



Acaricidal Evaluation of Aqueous Extracts of *Datura stramonium* against *Rhipicephalus (B.) microplus* Ticks in Udaipur, Rajasthan, India

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Author's contribution

The sole author designed, analyzed, interpreted and prepared the manuscript.

Article Information

DOI: 10.9734/CJAST/2022/v41i424001

Open Peer Review History:

This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: <https://www.sdiarticle5.com/review-history/94140>

Original Research Article

Received: 21/09/2022

Accepted: 26/11/2022

Published: 30/11/2022

ABSTRACT

The purpose of the current analysis was to determine the effectiveness of acaricidal *Datura stramonium* aqueous extracts. The effectiveness of acaricides against the tick *Rhipicephalus (Boophilus) microplus* in cattle was assessed from Udaipur District, India. Larval packet testing (LPT) and adult immersion testing (AIT) were employed in this study. *D. stramonium* extract was used at four different concentrations: 12.5%, 25%, 50%, and 100%, with one controlled group and two replications for each concentration. In both in-vitro tests, AIT and LPT, 100% aqueous extracts of *D. stramonium* showed the highest acaricidal efficacies. The *D. stramonium* aqueous extracts with the highest IO% at 100% concentration and 67.46% in AIT were found to have the highest efficacies. The 100% concentrations of aqueous extracts exhibited minimum reproductive index of 0.16. During the course of the study, there was a discernible decline in the reproductive index and an increase in the percent inhibition of oviposition in the Adult Immersion Test. Aqueous extracts of

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D. stramonium demonstrated the highest acaricidal efficacy at 100% concentration with 64% in the larval packet test. A rising trend in larval mortality was observed as plant extract concentrations rose.

Keywords: Tick; *datura*; larval packet test; adult immersion test.

1. INTRODUCTION

“India is a prominent agricultural economy and livestock and agriculture have closely associated. The main methods used for control of economically important tick species includes chemical compounds like organophosphates, pyrethroids and macrocyclic lactones which have been used for long time leading to the development of resistance in ticks. In Indian sub-continent, the acarid resistance is prevalent in genus *Boophilus*” [1]. Many researchers have focused on this aspect till date keeping in view environmental issues. The major focus has been on environment friendly natural products. “Immunological means to control diseases has been used as an alternative. Plant extracts and essential oils have showed significant activity against ticks species like *Rhipicephalus (Boophilus) microplus*” [2]. “Herbal plants and their extracts in different solvents have been used in the past. *Datura stramonium* has been selected for this study. This plant and its different parts possess high quantity of the tropane alkaloids atropine, hyoscyamine and scopolamine which acts as anticholinergic or deliriant” [3].

2. MATERIALS AND METHODS

The selection of plants was done based on 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. Powder of *Datura stramonium* was then processed for extract preparation using maceration methods as per Shyma et al. [1]. 400 ml of aqueous solvent were used to extract 100 g of powder. Extracts were kept in sealed containers at room temperature for two days, stirring frequently each day with a sterile glass rod. After that, mixtures were run through muslin cloth. A clear, colourless supernatant that indicated no more extraction from the plant material was possible was produced after repeating the extraction of the residue three to five times. The solvent from the extracts was then evaporated at 40 °C in a water bath. Finally,

extracts that were semi-solid were dried using a fan.

2.1 Tick Collection

Ticks were removed from animals early in the morning. They were then stored in glass vials that were clean, well-stoppered, and properly labelled at 70% alcohol. Ticks were mounted permanently using standard keys. Engorged female ticks were picked for Adult Immersion Test. Ticks collected were labeled and then kept individually in glass tubes with proper label covered by muslin cloth. Desiccators were used for oviposition of ticks. Desiccators were kept at room temperature having 85±5% relative humidity (RH). Collection of eggs was done after 7 days in incubation period. All tubes showing the 1st week egg production were properly marked for uniform batch of larvae, which were selected for larval packet Test. Hatching of eggs was done by under proper conditions of incubation. Larvae unfed for 14-21 days were used for Larval Packet Test.

