	16.50 ± 1.78^{Ad}	25.30 ± 1.4^{Bbc}	32.89 ± 1.2^{Cab}
	Group 5	20.06 ± 1.12^{Ac}	26.41 ± 0.81^{Bab}	30.66 ± 0.56^{Ca}
	Group 6	18.18 ± 1.23^{Acd}	27.78 ± 1.05^{Bab}	34.43 ± 1.21^{Cb}
	Group 7	18.74 ± 0.45^{Acd}	23.37 ± 0.57^{Bc}	30.77 ± 0.95^{Ca}
Total leucocytes count (10^3/µL)	Group 1	6.74 ± 0.62		
	Group 2	7.95 ± 0.76	7.3 ± 0.56	9.14 ± 1.16
	Group 3	8.32 ± 0.99	9.13 ± 0.54	7.78 ± 0.2
	Group 4	7.12 ± 1.11	6.98 ± 0.78	8.54 ± 0.78
	Group 5	7.4 ± 0.32	7.84 ± 0.91	8.86 ± 0.94
	Group 6	9.04 ± 0.51	7.59 ± 0.38	7.67 ± 0.53
	Group 7	7.97 ± 0.39	9.1 ± 0.36	8.57 ± 0.41

Values within the row with superscripts A, B, C and within column with superscripts a, b, c, d, e differ significantly ($p<0.01$).

Table 4: Cortisol level (ng/mL) in therapeutic, control and healthy groups (Mean ± SE)

Group	Day 0	Day 16	Day 28
Group 1	12.06 ± 0.91^{a}		
Group 2	21.65 ± 2.19^{Ab}	24.34 ± 1.26^{ABc}	$27.59 \pm 1.1B^{b}$
Group 3	21.14 ± 0.72^{Ab}	16.1 ± 1.32^{Bb}	11.42 ± 0.77^{Ca}
Group 4	23.16 ± 0.76^{Ab}	15.64 ± 0.97^{Bb}	10.3 ± 0.43^{Ca}
Group 5	20.16 ± 1.93^{Ab}	13.96 ± 1.16^{Bab}	10.23 ± 0.62^{Ba}
Group 6	22.27 ± 1.46^{Ab}	14.37 ± 1.09^{Bab}	11.72 ± 0.46^{Ba}
Group 7	21.76 ± 0.62^{Ab}	15.96 ± 0.39^{Bb}	11 ± 0.33^{Ca}

Values within the row with superscripts A, B, C and within column with superscripts a, b, c differ significantly ($p<0.01$).

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