Original Research Article

In-vitro acaricidal study of *Calotropis gigantea* methanolic extracts against (*Rhipicephalus (B.) microplus*) ticks in Udaipur, Rajasthan, India

Abstract

The present research was conducted to find out acaricidal efficacy of methanolic extracts of ***Calotropis gigantea*** against *Rhipicephalus (Boophilus) microplus* ticks in Udaipur (Rajasthan). Larval packet Test (LPT) and Adult Immersion Test (AIT) were used to evaluate acaricidal efficacy. A total of four concentrations of the ***Calotropis gigantea*** extract (12.5%, 25%, 50%, and 100%) were used along with one control group with twice replications for each concentration were used in the bioassay. The Highest efficacy in both in-vitro tests (AIT and LPT) was recorded in 100% methanolic extracts of ***Calotropis gigantea***. The *Calotropis gigantea* methanolic extracts displayed the highest IO% at 100% concentration, which was determined to be 67.46% in AIT. The methanolic extracts at 100% concentration had a minimum reproductive index of 0.16. During the study, it was clear that AIT had a lower reproductive index and a higher percent oviposition inhibition. Calotropis gigantea methanolic extracts in LPT demonstrated the highest efficacy at 100% concentration, which was of 64%. The Larval mortality increased trend with increasing concentrations of plant extracts.

Keywords: ***Calotropis gigantea***, ticks, larval packet test (LPT) and adult immersion test (AIT)

1. Introduction

"India is an agricultural country where both livestock and agriculture are closely associated with each other. Presently chemical acaricides like organophosphates, pyrethroids and macrocyclic lactones are used for control of the economically important tick species. The

incidence of acaricidal resistance has been widely reported in indian in one host tick Boophilus" (Shyma et al. 2013). "To overcome this resistance and other environmental issues linked with chemical control, constant efforts have been made. Sustainable immunological means have been developed for controlling ticks and tick-borne diseases. Ecofriendly anti-tick natural products have been produced as alternative to chemical acaricides. Medicinal plants and their extracts have shown significant activity against economically important tick species like *Rhipicephalus (Boophilus) microplus"*. (Sunil *et al*., 2013). "The plant extracts are usually made plants which have history of some pesticidal or medicinal properties and which have been traditionally used by the people. *Calotropis gigantea* (crown flower) is a species of *Calotropis* which is found in Cambodia, Indonesia, Malaysia, Thailand, Philippines, Sri Lanka, India, China, Pakistan, Nepal and tropical Africa. Its latex contains cardiac glycosides, fatty acids and calcium oxalate. The poisonous plant have active principles like uscharin, calotoxin, calactin and calotropin. It is also used as fungicide and insecticide properties" (Daniel, 2006).

2. Materials and Methods

"Plants were selected based on available scientific literature. Plant materials were brought to the laboratory and subsequently dried in room temperature for 8-10 days. The materials were dried completely. After drying, plant materials were powdered in mortar pestle and grinder. The powder of *Calotropis gigantea* parts was then processed for extract preparation by using maceration methods" (Shyma *et al.,* 2014). A volume of 400 ml of aqueous were used as solvents to extract 100 g of powder. The extracts were kept in airtight containers at room temperature for two days while being stirred frequently each day with a clean glass rod. Muslin cloth was used to filter these mixtures. When a clear, colourless supernatant appeared, it was determined that there was no more plant material to be extracted from. This process was repeated three to five times.The extracts were then subjected to water bath evaporation at 40℃ to for removing solvent. The semi-solid extracts were dried under a ceiling fan.

