Pharaonic Journal of Science
https://pjscience.org/
PJS SPBH

Original Article

Adulticidal, Larvicidal, and Repellent Effects of Ethanolic *Azadirachta indica* Seed Extract Against *Rhipicephalus microplus*

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DOI: https://doi.org/10.71428/PJS.2026.0307

Abstract

Ticks, especially *Rhipicephalus microplus*, are a major threat to livestock, as they cause blood loss, skin damage, poor weight gain, and the spread of tick-borne pathogens. Traditional control measures rely on synthetic acaricides, which have led to resistance after prolonged use, environmental pollution, and residues in animal products. The study evaluates the adulticidal, larvicidal, and repellent properties of an ethanolic extract of *Azadirachta indica* (Neem) seeds against *R. microplus*. A crude ethanolic extract (yield: 15.2%) was tested at concentrations ranging from 1.25–20% using adult immersion, larval immersion, and tick-climbing repellency assays. Dose-dependent acaricidal effects were observed to be significantly high with adult mortality of 85% and larval mortality of 92% recorded at 20% extract. The LC_{50} and LC_{90} values for adults were 6.23 and 18.75%, respectively, and the LC_{50} and LC_{90} values for larvae were 4.85 and 15.42%, respectively. Reproductive parameters were also adversely affected at the highest concentration: hatchability was 28%, and oviposition was inhibited by 88%. The highest repulsive percentage of 82.4 was recorded after 4 hours at 20% extract. These same findings indicate that the ethanolic extract of Neem seeds exhibits strong adulticidal, larvicidal, antireproductive, and repellent efficacy against *R. microplus*, thereby supporting its potential as a botanical acaricide candidate for further evaluation in tick management.

Keywords: *Rhipicephalus microplus*, *Azadirachta indica*, Ethanolic Extract, Adulticidal, Larvicidal activity

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Introduction

Ticks are hematophagous obligate ectoparasites that pose a major threat to livestock health and production worldwide [1]. One of the most economically significant ticks is *R. microplus*, or cattle tick, a highly destructive species that causes substantial economic losses in the livestock sector through blood loss, skin damage, and impaired weight gain [2]. The spread of tick-borne pathogens, including Babesia and Anaplasma species. Synthetic acaricides have long been used to control *R. microplus* [3]. However, their extensive and prolonged use has led to the emergence of resistant tick strains. It has also caused environmental pollution and the accumulation of residues in animal products, requiring safer and more sustainable methods. Botanical extracts have shown promise in recent years as bioactive agents for controlling ectoparasites, owing to their chemical constituents and relatively low toxicity to non-target organisms [4]. *A. indica*, which belongs to the family Meliaceae, has gained significant interest for its acaricidal, insecticidal, and repellent effects [5]. The bioactive compounds in neem seeds, leaves, and oil include azadirachtin, nimbin, and salannin that disrupt the growth, development, reproduction, and feeding of many arthropod pests [6]. The multimodal mechanism of action of these phytochemicals

Received: May 15, 2026. Accepted: July 20, 2026. Published: August 7, 2026

reduces the risk of resistance development and makes Neem a promising candidate for tick management [7]. Various studies have demonstrated the effectiveness of Neem extracts against tick species and have shown that they can control adults and larvae, reducing oviposition and hatchability [8,9]. Neem seeds are best extracted with ethanol, which yields higher bioactive compound yields and greater activity against ectoparasites [10]. Neem-based repellents have been useful in preventing ticks from attaching to hosts, serving as an alternative to direct acaricidal effects [11]. There have been very few studies on the effects of ethanolic Neem seed extracts, particularly regarding their combined adulticidal, larvicidal, and repellent properties against *R. microplus*, particularly under controlled conditions [12].

Given the growing concern about acaricide resistance and the need to implement environmentally friendly interventions, this research paper seeks to determine the effectiveness of ethanolic Neem seed extract against *R. microplus*. The experimental study examines adult and larval mortality, reproductive inhibition, and repellency, which will inform future use of Neem as a natural substitute for controlling ticks in cattle. By combining these bioassays, the study will help develop environmentally friendly, effective, and safe methods for controlling tick infestations with minimal use of synthetic chemicals.

