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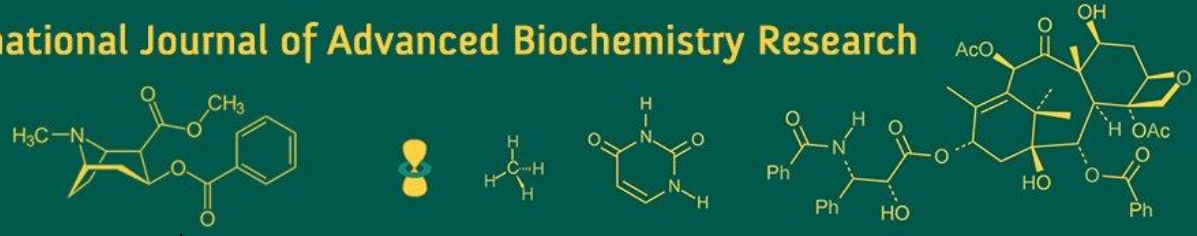

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Prevalence of tick infestation and *in vitro* larvicidal activity of polyherbal readymade spray and traditional plant extracts

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Abstract
Ticks and tick-borne diseases (TTBDs) pose a significant threat to livestock health, leading to economic losses of up to US$ 30 billion globally and US$ 498.7 million annually in India. Continuous use of chemical acaricides has resulted in resistant tick populations, environmental pollution, and harm to non-target organisms. In this study, 117 ticks were collected of which 72.65% were identified as *Rhipicephalus* spp. and 27.35% as *Hyalomma* spp. Age-wise tick infestation was highest in cattle over 48 months old (42.25%), followed by 13-24 months (22.54%), 1-12 months (19.72%), and 25-48 months (15.49%). Housing systems also influenced the prevalence of tick infestation, with higher infestation in loose housing (29.55%) compared to conventional housing (12.80%). The Larval Packet Test (LPT) was used to evaluate the efficacy of deltamethrin, polyherbal readymade spray, and herbal extracts against tick larvae. The Deltamethrin (2.0 mL/L) had the highest mortality (84.15%), followed by a polyherbal readymade spray (70.70%), a combination (1:1) aqueous extract of *Annona squamosa* and *Polyalthia longifolia* (65.53%), 7% aqueous extract of *Annona squamosa* alone (60.00%), and 7% aqueous extract of *Polyalthia longifolia* alone (51.72%). These results highlight the potential of plant-based alternatives for sustainable tick control. Integrating traditional herbal treatments with modern tick management strategies could offer an eco-friendly and effective solution to mitigate the impact of TTBDs on livestock productivity.

Keywords: Larval packet test (LPT), larvicidal activity, herbal acaricides

Introduction
Ticks and tick-borne diseases (TTBDs) pose a major challenge to livestock health and productivity, particularly in tropical and subtropical regions. These ectoparasites not only cause direct harm, such as blood loss, irritation, and reduced weight gain, but also serve as vectors for economically significant diseases like Babesiosis, Anaplasmosis, and Theileriosis. The economic burden of TTBDs is substantial, with global losses estimated at US$ 22 to 30 billion annually and an estimated US$ 498.7 million per year in India alone. Despite widespread reliance on chemical acaricides, their continuous use has led to the emergence of acaricide-resistant tick populations, causing environmental pollution and harming non-target species (Rajput *et al*., 2006; Singh *et al*., 2022; Sharma *et al*., 2012) [11, 14, 13].

To develop sustainable and effective tick control strategies, region-specific surveys are essential for assessing tick prevalence and resistance patterns. The extensive heritage of medicinal plants in India offers a promising avenue for alternative tick management approaches, with approximately 25,000 plant-based formulations widely used in traditional veterinary medicine. With over 1.5 million veterinary practitioners and 7,800 medicinal drug manufacturing units relying on plant-based treatments, the promotion of eco-friendly, non-toxic alternatives could help mitigate the impact of TTBDs while preserving environmental balance (Verma and Singh, 2008; Solomon *et al*., 2001) [20, 16]. A comprehensive strategy integrating traditional knowledge with modern scientific advancements is crucial to addressing TTBDs and sustaining livestock health and productivity in India.