2.2 Adult Immersion Test (AIT)

The Adult Immersion Test was done as per protocols of FAO, [4]. Engorged female ticks were thoroughly washed thrice using distilled water. Ticks were then kept to dry on filter paper. Ticks were placed in each crude extract of plant materials for five minutes as part of the experiment. Only distilled water was used in the control group. The ticks were then maintained on Whatman filter paper No. 1 in Petri dishes. Ticks that had been treated were kept in petri dishes for 24 hours at room temperature. Ticks were transferred in glass vials with muslin cloth covers after 24 hours. They were then kept in a desiccator with a relative humidity of 85.2 percent and put in a BOD incubator at 28.1 degrees Celsius. We closely monitored the oviposition and mortality of ticks. The percentage of adult tick mortality was calculated, and the treated ticks' egg weights were recorded and compared to those of the control group. The experiment was conducted in two replicates in each treatment group and the mean of two was estimated. Control groups were run singly. The

eggs were incubated in similar conditions. Hatched percentage was estimated visually.

$$\text{Reproductive index} = \frac{\text{Weight of egg laid (mg)}}{\text{RI (control group)}}$$

$$\text{Inhibition of oviposition, IO (\%)} = \frac{\text{RI (control group)} - \text{RI (treated group)}}{\text{Weight of adult females (mg)}} \times 100$$

2.3 Larval Packet Test (LPT)

The larval packet test was conducted according to FAO, [4] to evaluate the *In-vitro* acaricidal efficacy. An engorged female tick was collected from the cattle from the study area. They were then identified, cleaned, stored in a petri plates and maintained at 85-92% relative Humidity and temperature 27.0 ±1.0°C. Daily examination of female ticks was done until oviposition. A cotton plug was placed in the glass vials with the separated eggs, and the eggs were then allowed to hatch under ideal conditions. The seed ticks were kept for 14–21 days at a temperature of 27.0–1.0°C and 85–92% relative humidity. The larvae aged between 14 to 21 days were then subjected to larval packet test. Packets made with Whatman filter paper No. 1 (12 cm x 18 cm) were impregnated with 3 ml of compounds respectively and dried at room temperature for 2 hours. On a packet of filter paper with acaricide imbedded in it, 100 larvae were kept in total. White tape was used to seal the packets' tops. Then, all close packets were incubated for 24 hours at 27.0 1.0°C and 85–92% relative humidity. After a 24-hour period, mortality was observed and quantified by counting both dead and alive larvae. Tick larvae that were immobile were assumed to be dead and were not counted.

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

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

2.4 Preparation Working Solutions of Plant Extract

Using a standard protocol, working solutions for all plant extracts were created. For 15 to 20 minutes, dried powdered plant materials were left at room temperature. Weighing the necessary amount of extracts, they were then dissolved in distilled water. Four different dilutions were made at the rate of 12.5 mg/ml, 25 mg/ml, 50mg/ml and 100 mg/ml respectively.

2.5 Statistical Analysis

Enumerated data's from the experiment were statistically analyzed using SPSS, version 20.0. Analysis of variance was done as per the method of Snedecor and Cochran [5]. The mean were compared using Duncan's multiple range test [6] at 5% level of significance (P<0.5).

3. RESULTS AND DISCUSSION

3.1 Efficacy of *Datura stramonium* Aqueous Extracts in Larval Packet Test

The plants extract were kept in refrigerator then collected and kept in room temperature for 15-20 minutes. The quantity of extracts required were weighed and dissolved in distilled water. 4 different dilutions at the rate of 12.5 mg/ml, 25 mg/ml, 50 mg/ml and 100 mg/ml were prepared respectively. The peak mortality (42.67%) 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. Significant larval mortality was recorded with application of extracts of 50 mg/ml, 25 mg/ml and 12.5 mg/ml which were 31.67%, 14.33% and 7% respectively. Percent mortality rate increased with the increase in concentration level as shown in (Table 1).