Materials and Methods

Plant Material Preparation

A. indica seeds were obtained from the local market, air-dried, and ground into fine powder (48 h) using a seed grinder. The powder was placed in clean, dry glassware until extraction. The ethanolic extract was prepared by combining 300 g of Neem seed powder with 2700 mL of 95% ethanol (w/v, 1:10). The mixture was stirred intermittently, filtered, and the solvent was removed under reduced pressure at 27°C using a rotary evaporator (Xinchen® XZ-10L). The extract was kept at -4 °C until use. The formula used to calculate the percentage yield was:

$$\text{Yield (\%)} = \frac{\text{Weight of dry extract}}{\text{Weight of dry seed before extraction}} \times 100$$

Collection and Preparation of Ticks: A total of 300 *R. microplus* ticks were collected from cattle using blunt-ended forceps. Ticks were placed in 100mL ventilated plastic bottles and identified using a stereomicroscope according to standard identification guides. Of the collected ticks, 210 engorged adult females were used in the adult immersion test. The remaining engorged females were incubated at 27 ˚C and 85% relative humidity for oviposition and subsequent evaluation of reproductive parameters and larval emergence. Standard formulas were used to derive inhibition of oviposition (IO), egg hatchability (EH), reproductive index (RI), and reproductive efficiency (RE).

$$\text{IO (\%)} = \frac{(\text{IE}_{\text{control}} - \text{IE}_{\text{treated}})}{\text{IE}_{\text{control}}} \times 100$$

$$\text{EH (\%)} = \frac{\text{number of hatched eggs}}{\text{Total number of eggs}} \times 100$$

$$\text{RI (\%)} = \frac{(\text{Weight of eggs laid})}{(\text{Weight of adult females before treatment}) \times} 100$$

$$R\text{EI} = \frac{(\text{Egg mass weight} \times \text{\% egg hatching})}{\text{Engorged female tick weight}} \times 20000$$

$$\text{PE } (\%) = \frac{(\text{RE}_{\text{control}} - \text{RE}_{\text{treatment}})}{\text{RE}_{\text{control}}} \times 100$$

Bioassays

Adult Immersion Test (AIT), Larval Immersion Test (LIT), and Tick Climbing Repellent Assay (TCRA) were used to determine the acaricidal and repellent properties of ethanolic Neem extract.

Adult Immersion Test (AIT)

A population of 210 engorged female *R. microplus* ticks was separated into 7 subgroups: five treatment groups (20, 10, 5, 2.5, and 1.25% Neem extract), one positive control (0.1 % cypermethrin, K-CYPER), and one negative control (distilled water). The subgroups were repeated three times each with 10 ticks. The ticks were immersed in 5 mL of each test solution for 5 min, rinsed gently with distilled water, and placed on filter paper to dry. The ticks were later incubated at 27 °C and 85% RH. Death was registered at 6, 12, and 24 hours after treatment. The percentage of mortality was as follows:

$$\text{Adult mortality } (\%) = \frac{\text{Number of dead adult ticks}}{\text{Total number of adult ticks}} \times 100$$

Larval Immersion Test (LIT)

For the larval immersion test, approximately 100 unfed larvae (7-14 days old) were transferred into each 1.5 mL of a microcentrifuge tube containing the respective concentration of ethanolic neem seed extract. Neem extract concentrations (20, 10, 5, 2.5, 1.25% were tested, and a positive control (0.1% cypermethrin) and a negative control (distilled water) were also used. Tubes were shaken for 30 sec, then gently shaken for 5 minutes, and the larvae were placed on Whatman filter paper No. 1, folded, and incubated at 27°C and 85% RH. Deaths were observed after 48h and were calculated using the same formula.

$$\text{Larval mortality } (\%) = \frac{(\text{Number of dead larvae})}{(\text{Total number of larvae})} \times 100$$

Tick Climbing Repellent Assay (TCRA)

The repellent activity was tested on two vertical aluminum rods spaced 7cm apart, each covered with filter paper impregnated with Neem extract at five concentrations. Each concentration of 30 adult ticks was added, and positive (0.1% cypermethrin) and negative (distilled water) controls were used. Ticks that were not able to climb were counted after 30 min, and percentage repellency was calculated as:

$$\text{PR } (\%) = \left[\frac{(\text{Nc} - \text{Nt})}{\text{Nc}}\right] \times 100$$

Where: PR= percentage repellency, Nt = number in treatment, Nc = number in control

Statistical Analysis

All results, including adulticidal, larvicidal, reproductive indices, egg hatchability, and repellent activity, were analyzed using one-way ANOVA followed by Tukey's post hoc test at $P < 0.05$. The LC_{50} and LC_{90}, and the RC_{50} and RC_{90}, were determined using SPSS (version 25).

