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Exploring the Acaricidal Potential of *Calotropis procera* and *Lantana camara* Extracts Against *Rhipicephalus microplus* Ticks: A Sustainable Approach for Tick Control

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Abstract: Plant-based alternative control measures for tick management are gaining significant importance due to the increasing resistance of ticks to conventional chemical acaricides and the environmental concerns associated with synthetic pesticides. Natural plant extracts, rich in bioactive compounds such as alkaloids, flavonoids, and terpenoids, offer a sustainable and eco-friendly solution for tick control. This study investigates the acaricidal properties of aqueous and ethanolic extracts of *Calotropis procera* and *Lantana camara* against *Rhipicephalus microplus* ticks. The efficacy of these herbal extracts was evaluated using the Adult Immersion Test (AIT) and Larval Packet Test (LPT). The highest inhibition of oviposition (IO%) was observed for the ethanolic extract of *C. procera* at 100 mg mL^{-1} concentration (100%), while the lowest was recorded for the aqueous extract of *L. camara* at 6.25 mg mL^{-1} (17.33%). Maximum larval mortality (75.99%) was observed for the ethanolic extract of *C. procera* at 100 mg mL^{-1}, and the lowest mortality (14.11%) was seen for the aqueous extract of *L. camara* at 6.25 mg mL^{-1}. The LC50 (mg mL^{-1}) value of aqueous and ethanolic extracts of *L. camara* and *C. procera* in AIT were recorded as 78.46, 58.82, 31.57 and 28.00, respectively. Likewise, the LC50 (mg mL^{-1}) value of aqueous and ethanolic extracts of *L. camara* and *C. procera* in LPT were recorded as 406.27, 81.02, 106.88 and 32.26, respectively. These findings indicate that *C. procera* has significant potential as an eco-friendly alternative in integrated pest management strategies, offering a promising approach to reduce the economic burden of ticks and tick-borne diseases while promoting safer and more sustainable agricultural practices.

Key words: *Rhipicephalus microplus, Calotropis procera, Lantana camara,* adult immersion test, larval lacket test, acaricide

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India, with approximately 70% of its population relying on agriculture and livestock for their livelihood, is predominantly an agriculture-based economy. Livestock serves as a vital source of animal protein, draught power for agricultural activities, transportation, and also contributes to soil fertility through the use of manure. Ticks, as obligatory ectoparasites, inhabit the exterior of their hosts and provide considerable obstacles to animal production. The term 'Ectoparasite' derives from the Greek words 'ektos,' meaning outside, and 'parasites,' meaning parasite. Ticks are specialized hematophagous ectoparasites of mammals, birds, and reptiles, found globally (Furman and Loomis, 1984). In India, the economic losses attributed to ticks and tick-borne diseases (TBDs) are estimated at over \$1477 m yr^{-1}, impacting both small and large-scale farmers (Yadav and Upadhyay, 2024). The losses are mostly ascribed to diminished milk output, a critical source of revenue for rural farmers. Disease transmission diminishes livestock production, resulting in decreased weight increase, lower fertility, and, in extreme instances, animal mortality. Moreover, the expense associated with tick control treatments, including acaricide treatment, contributes to the financial burden. A prior research indicated that over 70% of cattle in India are infected with ticks, and the management of these pests constitutes over 20-30% of total veterinary expenses (Singh *et al*., 2020).

In the dairy sector, *R. microplus* is regarded as the most significant tick species. This tick species is recognised for inflicting significant costs on animal owners by spreading illnesses such as babesiosis and anaplasmosis. This tick results in a loss of 384.3 million USD to the Indian economy (Minjauw and McLeod, 2003).

The predominant and efficacious strategy for tick control in field circumstances is the administration of chemical acaricides. The heightened knowledge and enhanced socio-economic circumstances of farmers, along with state-sponsored animal health services in industrialized nations, have resulted in several instances of acaricide resistance (Baxter and Barker, 1999; Chen *et al*., 2007; Molento *et al*., 2013). Recent data demonstrate that the tick population in northern India has acquired resistance to the majority of existing chemical acaricides (Kumar *et al*., 2011; Shyma *et al*., 2012; Jyoti *et al*., 2015).