3.2 Efficacy of Aqueous Extracts of samples in Adult Immersion Test (AIT) (IO %)

Adult Immersion Test was used in present study to evaluate the acaricidal activity against *Rhipicephalus microplus*. Different concentrations of aqueous extracts of *Datura stramonium* were prepared as per FAO, [4]. Plant extracts that had been kept in the fridge were removed and left at room temperature for 15 to 20 minutes. Quantity of extracts required were weighed and dissolved in distilled water. 4 different dilutions at the rate of 12.5 mg/ml, 25 mg/ml, 50 mg/ml and 100 mg/ml respectively were prepared. Reproductive index decreased and inhibition of oviposition increased as per concentrations from 12.5 to 100 mg/ml. Inhibition of oviposition (IO %) at 100, 50, 25 and 12.5 mg/ml respectively of the extracts were measured to be 48.33, 38.07, 27.80 and 18.81% respectively. Mortality observed at different concentrations is shown in (Table 2).

Table 1. Efficacy of different concentrations of aqueous extracts of *Datura stramonium* against *Rhipicephalus (Boophilus) microplus* larvae by LPT

Extract Concentration (mg/ml)	Live larvae	Standard Error	Dead larvae	Standard Error	Percentage of Larval mortality	Standard Error
Control	100.000 ^e	0.000	0.000 ^a	0.000	0.000 ^a	0.000
100	57.330 ^a	1.202	42.670 ^e	1.202	42.670 ^e	1.202
50	68.330 ^b	3.283	31.670 ^d	3.283	31.670 ^d	3.283
25	83.670 ^c	0.882	16.330 ^c	0.882	14.330 ^c	2.333
12.5	93.000 ^d	1.528	7.000 ^b	1.528	7.000 ^b	1.528

* Significant difference at $P < 0.05$

Table 2. Acaricidal efficacy of aqueous extracts of *Datura stramonium* on *Rhipicephalus (Boophilus) microplus* by AIT using different concentrations

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	.725 ^{cd}	.001	.365 ^e	.002	.503 ^e	.002	.000 ^a	.000
100	.738 ^d	.005	.192 ^a	.002	.260 ^a	.004	48.333 ^e	1.149
50	.699 ^a	.001	.218 ^b	.001	.312 ^b	.001	38.079 ^d	.008
25	.704 ^{ab}	.009	.256 ^c	.001	.363 ^c	.004	27.803 ^c	1.199
12.5	.717 ^{bc}	.001	.294 ^d	.003	.408 ^d	.004	18.812 ^b	.488

* Means bearing different superscript in the same column differ significantly i.e. $P < 0.05$

A similar findings was reported by Shyma et al. [7] which shows acaricidal activities of methanolic *D. stramonium* extracts against *Rhipicephalus (Boophilus) microplus*. The inhibition of oviposition of 77.17% and larval mortality of 71% at highest concentration of 100 mg/ml was observed. The inhibition of oviposition of 75.52% and larval mortality of 65% at highest concentration of 50 mg/ml, followed by 73.15% inhibition of oviposition and 60.2% of larval mortality at 25mg/ml, and 70.61% inhibition of oviposition respectively and 22.6% larval mortality. Ghosh et al. [8] also endorsed a similar findings stated that acaricidal efficacy of 95% ethanolic extracts of *S. anacardium* fruits and *D. stramonium* leaves. the results showed that 50 and 20 percent, respectively, while 50% hydro-ethanolic extracts showed no acaricidal activity. Within 72 hours of application, 95% ethanolic extracts of *D. metel* resulted in 65.0% mortality at 10% concentration. With an increase in extract dose, the probit regression analysis of the *D. metel* extracts revealed a significant increase in the mortality rate of ticks that had been treated as well as a significant inhibition in reproduction.

4. CONCLUSION

The plant extracts under consideration showed varying degree of acaricidal efficacy against *Rhipicephalus (Boophilus) microplus*. *D. stramonium* exhibited highest activity based on their larval mortality, reproductive index and inhibition of fecundity. The study findings also suggest that these can be used in place of synthetic acaricides that are readily available in the marketplace. However, additional research on various tick species is required. To assess the potential of plant extracts, in-vivo testing must also be performed.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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Peer-review history:

The peer review history for this paper can be accessed here:
<https://www.sdiarticle5.com/review-history/94140>