Materials and Methods

Collection, Identification, and Processing of Plant Materials

This study involved the collection, identification, processing, and extraction of *Annona squamosa* and *Polyalthia longifolia* leaves, chosen for their traditional medicinal significance. The leaves were sourced from the Medicinal Plant Unit at the Veterinary College, Kamdhenu University, Anand, and their identification was authenticated by the Department of Genetics and Plant Breeding, B.A. College of Agriculture, Anand Agricultural University, Anand. Following collection, they were thoroughly washed with distilled water to eliminate impurities, shade-dried for approximately a week to preserve their phytochemical properties, and ground into a fine powder using a mechanical grinder. The powder was stored in airtight containers to prevent contamination. For aqueous extraction, 100 g of dried leaf powder from each plant was soaked in 1 liter of distilled water, with intermittent shaking every two hours over 48 hours. The extract was then filtered using muslin cloth, concentrated in a water bath at 50 °C until complete solvent evaporation, and stored as a dried extract in airtight glass containers at 4 °C to preserve its stability and potency for further experimental.

Collection and Identification of Ticks

Engorged ticks were collected early in the morning from cattle in both organized and unorganized dairy farms around Anand. Ticks were hand-picked or extracted using blunt-end forceps, focusing on areas like the ears, neck, thighs, and perianal regions. Collected specimens were placed in a ventilated container and examined under a stereomicroscope for identification based on key physical characteristics and standard identification keys from Soulsby (1982) [17]

Larva Packet Test for *In-Vitro* Study

The larval packet test (LPT) was conducted as per FAO (1984) [2] guidelines with slight modifications. Ticks were collected, cleaned, and incubated for oviposition. Hatched larvae were exposed to polyherbal readymade spray (*Acorus calamus*, *Allium sativum*, *Azadirachta indica*, *Curcuma longa*, *Ocimumten uiflorum*, and *Zingiber officinale*), 7% aqueous extract of *Annona squamosa* and *Polyalthia longifolia* individually and in combination (1:1) using impregnated Whatman No. 1 filter paper packets. After drying, packets were sealed and incubated at 27±3 °C and 85±5% relative humidity. Mortality was assessed after 24 hours, with larvae considered dead if unable to walk. Three replicates were maintained for plant extracts, a negative control (distilled water), and a standard control (Deltamethrin, 2 ml/L). Mortality was calculated using Abbott's formula (Abbott, 1925) [1].

$$\text{Corrected percent mortality} = \frac{\text{\% Test mortality-\% Control mortality}}{\text{100-\% Control mortality}} \times 100$$

Results and Discussion

Tick Morphological Identification

During the study, 117 tick samples were collected and morphologically identified, revealing 85 (72.65%) *Rhipicephalus* spp. and 32 (27.35%) *Hyalomma* spp. *Rhipicephalus* spp. was characterized by eyes, festoons, a short hypostome, hexagonal basum capitulum, and comma-shaped spiracles in females. *Hyalomma* spp. had eyes, a long hypostome, adanal shields in males, and triangular spiracles in females. Previous studies showed varying tick prevalence, with *Rhipicephalus* spp. being more common in some reports (Papa *et al*., 2009; Telmadarraiy *et al*., 2010) [8, 18], while others found *Hyalomma* spp. more prevalent (Gandhi *et al*., 2011; Mustafa *et al*., 2011) [3, 7], alongside the identification of *Boophilus* spp. (Gargili *et al*., 2011) [4].

Age-wise Prevalence of Ticks Infested Cattle

The study categorized 71 tick-infested cattle into four age groups, with the highest prevalence (42.25%) observed in those over 48 months old. Younger groups showed lower infestation rates: 19.71% (1-12 months), 22.54% (13-24 months), and 15.49% (25-48 months). These findings align with Kakar *et al*. (2017) [6] and Rony *et al*. (2010) [12], who reported higher infestation in older cattle. However, Kabir *et al*. (2011) [5] and Singh and Rath (2013) [15] found higher prevalence in younger animals.

Table 1: Age-wise prevalence of ticks infested cattle (n=71)

Age	No. of Animal	Percent
1 month to 12 months	14	19.72%
13 months to 24 months	16	22.54%
25 months to 48 months	11	15.49%
> 48 months	30	42.25%
Total	71	100%

Housing System wise Prevalence of Ticks Infested Cattle

The prevalence of tick-infested cattle varied significantly between different housing systems. During the study, 255 animals were observed, with 211 kept in conventional housing and 44 in loose housing. Among the loose housing group, 29.55% (13/44) had tick infestations, while 12.80% (27/211) of animals in conventional housing were infested. These results indicate a higher prevalence of tick infestations in animals housed in the loose housing system compared to those in conventional housing (Table 2).