Results

Yield of the Ethanolic Extract of Neem

The extraction yield of the ethanolic seed extract was 15.2%.

Adult Tick Mortality

The adult immersion test demonstrated that the ethanolic extract of *A. indica* had a strong dose-dependent activity as an acaricidal compound on engorged *R. microplus* ticks (Table 1, Figure 1). The highest adult mortality, 85.0 %, was observed at a 20% concentration after 24h, accounting for all deaths. The lowest mortality (12.0%) was observed at 1.25% concentration after 24 h. After 24 h, the LC_{50} and LC_{90} values for adult ticks were 6.23 and 18.75%, respectively (Table 2).

Table 1. Adult *R. microplus* mortality at various concentrations of ethanolic extract of *A. indica* seeds

Concentration (%)	6h (%)	12h (%)	24h (%)
1.25	4.0 ± 1.2^{d}	7.3 ± 2.1^{d}	12.0 ± 3.5^{d}
2.5	15.3 ± 3.2^{c}	25.6 ± 4.1^{c}	38.0 ± 4.8^{c}
5.0	28.6 ± 4.5^{b}	42.0 ± 3.7^{b}	55.6 ± 5.2^{b}
10.0	46.6 ± 3.7^{ab}	60.3 ± 4.6^{ab}	72.3 ± 5.0^{ab}
20.0	61.3 ± 5.1^{b}	75.0 ± 4.2^{b}	85.0 ± 4.5^{b}
Positive control	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}
Negative control	0.0 ± 0.0^{e}	0.0 ± 0.0^{e}	0.0 ± 0.0^{e}

Values are mean±SD. Different superscript letters within a column indicate significant differences ($P<0.05$).

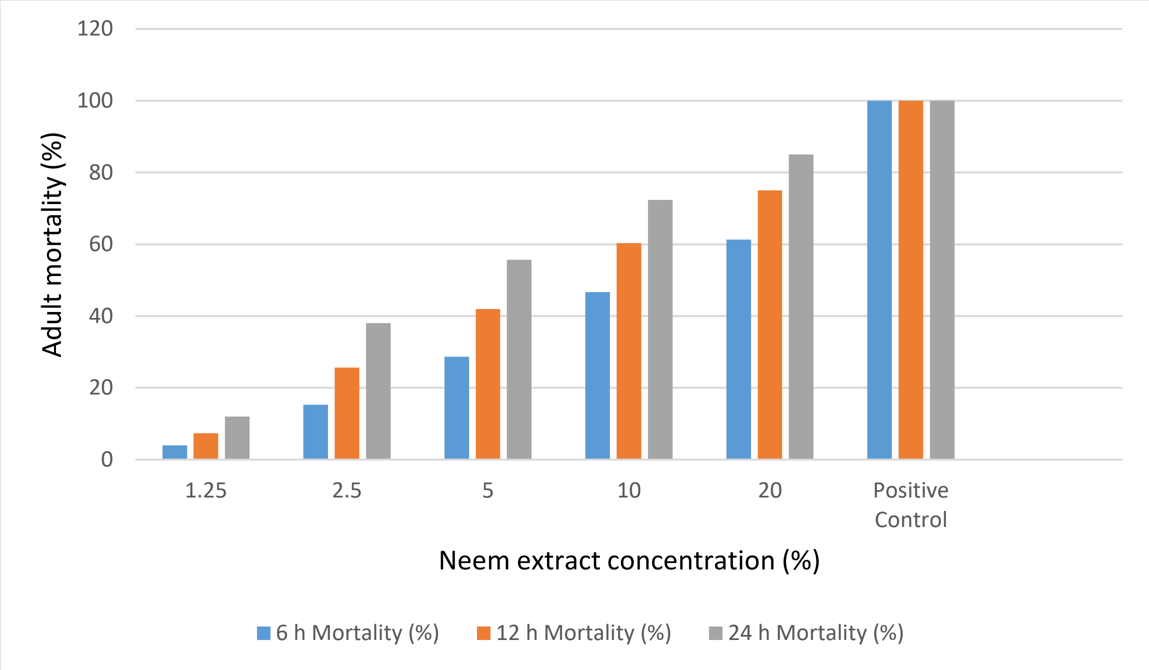

Figure 1: Adulticidal activity of ethanolic *A. indica* seed extract against *R. microplus* ticks at 6, 12, and 24h.