The escalating resistance of tick populations to traditional chemical acaricides has rendered the pursuit of alternative, environmentally acceptable, and sustainable treatments imperative in pest control. Plants have historically served as a source of natural acaricides, and their efficacy in addressing tick infestations is receiving considerable study. *C. procera* and *L. camara* are two plants that have attracted attention for their bioactive qualities and efficacy as natural acaricides. *C. procera*, referred to as the "madar plant," is extensively found in tropical and subtropical areas and has been historically utilised for its therapeutic attributes. Research indicates that several components of *C. procera*, such as leaves, flowers, and latex, have notable antibacterial, anti-inflammatory, and acaricidal properties (Sudha *et al*., 2018). Likewise, *L. camara*, famous for its vivid blossoms, possesses notable acaricidal characteristics. It has been utilized in traditional medicine for its medicinal properties, including anti-inflammatory, antioxidant, and anti-arthritic effects (Hassan *et al*., 2011; Raut *et al*., 2014). The active components of these plants have significant efficacy against several ectoparasites, including ticks, positioning them as promising candidates for the formulation of novel plant-derived acaricides.

Considering the aforementioned facts, this study was conducted to examine the acaricidal efficacy of aqueous and ethanolic extracts of *C. procera* and *L. camara* against *R. microplus* ticks to highlight the potential of these plants as natural substitutes for manmade insecticides, providing a safer and more sustainable method for tick management.

Materials and Methods

Healthy, disease-free plants were chosen for extraction, and fresh leaves of *C. procera* and *L. camara* were gathered from fields, roadsides, parks, and gardens in the Mathura region. The identification of gathered plants to the species level was conducted at the ICAR-National Bureau of Plant Genetic Resources (NBPGR), Pusa Campus, New Delhi, and an accession number was acquired following the submission of specimens to the repository. The leaves utilized for extract preparation were cleaned, laid out on paper sheets, and dried in a sheltered place at ambient temperature. Dried leaves were meticulously ground into a fine powder using a grinder, and the resultant powder was stored in a separate airtight container at 40°C until further usage.

Aqueous and ethanolic plant extraction procedure: Aqueous and ethanolic extracts of

C. procera and *L. camara* plants were extracted in Soxhlet Apparatus as per the standard protocol (Redfern, 2014) and were stored in refrigerator at 5°C temperature till further use.

The plant extracts were weighed and dissolved in water for making different dilutions. Different concentrations used in this study were 6.25, 12.5, 25, 50 and 100 mg mL^{-1}. A negative control containing only water without extract was also tested along with the plant extracts.

Rearing of tick larvae: Fully engorged adult female ticks were collected from field and washed with distilled water to remove any debris. The ticks were incubated for two weeks at 28°C at 85% relative humidity for egg laying. The eggs thus laid were collected in the vials and further incubated under the same conditions for next 14 to 21 days for rearing the larval stages (Fig.1 and 2).

Larval packet test: The LPT was conducted according to FAO (1984) guidelines with minor modifications. For preparing the packet, Whatman filter paper No.1 of 3.75- cm by 8.5-cm size was impregnated with 0.6 mL of crude plant extract in distilled water. Three packets of each concentration were used for this test. After the insertion of approximately 100 larvae, the top of each packet was sealed with aluminum clamps, and the packets were placed in a desiccators placed in BOD incubator maintained at 28±1°C and 85±5% RH (Fig. 3). Control group was treated with 0.6 mL of distilled water. The packets were removed after 24 h, and corrected per cent mortality was calculated by using given formula:

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

Adult immersion test: The AIT was conducted according to FAO (1984) guidelines. The engorged female ticks from the field were washed thoroughly with distilled water, weighing and were divided into groups of ten ticks. The ticks were immersed in crude extract of plant material for 5 min. The control group was immersed in distilled water. All the Petri dishes containing these treated ticks were kept for fifteen days in BOD incubator at 28±2°C and 85±2% relative humidity. The percent adult tick mortality and the weight of the eggs laid by the treated ticks were recorded in comparison with the control. The eggs were incubated at the same condition, and the percentage of hatched eggs was estimated. The percentage inhibition of oviposition was calculated using following formulae (Goncalves *et al*., 2007).

$$\text{Percentage Inhibition of Oviposition (IO\%)} = \frac{\text{RI (Control group)} - \text{RI(Treated group)}}{\text{RI (Control group)}} \text{x100}$$

$$\text{Reproductive Index (RI)} = \frac{\text{Weight of eggs laid (mg)}}{\text{Weight of adult engorged female tick (mg)}}$$

Fig. 1. Egg laying by female ticks in adult immersion test (AIT).

Fig. 2. Hatching of larvae from eggs.

Fig. 3. Extract imprignated packets for LPT.

Statistical analysis: The mortality percentage from each test was subjected to probit analysis to calculate lethal concentration (LC_{50} and LC_{99}) for respective extract using Graphpad Prism software.