Table 2: Housing system wise prevalence of ticks infested cattle

Housing System	No. of Cattle Observed	No. of Tick infested Cattle	Percent
Loose housing	44	13	29.55%
Conventional housing	211	27	12.80%
Total	255	40	

Patel *et al*. (2019) [10] observed a higher prevalence of tick infestations in animals kept on earthen floors (69.70%), followed by those on cement concrete floors (20.25%) and stone-paved floors (10.05%). The cracks and crevices in earthen or wooden floors provide hiding places and create favorable conditions for tick propagation. In contrast, animals in conventional housing systems have more space to move, which may reduce the likelihood of tick infestations.

In-Vitro Evaluation of Larvicidal Efficacy by the Larval Packet Test (LPT)

The Larval Packet Test (LPT) was performed following FAO (1984) [2] guidelines with minor modifications. A total of 100 larvae, aged 14 to 21 days, were placed in packets treated with different regimens, and mortality was observed after 24 hours using a hand lens.

Fig 1: Eggs laid down by tick

Fig 2: Hatched Larvae

Fig 3: Preparation of Packets

Fig 4: Counting of larvae after 24 hours

The test evaluated the larvicidal effects of distilled water as a control (Group 1), Deltamethrin at 2.0 mL per liter of water (Group 2), 7% aqueous extract of *Annona squamosa* (Group 3), a 7% aqueous extract of *Polyalthia longifolia* (Group 4), 1:1 combination of aqueous herbal extract of *Annona squamosa* and *Polyalthia longifolia* (Group 5), and a polyherbal readymade spray (Group 6). Mortality was calculated as the percentage of dead larvae relative to the total larvae in each packet.

The higher larval mortality was recorded with Deltamethrin at a concentration of 2.0 mL per liter of water, showing the most efficacy at 84.15±3.40%. This was followed by the polyherbal readymade sprayshowing efficacy at 70.7±3.5%. The 1:1 combination of aqueous herbal extract, which achieved a mortality rate of 65.53±2.50%. The 7% aqueous extract of *Annona squamosa* showed a mortality rate of 60.00±4.00%, while the 7% aqueous extract of *Polyalthia longifolia* resulted in 51.72±2.40% mortality after 24 hours.

Table 3: *In-vitro* evaluation of larvicidal efficacy by the larval packet test

Group	No. of Larvae in Packet	Larvicidal efficacy by Corrected percent mortality			Percentage (Mean ± SE) Mortality
		Replicate 1	Replicate 2	Replicate 3	
Group 1 (Distilled water as a control)	100	0	0	0	0
Group 2 (Deltamethrin at 2.0 mL per liter of water)	100	86.46	88.66	77.32	84.15±3.40
Group 3 (7% aqueous extract of *Annona squamosa*)	100	66.67	60.82	52.58	60.00±4.00
Group 4 (7% aqueous extract of *Polyalthia longifolia*)	100	52.08	55.67	47.42	51.72±2.40
Group 5 (1:1 combination of Group 5 and Group 6 extract)	100	69.79	65.98	60.82	65.53±2.50
Group 6 (Polyherbal readymade spray)	100	72.92	63.92	75.26	70.7±3.5

Parte *et al.* (2014) [9] and Varadharajan and Gnanasekar (2019) [19] found that the combination of aqueous extracts of *Azadirachta indica*, *Annona squamosa*, and *Polyalthia longifolia* was the most effective, followed by *A. squamosa* and *A. indica*, which showed similar efficacy. The extract of *Polyalthia longifolia* was less effective than the other two.

Conclusions

The research indicates a significant prevalence of *Rhipicephalus* spp. (72.65%) and *Hyalomma* spp. (27.35%) in cattle affected by ticks, with older cattle in loose housing systems showing greater rates of infestation. These results imply that both age and housing conditions play a crucial role in the tick infestation. The effectiveness of acaricide treatments evaluated through the Larval Packet Test (LPT), revealed that Deltamethrin resulted in the highest mortality rate among larvae followed by polyherbal readymade spray and single herbal extracts. These findings underline the necessity for focused tick control measures within cattle populations that are at higher risk. Further research is recommended to optimize herbal formulations for sustainable tick management.

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