

In-vitro study on acaricidal efficacy of aqueous extracts of *Datura stramonium* against cattle tick (*Rhipicephalus (B.) microplus*) in Udaipur (Rajasthan)

Abstract

The present research was designed to evaluate acaricidal efficacy of aqueous extracts of *Datura Stramonium* against cattle tick *Rhipicephalus (Boophilus) microplus* in Udaipur (Rajasthan). Acaricidal efficacy was evaluated Larval packet Test (LPT) and Adult Immersion Test (AIT). Four concentrations of the *D. Stramonium* extract (12.5%, 25%, 50%, and 100%) and one control group with ~~twice two~~ replications for each concentration ~~were used in the bioassay~~. The ~~H~~highest efficacy in both in-vitro tests (AIT and LPT) was recorded in 100% aqueous extracts of *D. stramonium*. The aqueous extracts of *D. stramonium* has highest IO% at 100% concentration which was 67.46% in AIT. The 100% concentrations of aqueous extracts showed minimum reproductive index ~~(of 0.16)~~. In AIT the decrease in reproductive index with increase in the percent inhibition of oviposition, was evident during the study. In LPT, aqueous extracts of *D. stramonium* has highest efficacy at 100% concentration which was 64%. ~~Larval mortality~~ increased with increasing concentrations of plant extracts.

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Keywords: *D. stramonium*, ticks, larval packet test (LPT) and adult immersion test (AIT)

1. Introduction

India is predominantly an agricultural country where livestock and agriculture are closely associated with each other. For control of the economically important tick species,

organophosphates, pyrethroids and macrocyclic lactones are used at frequent intervals leading to the development of resistance in ticks to most of these chemicals. In [the](#) Indian sub continent, the incidence of acaricide resistance has been widely reported in one host ticks of the genus *Boophilus* (Shyma *et al.* 2013). In order to tackle the problem of resistance and other environmental issues linked with chemical control, efforts have been made to develop sustainable immunological means for controlling ticks and tick-borne diseases through alternative ecofriendly anti-tick natural products. Amongst the natural products, plant extracts and essential oils have been shown to have significant activity against economically important tick species like *Rhipicephalus (Boophilus) microplus* (Sunil *et al.*, 2013). The plant extracts can be made from easily available local plants which have history of some pesticidal or medicinal properties and traditionally used by the people. [The p](#)Plants like *Datura stramonium* was taken into consideration during this study. All parts of *Datura* plants contain high levels of the tropane alkaloids atropine, hyoscyamine and scopolamine which are classified as deliriant, or anticholinergics (Arnett., 1995).

2. Materials and Methods

Plant materials were selected on the basis of available scientific literature and brought into the laboratory. After complete [drying](#), the plant material was powdered in mortar pestle and grinder. The powder of *Datura Stramonium* parts was processed for extract preparation by using maceration methods (Shyma *et al.*, 2014). 100 gm powder [from](#) [were](#) extracted using 400ml aqueous as solvents. The mixtures were kept for 2 days in tightly sealed vessels at room temperature and stirred several times daily with a sterile glass rod. These mixtures were filtered through muslin cloth. Further extraction of residue was done repeating 3-5 times until a clear colorless supernatant [extraction liquid](#) was present indicating that no more extraction from the

Comment [WU1]: Specify how the plant was dried i.e under which conditions? Is it room temperature, under the shade or sun and for how long?

plant material was possible. The extracted liquid was subjected to water bath evaporation at 40 °C to remove the solvent. The semi-solid extract was kept under a ceiling fan to dry.

3. Collection of ticks

The ticks were collected from animals and the cattle sheds during early morning from Vallabhnagar tehsil of Udaipur district (Rajasthan). The ticks collected were preserved in 70% alcohol in clean, well-stoppered glass vials and labeled properly. Permanent mounts of ticks were prepared as per standard keys. The damaged and discolored ticks were removed and remaining engorged female ticks were selected for AIT. The engorged female ticks collected from a particular area were labelled and kept individually in labeled glass tubes covered with muslin cloth. Ticks were put in desiccators maintained at room temperature and 85±5% relative humidity (RH) for oviposition. The eggs were collected after 7 days from commencement of incubation. Each tube containing the first week egg production was labeled to ensure the selection of more uniform batch of larvae for each LPT. The eggs laid were allowed to hatch under uniform conditions of incubation and 14- 21 days old unfed larvae were utilized for the performance of Larval Packet Test (LPT). [Provide the procedure for Larval Packet Test bioassay](#)

3.1. Preparation of working solutions of plant extracts

The working solutions of plant extracts were prepared as per standard protocol. Dried powder was kept in room temperature for 15-20 minutes. Required quantity of extracts were weighed and dissolved in aqueous for making four different dilutions at the rate of 12.5 mg/ml, 25 mg/ml, 50mg/ml and 100 mg/ml. In control group only distilled water was used.