Results and Discussion

C. procera and L. *camara* plants were identified to species level from ICAR- National Bureau of Plant Genetic Resources (NBPGR), Pusa Campus, New Delhi and obtained the accession number (*Lantana camara*-AC-50/2021 and *Calotropis procera*-AC-51/2021) after submitting the specimens to repository.

In vitro evaluation of different extract on larvae of R. microplus ticks: The highest mortality observed in LPT with aqueous *L. camara* extract was 37.24% at a concentration of 100 mg mL^{-1}, while the lowest mortality recorded was 14.11% at 6.25 mg mL^{-1}. Likewise, with aqueous *C. procera* extract, the highest mortality rate observed was 52.46% at a concentration of 100 mg mL^{-1}, while the lowest mortality rate recorded was 15.63% at 6.25 mg mL^{-1}. Table 1 and 2 presents the results of LPT using aqueous *L. camara* and *C. procera* extracts.

The ethanolic extract of *L. camara* exhibited the highest mortality rate of 50.09% at a concentration of 100 mg mL^{-1}, while the lowest mortality rate of 17.22% was observed at 6.25 mg mL^{-1}. Likewise, the ethanolic extract of *C. procera* exhibited the highest mortality rate of 75.99% at a concentration of 100 mg mL^{-1},

Table 1. In vitro effect of aqueous L. camara extract on R. microplus larvae

Concentration (mg mL^{-1})	Mean dead larvae ± SE (%)	Corrected % mortality	LC50 (mg mL^{-1})
6.25	15.58 ± 0.54	14.11	
12.5	19.47 ± 1.40	18.00	
25	22.13 ± 0.18	20.65	406.27
50	30.37 ± 0.96	28.89	
100	38.72 ± 0.07	37.24	
Control (Water)	1.45 ± 00	-0.01	

Table 2. In vitro evaluation of aqueous C. procera extract on R. microplus larvae

Concentration (mg mL^{-1})	Mean dead larvae ± SE (%)	Corrected % mortality	LC50 (mg mL^{-1})
6.25	18.10±1.74	15.63	
12.5	27.51±1.27	25.04	
25	37.34±1.53	34.87	81.02
50	45.02±0.60	42.54	
100	54.93±0.72	52.46	
Control (Water)	2.44±00	-0.02	

Table 3. In vitro evaluation of ethanolic L. camara extract on R. microplus larvae

Concentration (mg mL^{-1})	Mean dead larvae ± SE (%)	Corrected % mortality	LC50 (mg mL^{-1})
6.25	19.66 ± 0.49	17.22	
12.5	27.18 ± 0.87	24.75	
25	33.14 ± 1.56	30.70	
50	41.23 ± 0.70	38.79	106.88
100	52.53 ± 1.11	50.09	
Control (Water)	2.40 ± 00	-0.02	
Deltamethrin	65.06	62.62	

Table 4. In vitro evaluation of ethanolic C. procera extract on R. microplus larvae

Concentration (mg mL^{-1})	Mean dead larvae ± SE (%)	Corrected % mortality	LC50 (mg mL^{-1})
6.25	26.86±0.96	25.08	
12.5	36.68±0.39	34.89	
25	43.05±1.74	41.27	32.26
50	51.62±0.60	49.84	
100	77.77±0.38	75.99	
Control (Water)	1.76±00	-0.01	

while the lowest mortality rate of 25.08% was observed at 6.25 mg mL^{-1}. Table 3 and 4 presents the results of LPT using ethanolic *L. camara* and *C. procera* extracts.

This investigation demonstrated that ethanolic extracts of both plants had superior acaricidal activity regarding larval mortality compared to their corresponding aqueous extracts. The findings align with the prior study by Zaman *et al*. (2012), which indicated a 60% death rate of *R. microplus* larvae when treated with an aqueous leaf extract of *C. procera* at a dosage of 50 mg mL^{-1}. Similarly, Shyma *et al*. (2014) documented a 63.2% death rate of *R. microplus* larvae when exposed to a methanolic extract of *C. procera* at a dosage of 100 mg mL^{-1}. Adehan *et al*. (2016) documented a 35.79% death rate of *R. microplus* larvae when treated with ethanolic extract of *L. camara*. Khan *et al*. (2019) observed a 96% mortality rate of *R. microplus* larvae utilizing an aqueous leaf extract of *C. procera* at a concentration of 40 mg mL^{-1}.