Table 2. LC_{50} and LC_{90} values of ethanolic extract of *A. indica* seeds against adult *R. microplus* ticks.

Bioassay	Time	LC_{50} (%) (95% CI)	LC_{90} (%) (95% CI)	Slope±SE	χ^2
Adult immersion	24h	6.23 (5.1–7.2)	18.75 (16.8–20.9)	1.50±0.20	0.40
Larval immersion	48h	4.85 (4.0–5.7)	15.42 (13.5–17.8)	1.45±0.18	0.35

LC_{50} and LC_{90} values were estimated using probit analysis. Values in parentheses represent 95% confidence intervals

Larval Mortality

Larval immersion test also established the high larvicidal activity of *A. indica* extract (Figure 2). Mortality of the larvae increased with concentration, reaching 92% at 20% extract after 48 h. The LC_{50} and LC_{90} were 4.85 and 15.42%, respectively, indicating strong larvicidal activity.

Effectiveness of the Extract

The product effectiveness parameter was used to assess the efficacy of the ethanolic extract (Figure 3). The 20% concentration showed strong activity but remained significantly lower than the positive control (Figure 3)

Egg Hatchability and Reproductive Parameters

Neem extract markedly reduced egg hatchability, oviposition, and reproductive efficiency in a dose-dependent manner (Table 3). At the highest tested concentration, 20% egg hatchability decreased to 28.0% while inhibition of hatchability and oviposition reduction increased to 72.0 and 88.0%, respectively.

Repellency

The assay on the tick climbing repellent indicated high levels of repellent activity of *A. indica* extract (Table 4, Figure 4). The maximum repellency (82.4% after 4h and 67.2% after 8h) was recorded at 20% concentration, which was significantly higher than the lower percentage and the negative control.