3. Collection of cattle ticks (*Rhipicephalus (B.) microplus*)

The ticks were collected from different animals during early morning from Vallabhnagar. The collected ticks were preserved in glass vials that were clean, well-stoppered, and preserved in 70% alcohol. Only engorged female ticks were chosen for AIT and permanent mounts of ticks

were prepared according to standard keys. Ticks were collected from a specific area, labelled, and then kept separately in glass tubes with labels that were muslin-covered. Ticks laid their eggs in desiccators that were kept at room temperature and 85% relative humidity (RH). Eggs were collected seven days after the start of incubation. To ensure the selection of a more uniform batch of larvae for each LPT, each tube containing the first week's egg production was labelled. The larvae were only used for the Larval Packet Test after being left unfed for 14 to 21 days after the eggs were allowed to hatch under uniform incubation conditions (LPT).

Larval Packet Test (LPT)

The larval packet test was conducted according to FAO, (2004) "to determine the *In-vitro* acaricidal activity. Engorged female ticks were collected from the cattle from the study area. They were identified, cleaned, stored in a petri dish. They were maintained at 85-92% RH. and 27.0 ±1.0 ºC. dily examination of female ticks was done until oviposition. The eggs were separated and allowed to hatch in glass vials with cotton plug and kept in optimal conditions. The seed ticks obtained were maintained at 27.0±1.0ºC and 85-92% RH for 14-21 days". The larvae aged between 14 to 21 days were then subjected to larval packet test. Packets made of Whatman filter paper No. 1 (12 cm x 18 cm) was impregnated with 3ml of respective compounds and dried at room temperature for two hours. The total of 100 larvae are kept on acaricide impregnated filter paper packet. The top of the packet was sealed by white tape. All close packets were then incubated at 27.0 ± 1.0 ºC and 85-92% R.H for 24 hours., Mortality was observed and measured by counting the dead and live larvae after 24 hours. The non-motile tick larvae were presumed to be dead and were not counted.

$$\text{Percent mortality} = \frac{\text{Total number of dead larvae}}{\text{Total number of larvae}} \times 100$$

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

3.1. Preparation of of plant extracts (working solutions

The working solutions of all plant extracts were prepared using standard protocols. The dried powder of plant materials were kept at room temperature for 15-20 minutes. The quantity of extracts required were weighed and then dissolved in distilled. Four different dilutions were made at the rate of 12.5 mg/ml, 25 mg/ml, 50mg/ml and 100 mg/ml.

4. Statistical Analysis

"The enumerated data from the experiment was statistically evaluated analysis using SPSS, version 20.0. Analysis of variance" (Snedecor and Cochran, 1980) was done. The means were compared by Duncan's multiple range test (Duncan, 1995) at 5% level of significance ($P<0.5$).

5. Results and Discussion

5.1. Efficacy of methanolic extracts of *Calotropis gigantea* in Larval Packet Test (LPT)

The plant extracts kept in refrigerator were collected and kept in room temperature for 15-20 minutes. The quantity of extracts required were weighed and dissolved in distilled water. Four different dilutions at the rate of 12.5 mg/ml, 25 mg/ml, 50 mg/ml and 100 mg/ml were prepared. The peak mortality (57%) was observed at a concentration of 100 mg/ml. Four treatment groups and one control group were used for experiment. Control group showed no larval mortality. A significant larval mortality was recorded by application of extracts of 50 mg/ml, 25 mg/ml and 12.5 mg/ml which were 47.33%, 32.33% and 14.33% respectively. Percent mortality rate increased with the increase in concentration level as shown in (Table. 1).

Table 1: Efficacy of different concentrations of methanolic extracts of *Calotropis gigantean* against *Rhipicephalus (Boophilus) microplus* larvae by LPT

Concentration of extract (mg/ml)	Live larvae	SE	Dead larvae	SE	% Larval mortality	SE
Control	100.000^{e}	0.000	0.000^{a}	0.000	0.000^{a}	0.000
100	43.000^{a}	0.577	57.000^{e}	0.577	57.000^{e}	0.577
50	52.670^{b}	1.453	47.330^{d}	1.453	47.330^{d}	1.453

25	67.670[c]	0.882	32.330[c]	0.882	32.330[c]	0.882
12.5	85.670[d]	2.028	14.330[b]	2.028	14.330[b]	2.028