Comment [WU2]: Why didn't you have a positive control?

4. Statistical Analysis

The collected data from the experiment was subjected to statistical analysis using SPSS, version 20.0 for analysis of variance (Snedecor and Cochran, 1980). The treatment means were compared by Duncan's multiple range test (Duncan, 1995) at 5% level of significance ($P < .05$).

5. Results and Discussion

5.1. Efficacy of aqueous extracts of *Datura stramonium* in Larval Packet Test(LPT)

Comment [WU3]: Methodology for this bioassay is missing

The concentrations of aqueous extracts of leaves of *Datura stramonium* of varied ranged from 12.5 to 100 mg/ml. The peak mortality (42.67%) was recorded at a concentration of 100 mg/ml. A total of four treatment groups and one control group were used in experiment. In control group no larval mortality of ticks was observed. A significant larval mortality produced by application of extracts of 50 mg/ml, 25 mg/ml and 12.5 mg/ml were 31.67%, 14.33% and 7% respectively. With the increase in concentration level, the percent mortality rate also increased, as shown in (Table. 1).

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

Concentration of extract (mg/ml)	Live larvae	SE	Dead larvae	SE	% of Larval mortality	SE
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

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

5.2 Efficacy of *Datura stramonium* in aqueous extracts against *Rhipicephalus (Boophilus) microplus* in Adult Immersion Test (IO %)

Comment [WU4]: Methodology for this bioassay is missing

AIT was used in present study to determine the acaricidal activity against *Rhipicephalus (Boophilus) microplus* with various concentration of *Datura stramonium* of aqueous extract; FAO, (2004). The different concentrations of aqueous extract various from 12.5 to 100 mg/ml. In AIT the dependent decrease in reproductive index and increase in inhibition of oviposition was observed 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 48.33, 38.07, 27.80 and 18.81 % respectively. No mortality of ticks was observed at any concentration, as shown in (Table. 2).

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

Conc. of extract (mg/ml)	Live ticks weight (gm) (Mean)	(SE)	Weight of eggs (gm) (Mean)	(SE)	Reproduction 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 P<0.05

Shyma *et al.*, (2014) reported significant acaricidal activities of methanolic *D. stramonium* extracts against *R (Boophilus) microplus* by observing inhibition of oviposition of 77.17% and

larval mortality of 71% at highest concentration of 100 mg/ml, followed by 75.52% of inhibition of oviposition and 65% larval mortality at 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 and 22.6% larval mortality which are similar with the present findings. Ghosh *et al.*, (2015) in acaricidal screening observed 95% ethanolic extracts of *S. anacardium* fruits and *D. stramonium* leaves showed acaricidal efficacy of 50 and 20%, respectively, within 72h, while 50% hydro-ethanolic extracts exhibited no acaricidal activity while as 95% ethanolic extracts of *D. metel* caused significant mortality of 65.0% at 10% concentration within 72 hours of application. On analyzing the acaricidal activities of *D. metel* 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 in reproduction which are similar to our findings.

[Conclusion is missing](#)

6. References

1. Arnett AM. "Jimson Weed (*Datura stramonium*) poisoning". *Clinical Toxicology Review*. 1995;18(3):1-6.
2. Duncan DB. Multiple Range and Multiple F Tests. *Biometrics*. 1955;11(1):1-42. <https://doi.org/10.2307/3001478>
3. FAO. Resistance management and integrated parasite control in ruminants: Uidelines Animal production and health division, Food and agriculture organization of the united nation Rome, 2004, 25-77.
4. Ghosh S, Tiwari SS, Kumar B, Srivastava S, Sharma AK, Kumar S . Identification of potential plant extracts for anti-tick activity against acaricide resistant cattle ticks, *Rhipicephalus (Boophilus) microplus* (Acari: Ixodidae), *Experimental & Applied Acarology*. 2015;66(1):159-171.

5. Shyma KP, Gupta JP, Ghosh S, Patel KK, Singh V. Acaricidal effect of herbal extracts against cattle tick *Rhipicephalus (Boophilus) microplus* using in vitro studies. *Parasitology Research*. 2014;113:19-26.

6. Shyma KP, Kumar S, Sangwan AK, Sharma AK, Nagar G, Ray DD. Acaricide resistance status of *Rhipicephalus (Boophilus) microplus* and *Hyalomma anatolicum* collected from Haryana. *Indian Journal of Animal Sciences*. 2013;83:591-594.

7. Snedecor GW, Cochran WG. *Statistical Methods*. 7th Edition, Iowa State University Press, Ames, 1980.

8. Sunil AR, Amithamol KK, Juliet S, Nair SN, Ajithkumar KG, Soorya VC et al. Acaricidal effect of *Cassia fistula* Linn. leaf ethanolic extract against *Rhipicephalus (Boophilus) annulatus*, *Tropical Biomedicine*. 2013;30(2):231-237.

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