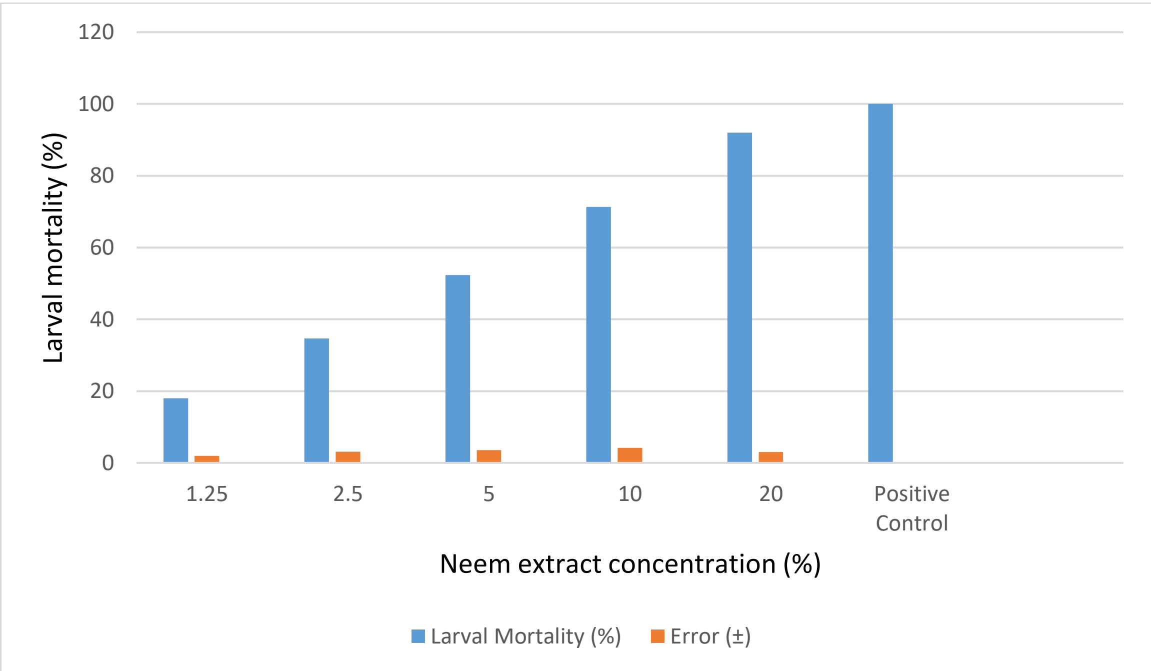

Figure 2. Larval mortality of *R. microplus* at various concentrations of ethanolic extract of *A. indica* seeds

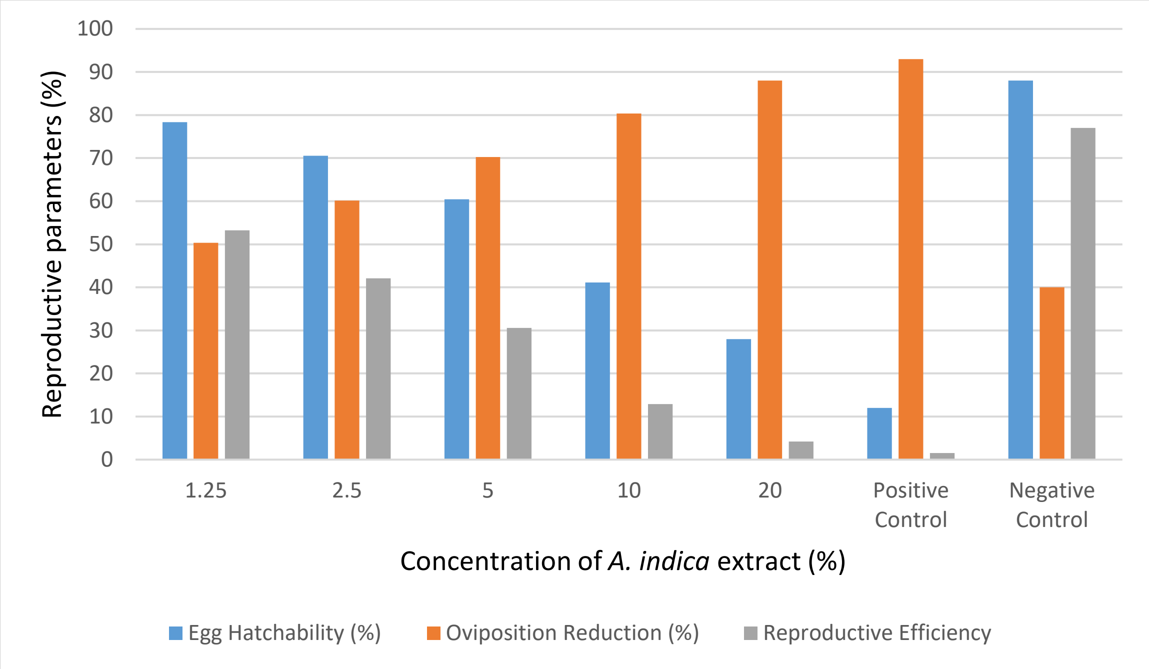

Figure 3: Effect of *A. indica* extract on reproductive parameters of *R. microplus*, including egg hatchability, oviposition reduction, and reproductive efficiency

Table 3. Egg hatchability, inhibition of hatchability, oviposition reduction, and reproductive indices of engorged *R. microplus* treated with ethanolic extract of *A. indica* seeds.

Conc. (%)	Hatchability (%)	Inhibition (%)	Oviposition (%)	RI	REI
1.25	78.3±2.1^{a}	21.7±2.1^{a}	50.3±2.5^{d}	32.1±1.2^{b}	53.2±2.3^{b}
2.5	70.6±2.9^{b}	29.4±2.9^{b}	60.1±3.1^{c}	28.4±1.5^{bc}	42.1±3.0^{bc}
5.0	60.4±3.2^{c}	39.6±3.2^{c}	70.2±2.8^{b}	21.9±1.2^{c}	30.6±2.5^{c}
10.0	41.1±2.5^{d}	58.9±2.5^{d}	80.3±3.2^{b}	15.4±1.1^{d}	12.9±1.3^{d}
20.0	28.0±2.0^{e}	72.0±2.0^{e}	88.0±2.2^{b}	7.6±0.9^{e}	4.2±0.8^{e}
Positive control	12.0±1.3^{f}	88.0±1.3^{f}	93.0±1.5^{a}	5.0±0.5^{f}	1.5±0.3^{f}
Negative control	88.0±2.0^{a}	12.0±2.0^{a}	40.0±1.8^{e}	44.0±2.1^{a}	77.0±3.1^{a}