Means bearing different superscript in the same column differ significantly P<0.05

5.2 Efficacy of *Calotropis gigantea* in methanolic extracts against *Rhipicephalus (Boophilus) microplus* in Adult Immersion Test (IO %)

"AIT was done in present study to evaluate the acaricidal activity against *Rhipicephalus (Boophilus) microplus.* Different concentrations of aqueous extracts of *Datura stramonium* were prepared" as per FAO, (2004). "The plant extracts kept in refrigerator were taken out and kept in room temperature for 15-20 minutes. The quantity of extracts required were weighed and dissolved in distilled water. Four different dilutions at the rate of 12.5 mg/ml, 25 mg/ml, 50 mg/ml and 100 mg/ml were prepared. In AIT the decrease in reproductive index and increase in inhibition of oviposition was recorded from concentration 12.5 to 100 mg/ml. A significant percentage inhibition of oviposition (IO%) at 100, 50, 25 and 12.5 mg/ml the extracts were measured to be 64.57, 60.79, 53.76 and 45.56 %respectively". Mortality of ticks observed at different concentrations is shown (Table. 2).

Table 2: Acaricidal efficacy of different concentrations Methanolic extracts of *Calotropis gigantean* against *Rhipicephalus (Boophilus) microplus* by AIT

Concentration of extract (mg/ml)	weight of Live ticks (gm) (Mean)	(SE)	Weight of eggs (gm) (Mean)	(SE)	Repro-duction Index (RI) (Mean)	(SE)	%IO (Mean)	(SE)
Control	0.725	0.001	0.365[e]	0.002	0.503[e]	0.002	0.000[a]	0.000
100	0.735	0.002	0.131[a]	0.002	0.178[a]	0.002	64.571[e]	0.245

50	0.718	0.002	0.142[b]	0.002	0.197[b]	0.003	60.797[d]	0.341
25	0.721	0.013	0.168[c]	0.002	0.233[c]	0.002	53.769[c]	0.639
12.5	0.706	0.002	0.194[d]	0.001	0.274[d]	0.001	45.562[b]	0.042

Means bearing different superscript in the same column differ significantly $P<0.05$

Shyma *et al.*, (2014) recorded 63.2% larval motility which were quite similar to our results. Zaman *et al*., (2012) evaluated "acaricide effect of *Calotropis procera* flowers by larval packet test and ear bag method. Lethal effects were observed on total larval mortality at 50 mg/ml. which are similar with the present findings. Combined aqueous herbal extracts of *Azadirachta indica* leaves and *Calotropis procera* flowers were used using adult immersion test, larval packet test and ear bag method. The extract exhibited lethal effects on egg laying (index of egg laying, 0.371404 ± 0.00435), hatching (22.35%)". Al-Rajhy *et al*., (2003) tested "extract from *Calotropis procera* and reported better efficacy than Azadirachtin on the basis of LC 50 values against adult stages of the camel tick, *Hyalomma dromedarii*. the probit regression analysis of the extracts showed that with the increase in the dose of extracts, a significant increase in the mortality rate of treated ticks with significant inhibition inreproduction" which are similar to our findings. Adil *et al.* (2019) reported that "same concentration of *Calotropis procera* and *T. ofcinale* treatments resulted in larval mortality of 96.0%±0.57 and 96.7%±0.88, respectively using the larval packet test (LPT). An increasing range of extract concentrations was tested to determine the LD50 and LD90 for *C. procera*, 3.21 and 21.15 mg/ mL, respectively, and *T. ofcinale*, 4.04 and 18.92 mg/mL", respectively which are similar to our findings.

Conclusion

It is concluded that AIT had a lower reproductive index and a higher percent oviposition inhibition. *Calotropis gigantea* methanolic extracts in LPT demonstrated the highest efficacy at 100% concentration, which was of 64%. The Larval mortality increased trend with increasing concentrations of plant extracts.

6. References

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