In vitro evaluation of different extract on adult R. microplus ticks: The use of aqueous *L. camara* extract in adult immersion test (AIT) demonstrated a maximum acaricidal efficacy of 56.88% inhibition of oviposition at a concentration of 100 mg mL^{-1}, while the minimum efficacy recorded was 17.33% inhibition at a concentration of 6.25 mg mL^{-1}. Likewise, with aqueous *C. procera* extracts, the highest oviposition inhibition (60.56%) was observed at a concentration of 100 mg mL^{-1}, while the lowest inhibition (25.08%) occurred at a concentration of 6.25 mg mL^{-1}. Table 5-6 presents the results of AIT using aqueous *L. camara* extract.

The ethanolic extract of *L. camara* demonstrated the highest acaricidal efficacy in AIT, achieving a 65.22% inhibition of oviposition at a concentration of 100 mg mL^{-1}, while the lowest efficacy recorded was 31.33% inhibition at a concentration of 6.25 mg mL^{-1}. The ethanolic extract of *C. procera* demonstrated the highest acaricidal efficacy, achieving 100%

Table 5. In vitro effect of aqueous L. camara extract on reproductive indices of R. microplus

Concentration (mg mL^{-1})	Weight of ticks (g)	Egg mass weight (g)	Reproductive index (RI)	% Inhibition of oviposition (%IO)	Larval hatching rate (%)	LC50 (mg mL^{-1})
6.25	0.342	0.273	0.798246	17.33	90	
12.5	0.335	0.262	0.78209	19.01	90	
25	0.341	0.243	0.71261	26.20	70	78.46
50	0.335	0.202	0.602985	37.55	60	
100	0.344	0.131	0.380814	56.88	50	
Control (Water)	1.02	0.985	0.967647	0	100	

Table 6. In vitro effect of aqueous C. procera extract on reproductive indices of R. microplus

Concentration (mg mL^{-1})	Weight of ticks (g)	Egg mass weight (g)	Reproductive index (RI)	% Inhibition of oviposition (%IO)	Larval hatching rate (%)	LC50 (mg mL^{-1})
6.25	0.269	0.195	0.724907	25.08	80	
12.5	0.282	0.182	0.645390	33.30	80	
25	0.271	0.163	0.601476	37.84	60	58.82
50	0.282	0.141	0.500000	48.32	50	
100	0.29	0.121	0.417241	60.56	40	
Control (Water)	1.02	0.985	0.967647	0	100	

Table 7. In vitro effect of ethanolic L. camara extract on reproductive indices of R. microplus

Concentration (mg mL^{-1})	Weight of ticks (g)	Egg mass weight (g)	Reproductive index (RI)	% Inhibition of oviposition (%IO)	Larval hatching rate (%)	LC50 (mg mL^{-1})
6.25	0.4645	0.2582	0.555867	31.33	80	
12.5	0.4691	0.213	0.454061	43.91	80	
25	0.4664	0.2034	0.436106	46.12	70	31.57
50	0.4735	0.1885	0.398099	50.82	60	
100	0.4657	0.1311	0.281512	65.22	40	
Control (Water)	0.483	0.391	0.809524	0.00	100	

Table 8. In vitro effect of ethanolic C. procera extract on reproductive indices of R. microplus

Concentration (mg mL^{-1})	Weight of ticks (g)	Egg mass weight (g)	Reproductive index (RI)	% Inhibition of oviposition (%IO)	Larval hatching rate (%)	LC50 (mg mL^{-1})
6.25	0.5822	0.1891	0.324802	35.84	70	
12.5	0.5899	0.1752	0.296999	41.33	70	
25	0.5883	0.1534	0.260751	48.49	60	28.00
50	0.5887	0.1358	0.230678	54.43	50	
100	0.5801	0.0000	0.00000	100.00	00	
Control (Water)	0.5882	0.2978	0.506290	0.00	100	

inhibition of oviposition at a concentration of 100 mg mL^{-1}. Conversely, the lowest efficacy was recorded at a concentration of 6.25 mg mL^{-1}, resulting in a 35.84% inhibition of oviposition. The outcomes of AIT utilising ethanolic *L. camara* and *C. procera* extract are presented in Table 7-8 and Figure 4.