Values are mean±SD. Different superscript letters within a column indicate significant differences (P<0.05).

Table 4. Percentage repellency of adult *R. microplus* ticks treated with ethanolic extract of *A. indica* seeds.

Conc. (%)	4 h (%)	8 h (%)
1.25	22.3 ± 2.1^{e}	17.2 ± 1.8^{e}
2.5	36.1 ± 2.5^{d}	29.3 ± 2.4^{d}
5.0	51.4 ± 3.2^{c}	43.2 ± 2.7^{c}
10.0	66.2 ± 3.5^{b}	52.1 ± 3.1^{b}
20.0	82.4 ± 4.0^{b}	67.2 ± 3.8^{b}
Positive control	98.2 ± 2.2^{a}	91.3 ± 4.1^{a}
Negative control	6.5 ± 1.5^{e}	4.2 ± 1.1^{e}

Values are mean±SD. Different superscript letters within a column indicate significant differences ($P<0.05$).

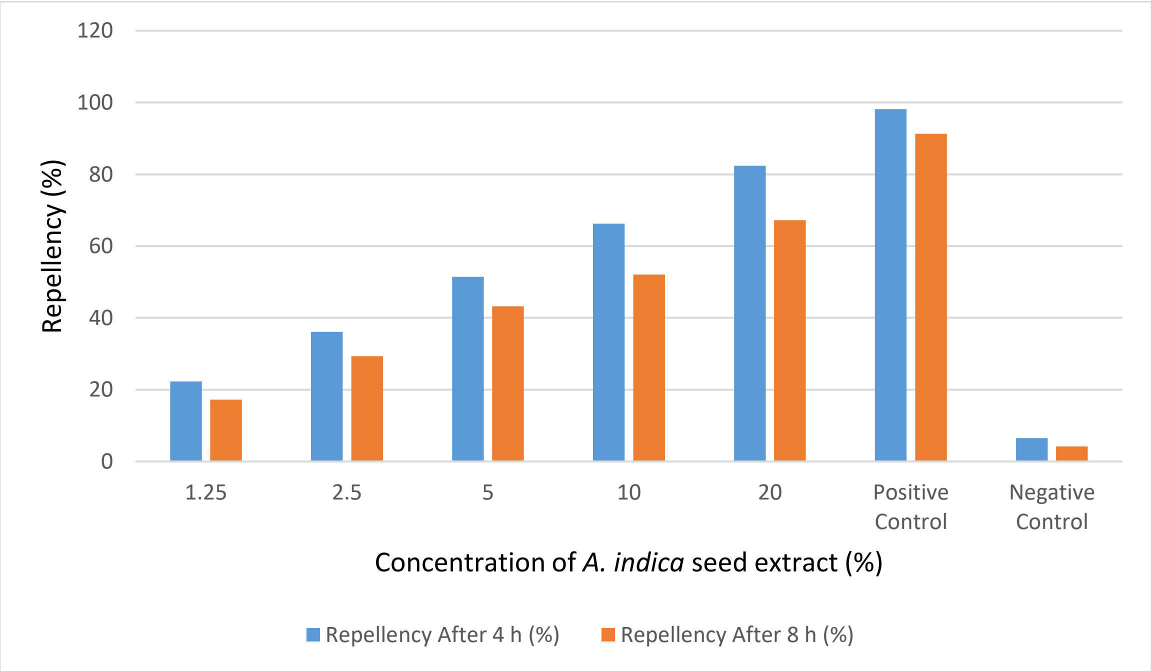

Figure 4: Repellent activity of ethanolic *A. indica* seed extract against adult *R. microplus* ticks at 4 and 8 h.

Discussion

The current research assessed the adulticidal, larvicidal, and repellent activities of the ethanolic extract of *A. indica* seed against *R. microplus* and found that these activities were dose-dependent across all concentrations tested. The crude and ethanolic extracts were 15.2%, respectively, which is comparable to prior literature, indicating that ethanol is an effective solvent for extracting bioactive phytochemicals such as azadirachtin, nimbin, and salannin from Neem seeds [13]. These are known to disrupt the development, reproduction, and feeding behavior of arthropod pests, thereby influencing the biological activity observed in both adult and larval ticks. The extract showed high acaricidal activity in the adult immersion test (AIT), with the highest mortality rate (85%) at a 20% concentration after 24 hours. The obtained LC_{50} and LC_{90} values (6.23% and 18.75%, respectively) suggest substantial activity under laboratory conditions and warrant further comparison with standard acaricides in field based study. Ethanolic extracts of Neem exhibited significant adulticidal activity against *R. microplus* with an LC_{50} of between 5-8 % [14]. Similarly, research on Hyalomma anatolicum and Rhipicephalus sanguineus has shown that Neem extracts cause high mortality in adult ticks in a dose-dependent manner [15]. The progressive increase in mortality with concentration indicates that Neem phytochemicals disrupt key physiological functions, ultimately causing mortality in ticks. Larval mortality reflected adulticidal activity, with up to 92 % mortality recorded at a 20 %extract concentration after 48 hours. The LC_{50} (4.85%) and LC_{90} (15.42 %) are also very low, which may be attributable to the thinness of their cuticle and increased exposure to phytochemicals. Larval mortality exceeding 85 % with ethanol extracts of Neem seeds, highlighting the potential of these extracts to control early life stages and reduce the overall tick population [16]. The larvicidal activity reinforces the combined management of *R. microplus*, as attacking larvae may substantially reduce the ability of the infestation to reproduce. Reproductive indices and mortality rates were also significantly affected. Hatching of the eggs dropped to 78.3% at 1.25% extract and 28% at 20, with oviposition loss being 88% at the highest concentration. The efficiency index of reproduction was reduced to 4.2 at 20%, indicating a potent anti-reproductive effect. The results are consistent with prior literature, indicating that Neem-derived azadirachtin disrupts vitellogenesis and embryogenesis in ticks. Oviposition and hatchability inhibition of eggs are especially important, as they prevent immediate infestations and reduce the emergence of the next generation of ticks, thereby improving the long-term efficacy of Neem-based treatments. Also prominent was repulsive activity. The tick climbing repellent assay showed that the highest percent repulsion at the 20 % extract concentration was 82.4 % at 4 hours and 67.2 % at 8 hours. Ethanolic extracts of Neem were more than 80 % repellent to adult *R. microplus* [17].

Neem compounds are highly repellent, which is why they are thought to disrupt ticks' chemosensory receptors, preventing host attachment. This property, together with its acaricidal activity, makes Neem extracts particularly useful in integrated pest management, with both preventive and curative effects. The current results help confirm and expand the existing literature on the acaricidal and repellent effects of Neem extracts. Neem provides an eco-friendly, biodegradable alternative to synthetic acaricides with a multi-target mode of action, thereby minimizing the risk of resistance. Although earlier research had primarily focused on adulticidal or larvicidal effects, this study provides a comprehensive evaluation by demonstrating both adulticidal, larvicidal, anti-reproductive, and repellent activities against *R. microplus*. These findings emphasize the promising applications of the ethanolic Neem seed extract as a natural and viable biological control agent against cattle ticks, particularly in areas with high rates of acaricide resistance.

Conclusion

The current paper has concluded that ethanolic *A. indica* seed extract is highly effective against R. microplus, with good adulticidal, larvicidal, anti-reproductive, and repellent properties that are dose-dependent. The extract had a significant impact by reducing tick survival, oviposition, egg hatchability, and host attachment through repellency. The extract showed meaningful activity relative to the positive group under laboratory conditions. Neem extract serves as a promising botanical candidate for integrated tick management, pending further validation through formulation studies, safety assessment, and field trials. The following research directions may include field testing, optimization of formulations, and integration with other plant-based products to enhance effectiveness and feasibility in livestock production systems.

Conflict of interest: NIL

Funding: NIL

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