The findings of AIT demonstrated that ethanolic extracts of both plants exhibited

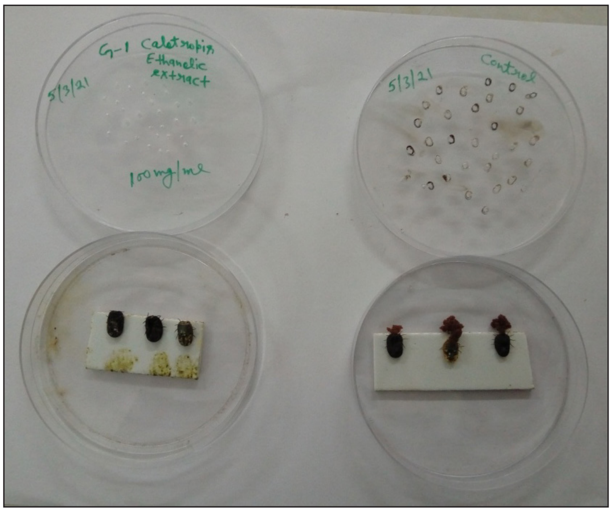

Fig. 4. Effect of 100 mg mL-1 concentration ethanolic C. procera extract on egg laying of female R. microplus tick.

greater acaricidal efficacy against the tick population than their aqueous extracts. The maximum percent inhibition of oviposition (%IO) recorded was 100% at a concentration of 100 mg mL^{-1} of ethanolic *C. procera* leaf extract against *R. microplus* ticks.

C. procera is a desert shrub commonly located in the semi-arid regions of Indian states, particularly Uttar Pradesh. The leaves of *C. procera* have demonstrated insecticidal activities (Chungsamarnyart *et al*., 1996; Elimam *et al*., 2009). The insecticidal properties of *C. procera* are attributed to digitoxin (Jain *et al*., 1996) and the cardiac glycoside cardenolide (Al-Rajhy *et al*., 2003). The contact and dipping LC50 values of cardenolide against adult Hyalomma dromedarii found in camels were recorded as 1185 and 1096 mg L^{-1}, respectively, and it functions by inhibiting Na+, K+-ATPase in ticks (Al-Rajhy *et al*., 2003).

Kulkarni *et al*. (2019) demonstrated that the ethanolic extract of *L. camara* exhibits greater efficiency than the aqueous extract of the same plant. The phytochemical analysis of the two extracts revealed the presence of phytosterols exclusively in the ethanolic extracts, suggesting that these components may account for the enhanced acaricidal efficiency observed. Moawad *et al*. (2017) documented increased acaricidal efficacy of various phytosterols against *R. microplus* ticks in Egypt. They recorded complete mortality of adult ticks in AIT utilizing ethanolic extracts of herbal origin.

L. camara is a large, scrambling evergreen shrub commonly referred to as wild sage or lantana weed. Dua *et al*. (1996) demonstrated that an extract of *L. camara* flowers in coconut oil offers protection against Aedes mosquitoes. The plant exhibits antibacterial, insecticidal (Abdel-Hady *et al*., 2005), and nematicidal activity (Qamar *et al*., 2005). It was also found to possess antioxidant (Basu and Hazra, 2006) and antifungal activity (Kumar *et al*., 2011). This plant has been further investigated for its repellent properties against mosquitoes (Ghisalberti, 2000). Moyo *et al*. (2009) and Kaur *et al*. (2015) investigated the effects of *L. camara* extract on ticks.

The LC50 results indicated that the ethanolic extracts of both plants exhibited greater acaricidal efficacy regarding the reproductive index compared to their corresponding aqueous extracts. Moyo and Masika (2009) documented a mean reduction of tick load by 58% when employing a 40% concentration of aqueous extract from *L. camara*. Ghosh *et al*. (2013) from Uttar Pradesh observed a mortality rate of up to 20% in adult *R. microplus* ticks 72 hours post-treatment with hydroethanolic root extracts of *C. procera*. Zaman *et al*. (2012) from Pakistan reported a 22.35% inhibition of egg laying in adult *R. microplus* ticks at a concentration of 50 mg mL^{-1} using an aqueous extract of *C. procera*. Shyma *et al*. (2014) from Gujarat reported a 71.34% inhibition of oviposition against *R. microplus* ticks using methanolic leaf extract of *C. procera*. Kaur *et al*. (2016) from Uttar Pradesh documented 74% and 78% mortality rates of adult *R. microplus* ticks when treated with leaf aqueous extracts of *C. procera* and *L. camara*, respectively, at a concentration of 3000 ppm. Khan *et al*. (2019**)** from Pakistan documented a 35.68% reduction in oviposition of engorged *R. microplus* ticks at a concentration of 40 mg mL^{-1}, utilising methanolic leaf extract of *C. procera*.

Conclusions

The outcomes of this experiment corroborated that the ethanolic extract of *C. procera* is highly effective against adult and larvae of *R. microplus* ticks. Further, studies are indicated for identification of active ingredients present in these extracts, that causes the mortality of tick, decrease in egg production and inhibition of hatching of the eggs. The anti-ovipositional and other acaricidal properties of the various extracts of these plants may make them a valuable component of developing sustainable strategy for integrated tick management.

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